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Simmons, Laura



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# Conservation genetics and population viability of the endangered swamp orchid *Phaius australis* (Orchidaceae) in a changing world

Catherine Laura Simmons, B.Sc. (Hons)

Faculty of Science, Health, Education and Engineering

University of the Sunshine Coast

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## Abstract

Human interference on the natural environment including habitat loss and fragmentation has resulted in a great loss of biodiversity and a major extinction crisis. The swamp orchid *Phaius australis* F.Muell. has a disjunct distribution along Australia's east coast in occurring in the ecotones of coastal wetlands and springs. The orchids are endangered due to past and current illegal collection, continued habitat loss, limited connectivity between populations and because they are an ecological specialist that occurs in ecosystems that are vulnerable to climate change. This study combines the use of genetic and demographic analysis across a 2 000 km latitudinal range to investigate the viability and persistence of *P. australis*.

Population genetic samples were collected at 34 locations from tropical north Queensland to the southern edge of the subtropics in New South Wales. Paired population demographic assessments were undertaken at 25 of these locations over three years to examine population dynamics, estimate population growth rates and determine a representative lifecycle for the species. A pollination experiment was carried out in a large stable population to test for evidence of self-pollination. Current population vulnerability and vulnerability to future rainfall and temperature changes have been modelled through population viability analysis (PVA) based on climate related vital rates and SimCLIM generated climate change predictions for two emissions scenarios.

Morphology assessments positively identified current *P. australis* population locations and population sizes in Australia. Populations in the southern extent of the species range are under greater human induced threats. NextGen developed microsatellite markers assisted with identifying genetic similarities with congener *Phaius bernaysii* and differences with Asia-Pacific *P. tancarvilleae*. It is likely that the scarce yellow *P. bernaysii* is the expression of a recessive allele of the more abundant purple *P. australis* in the one remaining population where the species co-occur.

Spatial patterns of genetic diversity indicate post-colonisation divergence from the tropics southwards to current climate niche limits. Genetic diversity is low across all populations ($A$=1.5, $H_e$=0.171), with little evidence of genetic structure or differentiation between regions or populations. Consistent with population genetic theory, the historic loss of populations has resulted in significantly lower genetic diversity in small populations compared to large ($P$, $A$, $He$; $p$<0.05). *Phaius australis* is not inbred, despite small population sizes. Instead, *P. australis* trends towards outbreeding in the majority of populations (mean $F$=-0.274). There is a great presence of cloned genotypes within populations and pollination experiments support that

spontaneous self-fertilisation is occurring in wild populations. Outbreeding could therefore be a result of heterozygote advantage observed in floral success or due to the proliferation of cloned individuals masking inbreeding effects.

Larger populations are more dynamic than small populations. At current locations under current climate, only large populations are predicted to increase greatly in population size over a 100-year PVA model duration. While some populations are predicted to decrease in size including one of high conservation value, none are predicted to go extinct from demographic processes.

The changes in climate that are predicted to occur in the coming 100 years include increases in mean maximum temperature of the hottest month (TMAX) and decreases in precipitation of the wettest quarter (PJFM). These changes may result in some locations across the species range having conditions that are less favourable for survival and reproduction than others. Minimal changes in population growth are predicted over a 100-year timeframe for all populations under the PVA model using the 'best case' emissions scenario (RCP 2.6) compared to the 'current climate' model. The 'worst case' climate change emissions scenario (RCP 8.5) PVA model predicts increased population growth at several southern populations where conditions are predicted to become more tropical and reduced population growth at locations where climate conditions are predicted to become hotter and drier.

The results of this study indicate a mostly positive future for the endangered *Phaius australis* in Australia. The ability to self-pollinate increases the natural survival ability of a species with low genetic diversity. Of high conservation significance are populations with unique alleles or genotypes, including the one known population of *P. bernaysii*. The PVA analysis indicates continued persistence, survival and growth of many populations in current locations under current and future climates and has identified some populations with declines in population growth. Small populations have limited population growth potential, combined with lower genetic diversity making them more vulnerable to stochastic events. Large populations within the same region are ideal sources of propagative material for future population augmentation or translocation to improve natural survival.

## Declaration of Originality

This thesis is my own account of research undertaken by me, wholly completed during the period of my candidature at the University of the Sunshine Coast. The thesis does not contain as its main content any work or material which is embodied in a thesis or dissertation previously submitted by me or any other person for a University degree or other similar qualification at this or any other higher education institution. To the best of my knowledge and belief, this thesis contains no material formerly published or written by another person, except where due and proper reference has been given in the thesis text.

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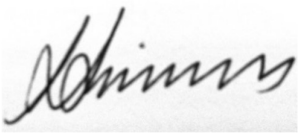

Catherine Laura Simmons

Date:

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# Chapter 1: General Introduction

## 1.1 Assessment of threatened plants in a changing world

The research in this thesis concerns the range and biological characteristics of an orchid species, to provide an informed view of how its occurrence may be impacted by factors ranging from human activities to climate change. This chapter explains the rationale for and the importance of this thesis by examining relevant literature and theory for threatened species conservation in a changing world. It introduces theory and current research related to habitat fragmentation, primarily that related to population size and isolation, as well as the effect that climate change may have on the survival of plant species. A brief introduction to the study species is given, and the aims and the outline of the subsequent research chapters are provided.

## 1.2 Climate change and global biodiversity

Climate change is increasingly recognised as one of the greatest global threats to biodiversity (Hilbert et al. 2007, Thuiller et al. 2008, Bellard et al. 2012). In 132 years from 1880 to 2012, average global temperature has risen by 0.85°C and precipitation patterns have changed, leading to longer periods of drought and higher frequency of extreme rain events (IPCC 2013a). Average temperatures in Australia are projected to increase by 0.6-1.5°C by 2030 and 1.0-5°C by 2100 with a higher frequency and longer duration of heat waves (IPCC 2013a, CSIRO and BOM 2014, Reisinger et al. 2014). Patterns of precipitation are predicted to continue to change. The predicted changes to precipitation vary across Australia, because of the ocean and atmosphere circulations that surround the continent (Reisinger et al. 2014). Precipitation is predicted to decrease by 5-30% in southern Australia, especially during the wet season (winter and spring). Precipitation in the north of Australia is predicted to become more variable and less predictable than precipitation patterns currently experienced (CSIRO and BOM 2014). Droughts are expected to become more frequent and more severe and extreme rainfall events are predicted to increase in frequency and severity (CSIRO and BOM 2014). The global sea level has risen by 0.19 m from 1901 to 2010 (IPCC 2013a). Future sea level around the Australian coastline is likely to rise 0.28-0.98 m by 2100, which will increase the frequency of extreme sea-level events such as storm surges (CSIRO and BOM 2014, Reisinger et al. 2014). The magnitude and the rate of future projected climate change are already greater and faster than historic climate change, a trend which is predicted to continue in the future (IPCC 2013a).

Physical changes in response to climate, such as sea level rise pose great threat to species in low lying and coastal areas. Altered soil salinity and inundation occasionally due to storm surge or permanently may render habitat unsuitable in such locations (Hughes 2003, Root and Schneider 2006, Karim and Mimura 2008, Kingsford and Watson 2011, Maschinski et al. 2011).

Predicted immediate responses of plants to climate change include shifts in phenology which have been observed more widely in the northern hemisphere than the southern hemisphere (Walther et al. 2002, Menzel et al. 2006, Parmesan 2006, Root and Schneider 2006, Laurance et al. 2011a, Pearson et al. 2014, Beaumont et al. 2015). Phenological shifts can lead to disrupted plant-pollinator interactions in flowering plants, resulting in a lack of seed set, thus altering population dynamics and survival (Jump and Peñuelas 2005, Parmesan 2006, Bellard et al. 2012). Some, but not all plants possess a level of reproductive assurance through an ability to spontaneously self-pollinate (autogamy), potentially assisting the survival of a plant species by decreasing its reliance on pollinators (Kalisz et al. 2004, Morgan and Wilson 2005, Willmer 2011). Plants that possess this biological trait may be less vulnerable to the current and predicted climate change induced shift in plant-pollinator interactions, overcoming the effect that this aspect of climate change may have on their survival (Sakai et al. 2001, Ramula et al. 2008, Willmer 2011, Suetsugu 2013). Therefore it becomes important to understand the reproductive mechanisms of rare and threated species when assessing risks to a species from changes in climate.

Climate change has the potential to threaten species beyond phenology and plant pollinator relationships because species distribution on medium spatial scales is primarily controlled by temperature and precipitation, termed a 'bioclimatic envelope' (Good 1931, Pearson and Dawson 2003, Hilbert et al. 2007, Kingsford et al. 2009, Kingsford and Watson 2011). Species with a spatial distribution across broad latitudes are likely to experience different climate conditions such as temperature and precipitation at different parts of their range. Temperature and moisture availability play critical roles in seed germination, growth and survival and reproductive output and success (Woodward and Williams 1987, Lawton 1993). Therefore, populations across a range may not have equal ecological advantages (Lawton 1993). Density and abundance in populations is therefore expected to vary across a plant species range, particularly if the range covers a broad climate (Lenoir et al. 2008, Thomson and Parker 2008, Winkler et al. 2009, Walck et al. 2011, Byrne et al. 2013). As a result, species may be more abundant in the centre of their geographical distribution, or in areas of optimal climate and decline towards the outer edges of suitability, which may also affect genetic

diversity (Hengeveld and Haeck 1982, Brown et al. 1996, Jump and Woodward 2003, Vaupel and Matthies 2012). Along the east coast of Australia a species will experience greater precipitation and warmer temperatures at the northern edge of species range than in the south. As a result, a plant that responds to greater moisture and warmer temperatures for growth or reproduction may have larger, denser populations that flower more abundantly in the northern populations compared to populations in the southern edge of the range. While there is uncertainty as to how true the abundant centre theory holds for all plants (Sagarin and Gaines 2002, 2006, Duffy et al. 2009, Abeli et al. 2014, Aikens and Roach 2014), there is an assumption that under predicted climate change, species may spatially track their optimal climatic niche in 'range shifts' (Thomas et al. 2004, Parmesan 2006, Gienapp et al. 2008, Bellard et al. 2012).

Range shifts are predicted to be more obvious on a local scale due to the differing performance in local habitats (Sletvold et al. 2013, Lenoir and Svenning 2015). A species may show signs of trailing edge retraction by becoming less vigorous and have a lower reproductive output as conditions become less than optimal in different parts of the current distribution under a changing climate (Jump and Peñuelas 2005, Hedhly et al. 2009, Bellard et al. 2012, IPCC 2013b). This can lead to lowered abundance and changes in spatial structure that are intermediately observable in altered recruitment and reproduction, declines in population growth and regeneration at parts of the species geographic distribution with less than optimal climate (Barry et al. 1995, Le Roux and McGeoch 2008, Lenoir et al. 2008, Thomson and Parker 2008, Jiguet et al. 2010, Dolanc et al. 2013). Conversely, increased density or abundance of floral outputs may occur in the parts of the range that become more climatically suitable, a leading edge expansion (Jiguet et al. 2010, Dolanc et al. 2013). Thus, plant species that have supressed reproductive or growth activity at latitudes and altitudes that are currently less favourable may benefit from increasing temperatures if they have the ability to expand (Pearson and Dawson 2003, Jump and Peñuelas 2005, Murphy et al. 2010). Evidence of leading edge expansion and trailing edge retraction have already been observed for some species (Walther et al. 2005, Dang et al. 2010, Catling and Oldham 2011, Shimazaki et al. 2011, Saintilan et al. 2014, Lenoir and Svenning 2015). However, several authors detail a lack of evidence of range shift or migration for other species (Kelly and Goulden 2008, Le Roux and McGeoch 2008, Corlett and Westcott 2013), thought to be due to generation turnover and the lag between climate change and migration (Huntley 1991, Davis and Shaw 2001, Malcolm et al. 2002, Neilson et al. 2005).

Many climatic factors and predicted changes are different for in the southern hemisphere compared to the north (IPCC 2013b). A large knowledge gap remains of how future climate change may affect plant species in the southern hemisphere and the tropical parts of the world, particularly low-lying areas (Hilbert et al. 2007, Laurance et al. 2011a).

### 1.3 Habitat fragmentation and biodiversity loss

Human population growth and expansion, particularly in the last half century, has had incredible impact on the natural world (NRMMC 2010, UNEP 2012). In Australia, urban population expansion in northern New South Wales and south-east Queensland has reduced high value natural areas to fragments of their pre-European settlement size (Groves 1994, Groves and Catling 2006, NRMMC 2010). Natural ecosystems are now trending towards small and isolated, sub-divided remnant patches surrounded by a matrix of different land-uses (Hobbs and Yates 2003, Lindenmayer and Fischer 2006). Habitat loss and fragmentation of natural ecosystems can have a devastating effect on the survival of species (Honnay et al. 2005). The number of flora species at risk of extinction worldwide is increasing with 1340 flora species listed under the Australian federal *Environment Protection and Biodiversity Conservation Act 1999* (the 'EPBC Act'). One of the greatest barriers to species movement to a new climatic niche is habitat availability and the legacy that fragmentation already has on impeding the survival of species in the future (Thomas et al. 2004, Araújo et al. 2006, Brook et al. 2008, Thuiller et al. 2008, Huntley et al. 2010, Fordham et al. 2012).

#### 1.3.1 Ecological and demographic effects of habitat fragmentation

Habitat loss reduces the number of species and plant population sizes because population size is correlated with patch area (Hanski and Gilpin 1997, Krebs 2009, NRMMC 2010). Loss of patch size can physically change the habitat over time so that it is no longer suitable for survival of particular species directly resulting in the decline of numerous species (Saunders et al. 1991, Harper et al. 2005, Swarts et al. 2009, Newman et al. 2013, Chung et al. 2014). A reduced habitat patch size also causes significant alterations to radiation and temperature balances, exposure to winds or severe weather damage, modification of water regimes, erosion and encroachment of weeds (Hobbs and Yates 2003, Harper et al. 2005, Lindenmayer and Fischer 2006, Brown et al. 2012). These effects make plant populations more susceptible to environmental and demographic variability (Lande 1993, Hobbs and Yates 2003). For

example, Jacquemyn et al. (2010) found that the threshold size for flower initiation was almost three times greater in shaded environments compared to light exposed environments which made the difference between a stable and growing population in some species. Similarly, small fragmented populations of *Silene otitis* had fewer juveniles and less seed set in Germany (Lauterbach et al. 2012). However, others have found no negative effects of patch size on species survival (Neal et al. 2010), requiring a species by species approach.

Fragmentation also increases the isolation and the distance between populations of species as well as the spatial arrangement of populations (NRMMC 2010). Plant pollinator interactions may be interrupted with fragmentation due to pollinators moving less among small, sparse and non-continuous patches (Young and Clarke 2000, Ghazoul 2005, Grindeland et al. 2005,Aguilar et al. 2006, Menz et al. 2013). Population dynamics can be affected via the constraints to successful sexual reproduction in plants resulting in decreased fruit set (Agren 1996, Mustajärvi et al. 2001, Broadhurst and Young 2006). For example, plant density in fragmented patches of the globeflower *Trollius europaeus,* was found to limit reproductive success through complex changes in the pollinator abundance (Klank et al. 2010). Unsuccessful fertilisation was strongly related to population size and density in fragmented populations of *Acacia dealbata* (Broadhurst and Young 2006). However other studies have reported no difference in reproductive success in isolated or fragmented populations with other factors more important (Grindeland et al. 2005) or land use changes creating a more desirable environment for pollinators (Newman et al. 2013). Thus, threatened species management based on broad generalisations may not be effective for a particular species and location without sufficiently detailed supporting scientific evidence and interpretation (Coates and Atkins 2001).

### 1.3.2 Habitat fragmentation and population genetics

Genetic variation and diversity enables a species to persist in the face of abiotic and biotic change and to cope with the challenges of pathogens and disease (Frankham 1995, Falk et al. 2006). A diversity of genotypes also provides a platform to enable survival and selection in altered conditions. Negative impacts to reproduction and survival have been associated with lost genetic diversity (Reed and Frankham 2003, Frankham 2005, Lauterbach et al. 2012, He 2013). Higher genotype diversity was found to increase the level of resistance to the pathogen *Phytophthora cinnamomi* for *Banksia* species in Australia (He 2013). Higher genetic diversity was associated with regeneration in an endemic Indian tree *Myristica malabarica* (Nagaraju et

al. 2013). Lauterbach *et al.* (2012) found that increased genetic diversity was correlated with increasing plant size in *Silene otitis*, an endangered plant in fragmented grasslands in Germany. Because the loss of genetic diversity can put species at risk of extinction, genetic studies are integral for threatened species management (Frankham 2005).

Genetic diversity within populations is maintained in equilibrium between many factors: breeding system, selection for fitness, genetic drift within populations, periods of time with low or large number of individuals and gene flow between populations (Falk and Holsinger 1991, Frankham 1995, Lowe et al. 2004, Frankham 2005). Genetic drift occurs as genetic diversity and variation is eroded over time via loss of alleles through chance mating and bottlenecks (Wright 1931, 1948, Nei et al. 1975, Ellstrand and Elam 1993). Inbreeding which can include mating within a similar gene pool, between close relatives, and self-fertilisation erodes heterozygosity, leading to the fixation of homozygous genotypes (Keller and Waller 2002, Frankham 2010a). When population size is reduced through environmental or human activities, genetic diversity and variation within the population is reduced due to a direct loss of genetically distinct individuals and consequent bottlenecking (Ford 1971, Nei et al. 1975, Ellstrand and Elam 1993, Young et al. 1996, Young and Clarke 2000, Lowe et al. 2004, Leimu et al. 2006). For example, genetic diversity in the endangered *Philotheca sporadica* decreased with decreasing population size and increasing isolation (Shapcott et al. 2015).

Small, fragmented populations often exhibit higher rates of inbreeding and therefore are more prone to the deleterious effects of inbreeding depression (Ellstrand and Elam 1993, Young et al. 1996, Leimu et al. 2006, Honnay and Jacquemyn 2007). Some orchids around the world have been found to have higher homozygosity in small populations (Gustafsson and Sjögren-gulve 2002, Swarts et al. 2009, Chung et al. 2012). Greater rates of homozygosity can result in exposure to deleterious recessive alleles that are usually masked by heterozygous dominants (Frankham 1995, 2010a). 'Inbreeding depression' or an inability to mask these disadvantageous alleles has been observed through reduced reproduction output or success, vigour or viability affecting the growth and persistence of populations (Agren 1996, Young et al. 1996, Young et al. 2000, Pullin 2002, Ramula et al. 2007, Frankham 2010a). However, Duffy et al. (2009) found no evidence of inbreeding depression in *Neotinea maculata* indicating that the theory may not apply in all circumstances and therefore be applied with caution.

Gene flow between populations, either by pollen or seed maintains the connection and some level of similarity between populations, reducing genetic differentiation and structure between populations (Wright 1943, Lowe et al. 2004, Falk et al. 2006, Cozzolino et al. 2007,

Qian et al. 2013). The long-distance dispersal of small seed is thought to allow high levels of gene flow and lower the genetic differentiation between populations (Ellstrand 1992, Hamrick and Godt 1996). This has been demonstrated for several species of orchid which have minute seeds (Peakall and Beattie 1996, Alexandersson and Ågren 2000, Devey et al. 2009, Swarts et al. 2009). However, in several other orchid species, the loss of connectivity between populations has allowed populations to differentiate (Hamrick and Godt 1996, Gustafsson and Sjögren-gulve 2002, Wallace 2002, Forrest et al. 2004, Duffy et al. 2009).

While much attention has been paid to latitudinal variation in species richness, little is known about variation in the diversity of genes in individuals or populations along latitudinal gradients (Gaston 2000). Founding populations often possess subsets of the full genetic diversity from the source population as they are dispersed (Wright 1943, Austerlitz et al. 1997, Excoffier et al. 2009). Refugial, isolated or older populations may be more distinct because they are older and have been isolated longer than leading-edge populations (Hampe and Petit 2005, Chung et al. 2012). The breeding system of a species also leaves a legacy on patterns of genetic diversity (Hamrick and Godt 1996, Lowe et al. 2004). Self-fertilisation can fix genotypic frequencies and lead to a reduction in genetic variation and the formation of family structures within populations (Wright 1931, 1948, Hamrick and Godt 1996, Leimu et al. 2006, Honnay and Jacquemyn 2007). A North American self-fertilising orchid species *Isotria medeoloides* was found to have strong genetic structure between populations (Stone et al. 2012). The way that genetic patterns are formed is vast, leading to challenges for management or recovery of rare species to decipher whether genetic diversity and structure are due to colonisation processes, a breeding pattern or to habitat fragmentation (Eckert et al. 2008).

### 1.4.1 Climate change: migration or persistence in situ?

Bioclimatic predictive models have been used to evaluate the projected climate change effects on a species specific occurrence by statistically associating a species' occupancy or abundance records to spatial environmental and climatic variables that are thought to affect a species distribution and migration ability (Thomas et al. 2004, Araújo et al. 2006, Elith and Leathwick 2009, Araújo and Peterson 2012). Several such recent studies have been undertaken in the southern hemisphere (Elith and Leathwick 2009, Delean et al. 2013, Vanderwal et al. 2013). However, the velocity of movement required by species to track the migration of a climatic niche may be much faster than previously thought and may exceed the generation time for long lived stationary species, such as perennial flora (Corlett and Westcott 2013, Vanderwal et

al. 2013). The fragmentation of natural areas further complicates the ability of species to move to new suitable habitat under predicted climate change because the occurrence of suitable, yet unoccupied habitat is reduced, with less stepping stones to connect and facilitate migration between large patches and larger populations (Huntley 1991, Hanski and Gilpin 1997). The predicted migration of species needs to go beyond the habitat suitability and availability and instead also examine the biological factors that may limit dispersal, movement and expansion of a species (Thomas et al. 2004, Araújo et al. 2006, Midgley et al. 2006, Fordham et al. 2012). Orchids, with their small, lightweight seed, may be able to migrate over large distances to new suitable habitat in response to climate change and create viable populations if exponential growth follows establishment (Arditti and Ghani 2000, Jacquemyn et al. 2009, Molnár et al. 2011).

Simulation models such as population viability analysis (PVA) based on species-specific data can be used to evaluate the growth or decline of a population and estimate the vulnerability of a population to extinction (Akçakaya and Atwood 1997, Caswell 2001, Akçakaya 2004, Keith 2004). PVA is a means of gaining a mechanistic understanding of the responses of species to different ecological processes, the impacts of human activities as well as identify biologically crucial viability parameters (Fieberg and Ellner 2001, McCarthy and Cary 2002, Akçakaya 2004, Keith et al. 2008). For example, Knight *et al.* (2009) quantified the levels of deer predation on life-stage classes of a forest understory plant that would be sufficient to cause the loss of the species from that habitat.

In a climatic context, the populations occurring in wetter environments with little disturbance were important for population growth of *Pedicularis furbishiae* (Menges 1990). Similarly, PVA highlighted that climatic cues were more important to the survival of *Cypripedium reginae* than the cessation of grazing (Kéry and Gregg 2004). In contrast, Simmons et al. (2012) found that populations of a palm distributed across 300-875 m did not exhibit upward migration of their climatic envelope in response to changed climatic conditions over the last 40 years. The most effective use of PVA is to evaluate, compare or rank the response of a species to an impact, undertaken by modifying the effect an activity is predicted to have on specific vital rates (Fieberg and Ellner 2001, McCarthy and Cary 2002, Akçakaya 2004, Keith 2004, Keith et al. 2008, Pearson et al. 2014). The PVA methodology is underutilised in species conservation management despite the successful outcomes it can provide (Menges 2000, Keith 2004, Driscoll et al. 2010).

Predictive global emission scenarios that are input to global climate models (GCM) are frequently utilised in predicting the future climate of a country, region or location (Perkins et al. 2007, Warrick 2009, IPCC 2013a, Perkins et al. 2014). With knowledge of how current climate affects the vital rates of a species, predictions can be made on how a species may biologically respond in future climates (Maschinski et al. 2012b, Pacifici et al. 2015). When climatically related vital rates are incorporated into a PVA, the comparative effect on population growth under changed conditions compared to current viability and expansion potential can be assessed (Maschinski et al. 2006, Andrello et al. 2012, Molano-Flores and Bell 2012, Shryock et al. 2014, Pacifici et al. 2015). Matrix modelling predicted slow population declines of the endangered *Purshia subintegra* in Arizona due to drier environmental conditions expected under climate change (Maschinski et al. 2006). Similarly matrix modelling of an arid-zone tree species *Acacia peuce* found an increased likelihood of extinction under future climatic change based on the impacts that low rainfall years have on demographic and reproductive rates (Raghu et al. 2013).

A species may have to persevere *in situ* where migration possibilities are limited by biology, life-history traits, or through a lack of suitable habitat in a fragmented landscape (Huntley 1991, Davis and Shaw 2001, Gienapp et al. 2008). High levels of genetic diversity and strong directional selection pressures may allow some degree of selection and adaptation to take place, over several generations (Lande 1988, Jump et al. 2009, Pfennig et al. 2010). However, spatially specific changes in climate are expected to be variable in the short-term, thus adaptive mechanisms may take generations to take place, far too slow for long-lived, sessile organisms to keep pace with rapid climate change (Bradshaw and McNeilly 1991, Jump et al. 2009, Benito Garzón et al. 2011, Hoffmann and Sgró 2011). Species with genetic and genotypic diversity that occurs across a broad range of environmental conditions may possess genotypes adapted for the entire range of environmental conditions (Bradshaw 1965, Bradshaw and McNeilly 1991). These species are thought to have greater survival potential than species with genotypes adapted to a narrow environmental range (Davis and Shaw 2001, Gienapp et al. 2008). When used in combination, genetic analysis and the mechanistic approach using demographic rates in PVA may provide a well-rounded assessment of a species risk to climatic changes (Pacifici et al. 2015).

### 1.4.2 Conservation management in a changing world

Conserving biodiversity is an essential part of safeguarding the biological life support systems on Earth as all living creatures, including humans, depend on these systems for the necessities of life (Frankel et al. 1995, Costanza et al. 1997, NRMMC 2010). Conservation of biological diversity into the future includes the *in situ* conservation of ecosystems, species and the genetic composition of the species within them as well as *ex situ* conservation as a last resort measure (Frankel et al. 1995, Bertzky et al. 2012, UNEP 2012). Thus, appropriately detailed knowledge of a species genetics, life history, biology and climate based ecology is required to undertake predictions and risk assessments on how species may respond to future climate change (Frankel et al. 1995, Scoble and Lowe 2010). Where a species may not be able to survive in current locations under future climate, populations may need to be re-created within historical range or perceived suitable habitat outside historic range (Thomas 2011, Dalrymple et al. 2012).

Restoration practitioners have been undertaking plant enhancements and translocations for years to develop self-sustaining populations of the threatened taxon (Maunder 1992, Hoegh-Guldberg et al. 2008, Weeks et al. 2011, Dalrymple et al. 2012). Existing guidelines for conducting reintroduction or translocation of plants to ensure populations have the highest possible chance of success can equally be utilised for assisted migration under climatic changes (Falk 1990, IUCN 1998, SERI 2004, Vallee et al. 2004, Weeks et al. 2011, Maschinski et al. 2012b). Assisted migration or managed relocation involve moving individuals to facilitate or mimic a natural range expansion for a species and is recognised as a last resort measure for species that face high risk of extinction under climate change (Thomas 2011, Maschinski et al. 2012b). The use of genetic data provides an evolutionary context that can be used to lessen negative genetic consequences such as inbreeding and increase evolutionary potential (ability to adapt) by choosing the founding individuals to be introduced (Rice and Emery 2003, Frankham 2005, Reusch et al. 2005, Neale 2012, Shapcott et al. 2015).

Extensive impacts are predicted for plant species under projected climate change and the current challenge is to mitigate the loss of species in the face of a changing world (Keith et al. 2008, Thuiller et al. 2008, Williams et al. 2008, Holt 2010). Threatened species are of particular concern because of their potentially small population sizes, ecological niche specialisation and because they are less resilient to demographic and environmental stochasticity (Falk 1990, Ellstrand and Elam 1993, Frankham 2005, Pearson et al. 2014). There is an urgent need to plan and prepare for the range of responses that an organism may have to climate change to

identify the most effective schemes for conservation that enable the survival of taxa under new conditions (Gaston 2000, Hughes 2003, Root and Schneider 2006, Hilbert et al. 2007, Hoegh-Guldberg et al. 2008, Williams et al. 2008, DECCW 2010).

## 1.5 Study species

Approximately 10% of the all native Australian orchid species are listed in a threatened species category under the Australian *Environmental Protection and Biodiversity Conservation Act* 1999 '*EPBC Act*' (Commonwealth; Clements and Jones 2008, DE 2015a). The swamp orchid *Phaius australis* F.Muell. is one such endangered terrestrial orchid that produces tall inflorescence stalks with multiple large flowers time (Dockrill 1992, Jones 2006, Freeman et al. 2011; Figure 1.1). *Phaius australis* occurs in the ecotones between freshwater swamp wetlands and the margins of paperbark and sclerophyll forest, littoral rainforest or open swamps with permanently wet freshwater soils on the east coast of Australia (Sparshott and Bostock 1993, Benwell 1994, Jones 2006, Stephens and Sharp 2009; Figure 1.2). *Phaius bernaysii* Rowland ex Rchb.f. is physically identical to *P. australis* other than its yellow-flowers and occurs in South East Queensland (Sparshott and Bostock 1993, Benwell 1994).

Figure 1.1. Swamp orchids from left *P. australis* plant, middle top *P. australis* flower and bottom *P. bernaysii* flower all from Stradbroke Island; right small *P. australis* colony in Yamba.

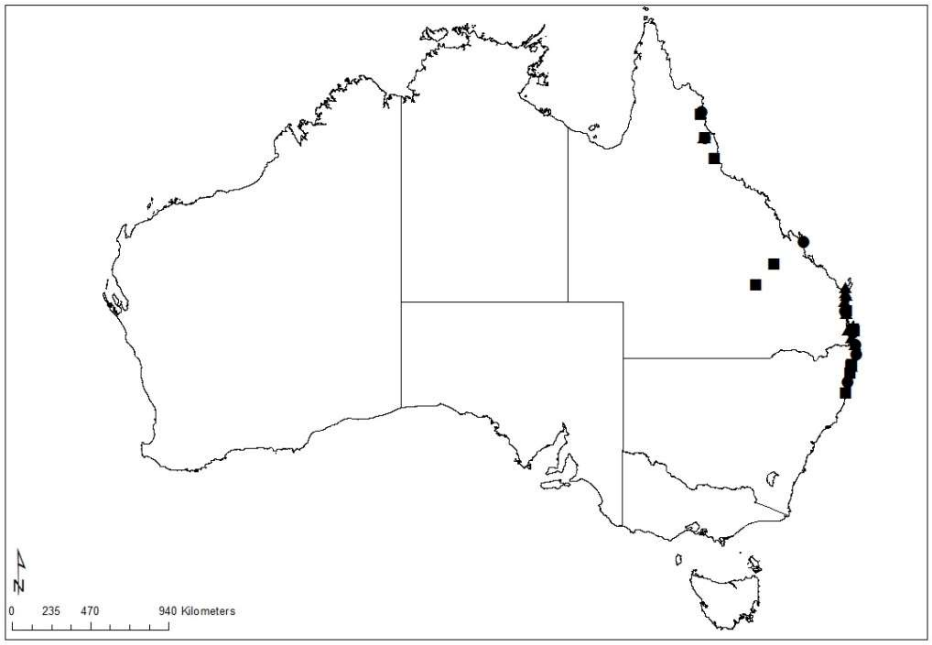

Figure 1.2. Geographic distribution of *P. australis* (DE 2015a).

Originally described as *Phaius grandifolius* in 1858 from material collected on Lady Elliot Island, the swamp orchids have been taxonomically in flux until early in the 21st century. *Phaius australis* is very closely related to south-east Asian species, *Phaius tankervilleae* (Banks ex L'Herit.) which has a variety of synonyms and *P. wallichii* Lindl. (Cribb et al. 2004, Clements and Jones 2008). *Phaius australis* and *P. tankervilleae* were both historically described as occurring in Australia, with subtle morphological differences utilised to differentiate the two species (Stanley and Ross 1983, Dockrill 1992). This document uses the spelling '*Phaius tancarvilleae*' to refer to populations of *Phaius tankervilleae* Banks ex L'Herit. in Australia, as this was the latest accepted nomenclature prior to revision of the genus in Australia in 2008 (Clements and Jones 2008, CHAH 2010, DE 2015b). Clarification of taxonomy leads to improved conservation management (Hopper 2009).

## 1.6 Thesis aims and objectives

The overall objective of this thesis is to investigate the potential long-term viability and extinction risk of the endangered swamp orchid *P. australis* currently and under future climate change scenarios by combining population genetics, dynamics and population viability analysis (PVA) with future climate change predictions. The PVA approach relies on the collection and

quantification of sufficient biological data over multiple seasons or years, which is seldom available or used (Ellner et al. 2002, Keith et al. 2008, Pearson et al. 2014).

Specifically this study aimed to:

(1) Clarify the taxonomy of *P. australis* and determine the current distribution, size and threats to its populations in Australia to test if the species is in decline.

(2) Test if fragmentation, particularly population size and isolation, has negatively impacted on population demography and reproduction of *P. australis* across its range.

(3) Test if climatic differences across the species' range may affect the reproduction and population dynamics of *P. australis* consistent with the abundant centre hypothesis.

(4) Test if fragmentation, climate and/or breeding system have negatively impacted on the levels of genetic diversity and structure of *P. australis* consistent with current hypotheses.

(5) Use population viability modelling to investigate if recent climatic changes are impacting on species' population dynamics and predict the viability of populations under future climatic conditions.

The results of this study will have application for the long-term conservation and management implications for *P. australis* taking into account possible climate change impacts. Each chapter is structured independently for later submission for publication, thus some repetition of core material and concepts is unavoidable.

Chapter 2 reports the development of microsatellite loci for *P. australis* and tests for cross-amplification to *P. bernaysii* and *P. tancarvilleae*. It aims to clarify the taxonomic differentiation between *P. australis*, *P. bernaysii* and *P. tancarvilleae* using a combination of approaches. It then aims to determine the current distribution, threats, size and morphology of current *Phaius australis* populations.

Chapter 3 addresses the range wide population genetic diversity, structure and connectivity of *P. australis*. The patterns of population genetics are then examined in the context of three competing hypotheses: the historic southward colonization of the species across a climatic range, the abundant centre hypothesis as the species has colonised, or that fragmentation has affected density and isolation resulting in a loss of genetic diversity.

Chapter 4 reports the study of the breeding system, reproduction and the potential occurrence of inbreeding in *P. australis*.

Chapter 5 reports the population dynamics of *P. australis* across the species' climatic range. This chapter also provides the results of the long-term survival of *P. australis* under current conditions and two potential climate change scenarios.

A summary of the research findings, significance of the research, implications for conservation management of the species and future research directions are provided in Chapter 6.

# Chapter 2: What and where is *Phaius australis*? Establishing microsatellite molecular markers for population genetic analysis of endangered swamp orchids *P. australis* and *P. bernaysii*.

## 2.1 Introduction

The family Orchidaceae is the most successful flowering plant group with over 26 500 recognised species worldwide (Dixon et al. 2003, WCSP 2015). The success of the family is due to adaptations to the environment and unique reproductive and survival techniques leading to the diversity of species and the habitats they occupy (Arditti 1992, Otero and Flanagan 2006, Swarts et al. 2010). Ecological specialisation has, however, often resulted in a naturally rare, discrete or patchy occurrence of many orchid species within landscapes that can make them naturally vulnerable to extinction (Dixon et al. 2003, Smith and Read 2008, Swarts et al. 2010). Like many novel plant groups, orchids can inspire people to cultivate and collect, on occasion leading to exhaustive sourcing and removal of rare occurring specimens from the wild to the point of extinction (Koopowitz 2001, Dixon et al. 2003).

Land-use change has destroyed both the quantity and quality of habitat, reducing connectivity and increasing isolation between already fragmented, patchy or ephemerally distributed orchid species (Thrall et al. 2000, Hobbs and Yates 2003). Continuing degradation alters the fine environmental conditions required for survival, and increases the exposure of ecological specialists to severe conditions (Saunders et al. 1991, Hobbs and Yates 2003, Lienert 2004). Such land-use change can lead to increased extinction risk through the ecological, genetic and demographic effects of reduced population or patch size as well as reduced gene flow between populations (Young and Clarke 2000, Newman et al. 2013). This is turn could decrease the potential for a species to persist in the face of abiotic and biotic environmental change (Falk et al. 2006). Orchids currently comprise 17% (218) of all total threatened flora species listed under the Australian Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act; Jones 2006, DE 2015c).

Australia's tallest terrestrial orchid is the Swamp Orchid, *Phaius australis* F.Muell. (DE 2015a). It occurs in disjunct populations in marginal swamp wetlands and littoral rainforests distributed across a 2 000 km latitude gradient along the east coast of Australia, from north coast New South Wales to north Queensland (Dockrill 1992, Clements and Jones 2008). This distribution coincides with locations desirable for urban development, thus, populations have become highly fragmented (Benwell 1994, Jones 2006, DE 2015a). While *Phaius* orchids were probably always confined to limited areas, in 1994 it was estimated that 95% of the

populations have been lost since European settlement (Benwell 1994). This species is one of the most desirable for collections, becoming first protected under the Native Plants Protection Act of 1930 (Qld). Continued threats and vulnerability to climate change have resulted in its current listing as 'endangered' under the EPBC Act (Koopowitz and Hawkins 2012, DE 2015c). The loss of remaining individuals and populations with small, fragmented populations could result in reduced genetic diversity (Ellstrand and Elam 1993, Lienert 2004, Neale 2012). The development of microsatellite loci for later genetic diversity analyses would be beneficial for the species conservation as it has been for other orchids (Duffy et al. 2009, Swarts et al. 2009), particularly with future climate change and potential for assisted migrations or population enhancements (Hilbert et al. 2007, Kingsford and Watson 2011).

*Phaius australis* has large 3-7 cm ovoid pseudobulbs above the ground, with 4-7 large, plicate, robust leaves to 2 m tall (Stanley and Ross 1983, Stephens and Sharp 2009). The yellow swamp orchid *Phaius bernaysii* Rowland ex Rchb.f. is a congener from South East Queensland and is also listed as federally 'endangered' on the EPBC Act (Sparshott and Bostock 1993, Benwell 1994, DE 2015a). *Phaius bernaysii* is distinguished from *P. australis* by its yellow-green flowers, compared to the deep purple and cinnamon brown flowers of *P. australis*. The plants are identical when not in flower. Both species have the ability to produce multiple inflorescences per plant that can be over two metres tall, with four to twenty flowers that are produced in August-October (Benwell 1994, Jones 2006, Freeman et al. 2011). Flowers are 7-10 cm across but can be as large as 17 cm, opening sequentially from the base to the top of the flower spike one or two at a time (Dockrill 1992, Bishop 2000). No pollinators are known for *Phaius australis* however the species has been observed to spontaneously self-pollinate (autogamy) in natural populations, due to the lack of a rostellum to separate male and female flower parts (Benwell 1994, Jones 2006).

*Phaius australis* is very closely related to *Phaius tancarvilleae* Banks ex L'Herit., found in the Pacific Islands and parts of south-east Asia, and *P. wallichii* Lindl. (Clements and Jones 2008, DE 2015a). These names have been applied interchangeably to the populations now recognised as *P. australis* in Australia on the basis of differing floral morphology however, *P. tancarvilleae* is still recognised to some degree within the orchid growing community in Australia (DE 2015c). Little is known of the numbers of individuals and populations across the species range and observations of some variation in the flowers have been described in the widespread *P. australis* (Benwell 1994, NPWS 1998, Bishop 2000). Despite all reclassifications, a previous study using ITS molecular markers found no difference between Australian *P. australis* and

Asian *P. tancarvilleae* (Harrison et al. 2005). However, differences between the species may be identified based on the use of population genetics analysis using microsatellite loci which are known for their highly polymorphic nature (Gustafsson 2000, Bory et al. 2008).

*Phaius bernaysii* was suggested as *P. australis* var. *bernaysii* (Hooker 1873, Nicholls 1950). Flower colour polymorphism is known in orchids around the world (Pellegrino et al. 2005). There are three possibilities to explain the occurrence of *P. bernaysii*, particularly the lack of geographic isolation (allopatric speciation) between *P. australis* / *P. bernaysii* in a very small geographic area (750m$^2$): sympatric speciation (evolved reproductive isolation), genetic differentiation or the expression of different colour phenotypes.

This study investigates the current distribution, threats, size and morphology of current *Phaius australis* populations. The aims of this study are (a) to identify how many *P. australis*, *P. tancarvilleae* and/or *P. bernaysii* populations are left in the wild; (b) to critique floral morphology in the field to assess if the populations morphologically resemble one species (*P. australis*) or many species (*P. australis, P. bernaysii, P. tancarvilleae*) for conservation management purposes; (c) examine if populations of the species are in decline or currently threatened; (d) use population counts, floral synchrony and floral output studies to identify possible sympatric or allopatric differences between *P. australis* and *P. bernaysii*; (e) characterise 10 polymorphic microsatellite loci for *P. australis*; and (f) cross-amplify microsatellite loci and utilise allelic patterns to determine taxonomic boundaries between *P. bernaysii* and *P. tancarvilleae* to identify molecular differences between the three species (*P. australis, P. bernaysii, P. tancarvilleae*).

## 2.2 Methods

The expected distribution and population abundance of *Phaius australis* and *P. bernaysii* was determined using records across the period of 1930-2008 from the Queensland Herbarium, Brisbane (BRI), the National Herbarium of New South Wales, Sydney (NSW) and the Australian National Herbarium, Canberra (CANB). Additional presence records for Queensland were extracted from Wildlife Online (DSITIA 2012), communication with park rangers and community groups, and flora census (WA Herbarium 2013, NT Herbarium 2011). Populations of *P. australis* are recorded in scattered locations from North Queensland to mid-north coast New South Wales while *P. bernaysii* is historically recorded from five locations in South East Queensland (Figure 2.1).

Over 70 unique documented historic population locations were ground-truthed for the presence of current populations of *Phaius australis* and three for *P. bernaysii*. Historic populations of *P. tancarvilleae* based on formerly described flower morphology differences (Table 2.1) were identified at six existing *P. australis* sites in the north, two in the mid and five sites in the south. Seven additional locations were investigated using where populations had not historically been located but ideal habitat was identified.

Ten locations, five in New South Wales and five in Queensland, were not ground truthed due to logistical difficulty or complete habitat destruction since the original records were made. Populations were investigated on foot in the flowering season between August and October 2012 to confirm the species' continued presence post original collection, to document species phenology and basic floral morphology. Data was accumulated for the number of historic populations that were reconfirmed, new populations, unconfirmed and not found, as well as population size at the time of the survey. It was noted if populations had apparently increased or decreased since the original records were documented. Current population sizes and changes in population size were compared. This information was later used to develop a sampling design for a larger species-wide population genetic and demographic study for the species. At each site, the presence of any listed threats recorded on the Commonwealth Environmental Protection and Biodiversity Conservation Act 1999 ('EPBC Act') and any additional threats to the species were recorded and tallied.

*Field Surveys*

Population sites were located in the field utilising a combination of GPS coordinates from historic records, maps, verbal descriptions or local guides. Searches were undertaken by systematically searching the entire area of potential habitat in a series of continuous parallel belt transects 10 m wide (Cropper 1993). The geographical extent of the population at each site was estimated by recording the perimeter boundary points using a handheld GPS device (eastings and northings; Datum WGS84, UTM zones 55, 56, 57), then converted to area ($m^2$) to elucidate the area of occupancy at each population.

All *Phaius* plants encountered were documented across the geographic range within a site. At all sites, the starting location of each population was recorded using the GPS.

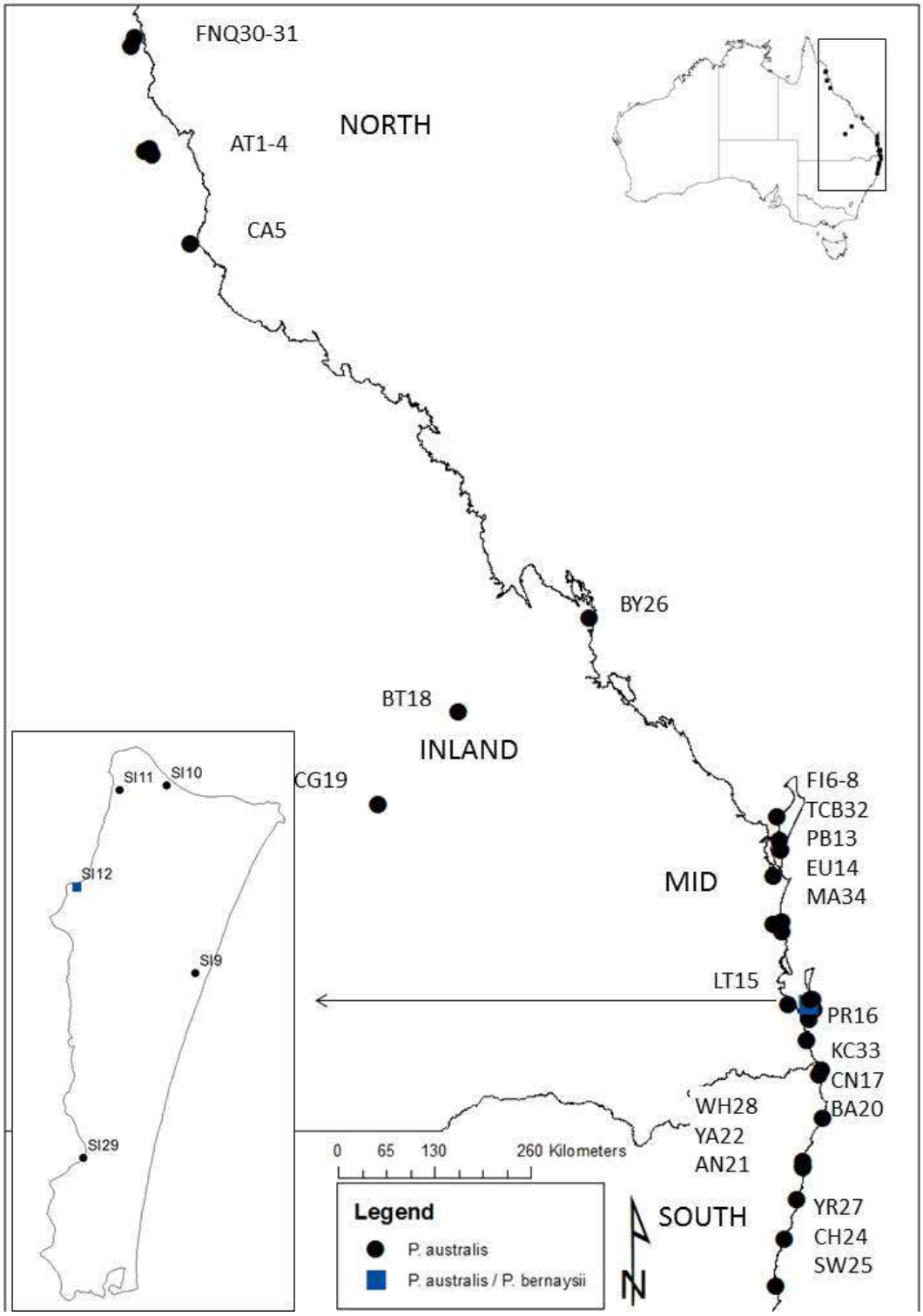


Figure 2.1. Map overview of the *Phaius australis* and *P. australis* / *P. bernaysii* distribution across the east coast of Australia (inset: North Stradbroke Island sites). Numbers indicate populations in order of sampling with letters abbreviating place names.

The location of each individual plant within a site was mapped relative to the start location using compass direction (degrees) and distance (metres) between clumps and plants measured with a LaserDisto measure (Leica geosystems) for up to 100 plants and later converted to XY coordinates. In sparse, smaller populations, the location of each individual was marked with a GPS. For more extensive and/or dense populations beyond 100 individuals, plants were mapped in clumps with plant and shoot number estimated.

A total of 617 leaf samples were collected for later detailed genetic analysis of *P. australis*; no populations of *P. bernaysii* were located in isolation of *P. australis*. A representative nine leaf samples of *P. bernaysii* were collected from one mixed *P. australis* / *P. bernaysii* population at Myora Springs (North Stradbroke Island, SI12; Figure 2.1) where only positively identified flowering plants were sampled. This small sample size represents the only known *P. bernaysii* plants at the time of sampling, though additional plants were located in the year following the collection of genetics samples. Samples for genetic studies were collected from 30 individuals per population, approximately every third plant, in order to capture variation across the whole spatial occupancy of the population distribution and across all size classes including recruits, intermediates and sexually mature individuals. Where populations were smaller than 30 individuals, samples were collected from every individual. A single opportunistic sample was obtained from an Asian *P. tancarvilleae* cultivar from a known nursery source (pers. comm. S. Ogbourne) to test for cross amplification of microsatellite markers. Genetics samples consisted of approximately 10 x 10 cm of green, healthy leaf material, placed in a sealed plastic bag labelled with the plant number. Surface mould, rust and foreign material was cleaned from the leaf, then samples were placed in labelled plastic clip-seal bags with Grade 12, 28-200 mesh silica gel (Sigma-Aldrich). Samples were stored at room temperature away from light in the University of the Sunshine Coast Laboratory prior to DNA extraction.

*Floral morphology and fecundity*

To investigate if the current taxonomy is suitable for future species-wide analysis, the floral morphology of 15 randomly collected flowers was critiqued in the initial surveys (2012) according to the 10 published differences between *P. australis* and *P. tancarvilleae* (Table 2.1; Stanley and Ross 1983, Benwell 1994). Where populations had less than 15 open flowers, all flowers were critiqued. In order to determine if plants were yellow *P. bernaysii*, or cinnamon purple *P. australis*/*P. tancarvilleae*, the following reproductive variables were recorded for each individual in each populations: number of flowers per inflorescence and the colour of the

flowers. Each flower trait was assigned a score of 1, if resembling the *P. australis* trait; 2, if resembling the *P. tancarvilleae* trait. Repeated floral morphology surveys aiming to capture morphological differences between years were undertaken at a subset of populations on an ad hoc basis the following year (2013) as part of additional studies. The percentage frequencies of flowers within each population resembling *P. australis*, *P. tancarvilleae* or intermediate traits were calculated for each site, each year. *Phaius bernaysii* was critiqued as per *P. australis* because the plants of *P. bernaysii* are identical to *P. australis* in form and for each floral trait except for colour.

Floral morphology for populations were summarised as a percentage of flowers resembling *P. australis*, intermediate or *P. tancarvilleae*. Flowers were assigned to categories based on the combination of all traits where *P. australis* was assigned if >70% of total combined features were *P. australis* traits, intermediate if >70% of traits intermediate and *P. tancarvilleae* if >70% of traits were *P. tancarvilleae* traits. A sub-set of accessible populations was revisited three months post-flowering to assess the output of fruit set and the average number of flowers per inflorescence. The frequency of fruit set was calculated as the proportion of fruit set per inflorescence, then averaged across each site.

Table 2.1. Attributes assessed according to published and reported differences between *P. australis* and *P. tancarvilleae* (from Benwell (1994), references (1) Benwell (1994), (2) Dockrill (1992) (3) Harden (1990), (4) Stanley and Ross (1983), (5) Nicholls (1950).

| **Attribute** | ***P. australis*** | ***P. tancarvilleae*** |
|---|---|---|
| Perianth colour [2, 3, 4] | Red-brown with yellow veins | Cinnamon brown |
| Labellum shape [2, 3, 4] | Lateral lobes erect, incurved | Lateral lobes overlapping, tightly tubular |
| Labellum colour [2] | Red-brown & yellow | Yellow, crimson mauve or white suffused mauve |
| Column shape [2, 5] | Shorter & stout | Longer & slender |
| Rostellum [2, 5] | Absent | Present |
| Connective disc [5] | Absent | Present |
| Anther texture [5] | Echinulate | Smooth |
| Stigmatic cavity appendages [2, 5] | Present | Absent |
| Additional anthers [5] | Often present | Absent |
| Leaf Width [1] | Narrower | Broader |
| No. flowers per spike [3] | 10-20+ | 4-10 |
| Fruit set frequency [3] | Common (great than 30%) | Rare |

Additional floral studies were undertaken at the mixed *P. australis* / *P. bernaysii* population at Myora Springs, North Stradbroke Island in the flowering season following initial surveys to assess if floral synchronicity isolates the two species reproductively (sympatric speciation). Each plant in the population was recorded according to colour (purple, yellow or unknown where no inflorescence) to estimate the ratio of *P. bernaysii* to *P. australis* in the wild. Then the maturity of plant (reproductive, non-reproductive), the abundance of flowers/fruit on each

inflorescence (0, <5, 5-10, 10+) and floral synchrony were recorded according to maturity of flowers on each inflorescence (majority buds, flowers, old flowers or early fruit set). Yellow flowering plants (*P. bernaysii*) were tagged with biodegradable flagging tape and the population was revisited in January 2014 to assess fruit set frequency. Abundance of fruit set was recorded according to three categories (0 fruit set, <5 fruit, >5 fruit). The proportion of each maturity category (floral synchronicity) and fruit set abundance were calculated for both species. To test for reproductive isolation between the species and the possibility of reproductive advantage in either species supporting allopatry, differences in the floral synchronicity, the floral output and fruit set between the two species were tested using Chi-square contingency table tests in SPSS (IBM 2010).

The proportion of plants of each colour was calculated to give the ratio of cinnamon-pink *P. australis* to yellow *P. bernaysii.* As *P. bernaysii* could potentially be an expressed recessive phenotype (albino) of *P. australis*, the percentage of the population predicted to be heterozygous for the phenotype was calculated using the Hardy-Weinberg equation ($p^2 + 2pq + q^2 = 1$).

*Laboratory methods*

Approximately 30-50 mg of finely cut plant tissue and a 3 mm tungsten bead was placed inside Eppendorf 2.0 mL Safe-Lock tubes. Samples were frozen using liquid nitrogen for 30 secs and ground using a Retsch MM200 Tissue Lyser grinding mill (Qiagen). Total genomic DNA was extracted from the leaf tissue using QIAGEN DNeasy® Plant Mini Kits (QIAGEN Valencia, CA, USA) following manufacturer's instructions: with some alterations: 500 µL of buffer AP1, 162.5 µL of buffer AP2 were used. Genomic DNA was eluted in a final volume of 200 µL AE buffer and compared with known concentrations (5 ng/µL, 10 ng/µL, and 20 ng/µL) of Lambda EcoR1/HindIII digest molecular weight marker (Fisher-Biotech) to estimate yields using 1.5 % agarose gel electrophoresis; viewed under UV light using a Syngene gel documentation system and Syngene GeneSnap software.

To generate a microsatellite library of suitable primers for this species, DNA (5 µg) from 4 individuals was sent to the Australian Genome Research Facility Ltd (AGRF, Brisbane, Australia; http://agrf.org.au/) for microsatellite (SSR) marker development using Next-Generation 454 pyrosequencing and used to construct a random library that was sequenced using GS-FLX Titanium chemistry Illumina HiSeq (Roche Applied Science; Mannheim, Germany). Sequences

were trimmed for length and quality using the CLC Genomics Workbench v6 software. A total of 53 176 reads were obtained with an average length of 381 bp and searched for microsatellite loci having a minimum of six repeats for di-nucleotides, and four repeats for tri- and tetra-nucleotides, using the QDDv2b pipeline (Meglécz et al. 2010) and PRIMER 3 (Rozen and Skaletsky 2000).

The resulting 2 102 unique loci were sorted based on PCR product size, repeat class, repeat length, GC content and multiplexing potential. A selection of 61 unlabelled microsatellite primer pairs was thus selected for evaluation of amplification and optimisation. Fifteen *P. australis* DNA samples from individuals across the species range (positive control) and a blank sample (negative control) were used in initial trials. Polymerase chain reaction (PCR) amplification was performed using reaction volumes of 12 µl containing approximately 25 ng of *P. australis* genomic DNA, 1 x reaction buffer (67 mM Tris-HCl (pH 8.8), 16.6 mM $(NH_4)_2 SO_4$, 0.45% Triton X-100, 0.2 mg/mL gelatine; Fisher Biotech), 200 µM of each dNTP, 2 mM MgCl2 (Fisher Biotech), 0.2 µM BSA (Fisher Biotech) 0.2 µM forward primer, 0.2 µM reverse primer and 0.5 U F1 Taq polymerase (Fisher Biotech).

Amplification was performed on a Eppendorf Mastercycler Nexus Gradient with the following cycling conditions: denaturation at 95 °C for 3 mins; 35 cycles of 94 °C for 30 secs, specific annealing temperature (Table 2.2) for 30 secs, 72 °C for 45 secs; final elongation step at 72 °C for 10 mins. PCR products were run on 1.5% agarose gels containing ethidium bromide and visualised under UV light to check for selective amplification of fragments within the expected size range. Twenty-five primer pairs that consistently amplified single bands within the expected size range were selected for further optimisation with the forward primer of each pair being end-labelled directly with one of four fluorescent dyes (VIC, NED, PET, Applied Biosystems, FAM, Geneworks, Table 2.2) to enable multiplexing of several loci in fragment analysis.

PCR amplification of the 26 loci was conducted for all 627 individuals, multiplexed for the same individuals according to different dye sets and size ranges to avoid overlap in two mixes, and then separated by capillary electrophoresis on an AB 3500 Genetic Analyser (Applied Biosystems). Fragment sizes were determined relative to internal lane standard (GS-600 LIZ; Applied Biosystems) then banding patterns were manually checked in GENEMAPPER v4.1 software (Applied Biosystems) that scored bands that fitted the expected size range and patterns of inheritance. Some loci (Pa05 and Pa40) did not yield consistent results and these were eliminated from further analysis. A resultant 13 polymorphic primers (*ml-Pa03*, *ml-Pa19*,

*ml-Pa44*, *ml-Pa59*, *ml-Pa31*, *ml-Pa49*, *ml-Pa40*, *ml-Pa24*, *ml-Pa12*, *ml-Pa27*, *ml-Pa14*, *ml-Pa47*, *ml-Pa02*) were scored for *P. australis*. The presence of null alleles, scoring errors, and large allele dropouts were checked for all loci using MICROCHECKER v2.2.3 (Van Oosterhout et al. 2004). The independence of genotypes between loci or linkage disequilibrium was tested using Fisher's exact tests, as implemented in GENEPOP v1.2 and a sequential Bonferroni correction was applied for multiple tests.

*Analysis*

The multi-locus genotypes were used to characterise the microsatellites by calculated allelic frequencies, mean number of alleles per locus (*A*), mean expected heterozygosity (*He*), mean observed heterozygosity (*Ho*) for each locus using GenAlEx 6.5 (Peakall and Smouse 2012). Genotypic frequencies for each locus of *P. australis* samples were tested for deviation from Hardy-Weinberg equilibrium (*HWE*) using chi-square goodness-of-fit tests GenAlEx 6.5 (Peakall and Smouse 2012). Multi-locus genotypes were used to calculate allelic frequencies and the mean number of alleles per locus (*A*) for the small number of samples of *P. bernaysii* and *P. tancarvilleae*. Allelic frequencies and the mean number of alleles per locus were compared between the three species to identify species differentiation or lack thereof.

To investigate the taxonomic boundaries between *P. australis*, *P*. *bernaysii* and *P*. *tancarvilleae* based on allelic patterns, all populations and all plants within populations were classified by historical nomenclature (Table 2.5). Nei's standard genetic distance measures were calculated between all pairs of historical populations in GenAlEx 6.5 (Peakall and Smouse 2012). This was imported into PRIMER 6.1.5 (PRIMER-E Ltd 2006) and hierarchical cluster analysis (UPGMA - unweighted pair group method with arithmetic averaging) was undertaken to investigate genetic relationships among populations. A Principle Components Analysis was undertaken with 9999 bootstrap permutations in GenAlEx 6.5 (Peakall and Smouse 2012) to identify where the *P. bernaysii* and *P. tancarvilleae* morphs cluster within the *P. australis* samples and identify any species boundaries through genetic differentiation. To test if there was any admixture between the three species a Bayesian model-based clustering method was performed using the program STRUCTURE 2.3.4 (Pritchard et al. 2000, Falush et al. 2007) based on multilocus (13 polymorphic loci) genotype data (K=3; *P. australis*, *P. bernaysii*, *P. tancarvilleae*). A burn-in period of 250 000 steps, and 750 000 Markov Chain Monte Carlo reps were selected for 20 iterations per K value, along with admixture model and correlated allele frequencies.

Table 2.2. Details of 25 microsatellite loci characterised for *P. australis*. Primer names, repeat motif, primer sequence (F, forward; R, reverse), optimal annealing temperature (*Ta*), allele size ranges (bp), genbank accession numbers, and florescent dye labels are given.

| Primer | Repeat Motif | Primer sequence (5'-3') | *Ta (°C)* | Size Range | Genbank Accession | Flourescent Dye |
|---|---|---|---|---|---|---|
| ***ml-Pa08*** | $(CT)_5$ | F: GGAGGAACTAATGGCGCTCTA<br>R: CAGGAACTGAAGGCTGTTGC | 56 | 100 | KR698092 | FAM |
| ***ml-Pa05*** | $(CT)_5$ $(CT)_3$ | F: ATTTGCCTCAATGGTTCCCT<br>R: TCGAAGAACCATTACGAGAGC | 56 | 116-168 | KR698091 | FAM |
| ***ml-Pa03*** | $(AT)_8$ | F: TAGAGGTAATCCAGGCCTCT<br>R: ATAATATGTGAATTAGATAGCTTGGGC | 51.8 | 232-240 | KR698090 | FAM |
| ***ml-Pa19*** | $(AT)_5$ | F: GGAGCTATGCAACTGACTATCACA<br>R: TCATTAGTTGTGTGCTCTTGC | 56 | 298-300 | KR698095 | FAM |
| ***ml-Pa60*** | $(AT)_5$ | F: TTCCTTCTCATGCATCAATACTT<br>R: TAAGCCTCCTATGCCTTCCA | 56 | 115 | KR698112 | NED |
| ***ml-Pa44*** | $(AT)_5$ | F: CCATTGGGTCACTTCCTCTG<br>R: TCAGAGAACAACCATGCCAA | 56 | 188-192 | KR698104 | NED |
| ***ml-Pa59*** | $(AT)_5$ | F: ACCCAATTAGAAGCAAACTTGAAGA<br>R: TGGTTAGAGAACATCTCATTGGG | 56 | 228-232 | KR698111 | NED |
| ***ml-Pa55*** | $(AT)_7$ | F: AAGGAGGTTAGGATTCAACACTT<br>R: TGCTGAGTATGAAGCCCTGA | 56 | 137-143 | KR698108 | PET |
| ***ml-Pa41*** | $(AT)_6$ | F: GCTCTTAAGAAGCACTTCAAGTCAA<br>R: CCCAAACTCCTCTTCCAATC | 56 | 192 | KR698103 | PET |
| ***ml-Pa31*** | $(AG)_5$ | F: TAGAGGTAATCCAGGCCTCT<br>R: GATAGCTTGGGCCATTCAAA | 59.7 | 221-229 | KR698098 | PET |
| ***ml-Pa61*** | $(AG)_5$ | F: AAGAATGGAGACGATCCCGT<br>R: GCAAGATTTCAAAGGCTGCT | 56 | 129 | KR698113 | VIC |
| ***ml-Pa49*** | $(CT)_5$ | F: GATGCAGGAATGGGAAACAG<br>R: ATATTGGAACCACTCGACGG | 56 | 195-199 | KR698107 | VIC |
| ***ml-Pa45*** | $(AT)_5$ | F: TGTAGGGCTACTGGTCCGTC<br>R: AGCCCTGCATCTTGGGTATT | 56 | 269 | KR698105 | VIC |
| ***ml-Pa56*** | $(ACT)_5$ | F: ACACGCACATGCATATCAAA<br>R: ATTGTGTGCGGCTCTCATTC | 56 | 101 | KR698109 | FAM |
| ***ml-Pa40*** | $(AG)_5$ | F: ATTTAGACCACCTCCACCCG<br>R: TGAATTGCATGGATGGATGA | 56 | 166-168 | KR698102 | FAM |
| ***ml-Pa24*** | $(TTC)_{11}$ | F: ACGTGGCGGAGGAGAAGT<br>R: TCCTTAATAGATCTGAAGCACAAA | 56 | 235-241 | KR698097 | FAM |
| ***ml-Pa12*** | $(AT)_8$ | F: TTGGTTGCTCCTTCTTGACC<br>R: TGAGGCCTCCTTAATCTCTAGC | 59.7 | 315-317 | KR698093 | FAM |
| ***ml-Pa39*** | $(AC)_5$ | F: TTGAACACACCTTGCATTTGA<br>R: CACCTCATGCAACTCTCAACA | 56 | 140 | KR698101 | NED |
| ***ml-Pa27*** | $(AT)_7$ | F: CAAACTAAAGGAAGGTGAGCCA<br>R: GGCATTGGTGAGTAGGCAGA | 56 | 209-215 | KR698098 | NED |
| ***ml-Pa14*** | $(AT)_{10}$ | F: CAAACTAAAGGAAGGTGAGCCA<br>R: TCCAAGCCTTAGAAAGGGC | 59.7 | 276-290 | KR698094 | NED |
| ***ml-Pa57*** | $(ACT)_5$ | F: TGACCATCCCACAATGTTGA<br>R: TCTCCACTACCATAACCACCAC | 56 | 157-159 | KR698110 | PET |
| ***ml-Pa02*** | $(AT)_8$ | F: TGAAGCCAAAGGATGAACAA<br>R: GGAAGCATTATGATTGATGACG | 56 | 198-202 | KR698089 | PET |
| ***ml-Pa21*** | $(TA)_3(TC)_4$ | F: CCATACATAGGGTCATCAATCCA<br>R: GTTAAGGCCTCCTATTCCCG | 59.7 | 158 | KR698096 | VIC |
| ***ml-Pa46*** | $(CT)_5$ | F: TTAAAGGACGGAAACGCAGA<br>R: GCCTACCGATCGATTGAACA | 56 | 231 | KR698106 | VIC |
| ***ml-Pa33*** | $(AG)_5$ | F: CCCAATTAGAAGCAAACTTGAA<br>R: AGGAGGAAATGTAAGAGCCCA | 51.8 | 333 | KR698100 | VIC |

## 2.3 Results

*Population surveys*

Thirty-six extant populations of *P. australis* were found across a latitude span of approximately 1 800 km from Rossville near Cooktown in North Queensland to South West Rocks in New South Wales and were confirmed via lodgement of specimens at herbaria (Figure 2.1). Population size ranged from one individual to several hundreds of individuals (Table 2.3). Four of the current populations are new records for herbaria. Twenty-six historic locations where no current *Phaius* populations were located are now considered locally extinct as no plants were found when ground-truthing for this study (Table 2.3). An additional six unfound populations were due to land-use change since the mid-1900's and thus are confirmed as locally extinct (Table 2.3).

Seven of the thirteen populations formerly identified as *P. tancarvilleae* were located in the surveys (Table 2.3). Only one population of *Phaius bernaysii* was located, due to a housing development at one historic location and no plants of *P. bernaysii* or *P. australis* located at two other historically known locations (Table 2.3). The only remaining population of *Phaius bernaysii* is near Myora on North Stradbroke Island (SI12) and was completely intermixed with *P. australis* with plants as identified by flowers. In no populations were pollinators observed on flowers.

Thirteen populations were composed of less than 10 individuals and fewer populations with more than 50 individuals in the south region (New South Wales; Table 2.3). Eighty percent of populations in New South Wales with known historic population sizes have decreased in size since first documented, compared to 73% of those in Queensland (Table 2.3).

Threats identified to populations during the timeframe of the study included the cutting and removal of 60%of the flower stalks at one population in the north region (AT3) over a three day period. Since the surveys in 2012-2014, all of the plants in two populations on the Tweed Coast in the south region have disappeared. An individual *P. bernaysii* plant was dug up from the population on Stradbroke Island (SI12); all plants within two small populations (less than 30 individuals) completely disappeared in the south region. Invasive plant species were recorded at all populations (Table 2.4). Sea level rise is a potential additional threat to low lying coastal populations (mid and south) and future climatic changes such as precipitation and temperature changes are likely to be far more widespread (Table 2.4). Browsing and trampling impacts by feral pigs occurs at WH28 (south) and directly adjacent to CG19 (inland).

Table 2.3. Details of *Phaius* spp. population surveys undertaken in 2012 with regions and taxa given; total number of populations across all taxa and the size of the populations at the time of the surveys. Where initial population size was known, the change in population size is described as increased or decreased.

| | | | | | | | | Current population sizes | | | | Change in size | | Threats within region |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Region | Taxa | Historic | Located | Not Located | Un-confirmed | New | Total | <10 | 10-50 | 50-100 | >100 | Increase | Decrease | |
| North | *Pa* | 6 | 2 | 3 | 1 | 0 | 2 | 3 | 1 | 1 | 2 | | | Habitat modification |
| | *Pb* | 0 | - | - | - | 0 | 0 | | | | | | | Illegal removal |
| | *Pt* | 6 | 5 | 1 | - | - | 6 | | | | | | | (flower stalks) |
| | Total | 12 | 7 | 4 | 1 | 0 | **8** | 3 | 1 | 1 | 2 | 2 | 5 | Invasive plants |
| Mid ^ | *Pa* | 28 | 12 | 12 | 4 | 3 | 15 | 6 | 1 | 1 | 9 | | | Illegal removal (single |
| | *Pb* | 3 | 1 | 3 | - | 0 | 1 | | | 1 | | | | plants/flower stalks) |
| | *Pt* | 2 | 1 | 0 | 1 | - | 1 | | | | | | | Habitat modification |
| | | | | | | | | | | | | | | Invasive plants |
| | Total | 34 | 14 | 15 | 5 | 3 | **17** | 6 | 1 | 2 | 9 | 5 | 7 | Sea level rise |
| South & | *Pa* | 17 | 10 | 5 | 2 | 1 | 11 | 4 | 3 | 1 | 2 | | | Illegal removal (single |
| | *Pb* | 0 | | | | 0 | 0 | | | | | | | plants/entire population) |
| | *Pt* | 5 | 1 | 3 | 1 | - | 1 | | | | | | | Habitat modification |
| | | | | | | | | | | | | | | Invasive plants |
| | Total | 22 | 11 | 8 | 3 | 1 | **12** | 4 | 3 | 1 | 2 | 1 | 4 | Sea level rise |
| Inland $ | *Pa* | 2 | 2 | 0 | - | 0 | 2 | | 1 | | 1 | | | Feral animals |
| | *Pb* | 0 | - | | - | 0 | 0 | | | | | | | (pigs, cattle) |
| | *Pt* | 0 | - | 0 | - | - | 0 | | | | | | | |
| | Total | 2 | 2 | 0 | 0 | 0 | **2** | | 1 | | 1 | 1 | 1 | |
| Grand Total | | 70 | 34 | 27 | 9 | 4 | **39** | 13 | 6 | 4 | 14 | 9 | 17 | |

^ Unknown historic population sizes at several locations. & Several unknown previous population sizes, current population size unknown for 2 populations. $ Since initial surveys, population size decreased for 1 location.

Table 2.4. Tallied number of *Phaius* spp. sites where threats listed on the EPBC Act 199 Conservation Advice were observed during surveys.

| Threat category | No. of Sites Present |
|---|---|
| Invasion by weeds | 34 |
| Human intrusions and disturbance due to recreational activities and development | 7 |
| Habitat shifting, loss or inundation associated with climate change | 7 |
| Disturbance and browsing due to trampling by feral pigs and domestic livestock | 6 |
| Illegal collection for horticulture or cut flowers | 5 |
| Road works | 4 |
| Potential for inappropriate fire regimes | 3 |
| Habitat loss through clearing and altering drainage due to: | |
| development | 3 |
| timber harvesting | 1 |
| mining | 1 |

Similar impacts from domestic and wild livestock were recorded at inland population BT18. Land and housing developments were observed to reduce population sizes at EU14 in the mid region and BA20 in the south, while a north population (AT3) lost over three-quarters of individuals in the population through road clearing and changed drainage. Direct threat of habitat destruction from mining may have reduced over time as the focus of such operations diverted to other locations on North Stradbroke Island.

*Floral morphology as a distinction between species*

Floral traits varied within flowers on the same plant and population as well as between years (Table 2.5). The only trait that did not change across any flowers in any population in both years was the universal absence of a rostellum, a trait associated with *P. australis*. The Lota population (LT15) had 62.5% *P. australis* traits, 25% intermediate traits and 12.5% *P. tancarvilleae* traits in 2012, while in 2013, this population had 85% *P. australis* traits, 15% intermediate traits and no flowers with a majority of *P. tancarvilleae* traits (Table 2.5). Likewise, despite the majority of flowers possessing *P. australis* traits in the Angourie population (AN21), there were more flowers with a majority of the *P. tancarvilleae* floral traits in 2013 compared to 2012 indicating inconsistent floral morphology within a site (Table 2.5).

The variation in labellum shape contributed to moderate *P. tancarvilleae* attribute scores for some *P. australis* sites with some flowers possessing a tubular labellum and others possessed an open / flat labellum (sites PB13, SI11, SI12, YR27, CG19, SI9; Table 2.5).

In most cases, the possession of a tubular labellum, the lack of yellow veins on the perianth and labellum colouration contributed to higher resemblance towards *P. tancarvilleae* traits. Stradbroke Island populations (SI9-12, SI29) consistently had the highest scores for possessing a greater suite of *P. australis* traits (Table 2.5). In most cases, floral output was greater than 10 flowers and fruit set was greater than 30% of flowers on an inflorescence resembling that described for *P. australis*, though there were notable exceptions in the Southern region (Table 2.5).

Despite variation between flowers within populations and variation between years, there was generally greater resemblance to *P. australis* rather than *P. tancarvilleae* (Table 2.5). Even those populations that were formerly identified as *P. tancarvilleae* had low to moderate scores for *P. tancarvilleae* traits (AT1, AT2, AT3, BY26, PR16, WH28, KC33; Table 2.5). This is a result that is consistent with current accepted taxonomy that in Australia all plants are *P. australis* (CHAH 2010). Based on the floral morphology results, all populations surveyed in this study without yellow flowers in Australia should be considered a *P. australis*. It also demonstrates considerable variability in floral morphology across the species range.

The flowers of *P. bernaysii* that were critiqued possessed an equally high resemblance to *P. australis* morphology as flowers of *P. australis* within the same site (90% SI12 and SI12[&], both years; Table 2.5). With the lack of geographic separation between the two species, the yellow colouration of the sepals, petals and labellum is the only way for identifying the only remaining *P. bernaysii* plants in the wild. A total of 560 adult sized flowering plants were counted in Myora Springs (SI12) with positive identification of 58 *P. bernaysii* plants interspersed within another 502 *P. australis* plants.

There was no significant ($\chi^2$, $p$>0.05) difference in the timing of buds, flowers, early fruit between *P. australis* and *P. bernaysii* (Figure 2.2a). Thus the two species are not separated by timing of reproduction (sympatric differentiation) within the same geographic area. As a result, it is hypothesised that either the flower colour is the expression of an albino phenotype that occurs as a recessive allele within the population or that the two species are separated genetically. If *P. bernaysii* is in the expression of a recessive allele based on Hardy-Weinberg calculations, the allele could be present at a frequency of 0.32 in the populations where 10.4% of the population is *P. bernaysii* homozygous form, thus 43.7% could be heterozygote carriers.

Table 2.5. Total percentage of flowers assigned as *P. australis* (*Pa trait*), intermediate (*Int. trait*) or *P. tancarvilleae* (*Pt trait*) critiqued as per Table 2.1 for two years (2012, 2013) based on a flower assigned as *P. australis* if total score is >70% *P. australis* traits, intermediate if >70% of traits intermediate and *P. tancarvilleae* if total score is >70% *P. tancarvilleae* traits. Floral output = the average number of flowers per inflorescence, fruit set = the average percentage of fruit set per inflorescence. Historical nomenclature given as *Pa*= *P. australis*, *Pt*= *P. tancarvilleae* or *Pb*= *P. bernaysii*. – indicates no data [&] *P. bernaysii* with yellow flower colour.

| | | | *Morphology 2012* | | | *Morphology 2013* | | | *Floral Output* | | *Fruit Set %* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Region* | *Pop.* | | *Pa trait* | *Int. trait* | *Pt trait* | *Pa trait* | *Int. trait* | *Pt trait* | *2012* | *2013* | *2012* |
| North | FNQ31 | Pt | - | - | - | 62.5 | 12.5 | 25 | 20.3 | 15 | 52.5 |
| | AT1 | Pt | 58.8 | 12.4 | 28.8 | 50 | 12.5 | 37.5 | 12 | 17.1 | 23.9 |
| | AT2 | Pt | 62.5 | 0 | 37.5 | 62.5 | 0 | 37.5 | 12.3 | 15.4 | 10 |
| | AT3 | Pt | 60.9 | 12.5 | 26.6 | 62.5 | 12.5 | 25 | 8.9 | | 11.3 |
| | AT4 | Pt | - | - | - | - | - | - | 0 | 0 | - |
| | CA5 | Pa | 0 | 100 | 0 | - | - | - | 3.5 | 0 | 3 |
| Mid | BY26 | Pt | 87.5 | 0 | 12.5 | - | - | - | 12 | - | 0 |
| | FI7 | Pa | 25 | 75 | 0 | - | - | - | 7.5 | - | 44.2 |
| | FI6 | Pa | 0 | 87.5 | 12.5 | - | - | - | - | - | 23.6 |
| | FI8 | Pa | 12.5 | 87.5 | 0 | 12.5 | 87.5 | 0 | 3.5 | 15.3 | 58.8 |
| | TCB32 | Pa | 0 | 87.5 | 12.5 | - | - | - | 22.9 | - | 67 |
| | PB13 | Pa | 81.2 | 0 | 18.8 | 82.5 | 0 | 17.5 | 13 | 17.5 | 52.6 |
| | EU14 | Pa | 25 | 75 | 0 | 0 | 100 | 0 | 10 | 15 | 0 |
| | MA34 | Pa | 12.5 | 87.5 | 0 | 12.5 | 87.5 | 0 | 11.3 | - | 63 |
| | LT15 | Pa | 62.5 | 25 | 12.5 | 85 | 15 | 0 | 11.3 | - | 67.4 |
| | SI10 | Pa | 97.9 | 0 | 2.1 | 100 | 0 | 0 | 11 | 11.7 | 11.3 |
| | SI11 | Pa | 80 | 0 | 20 | - | - | - | 15.4 | - | |
| | SI12 | Pa | 90 | 0 | 10 | 77.5 | 0 | 22.5 | 4.2 | - | |
| | SI12 [&] | Pb | 90 [&] | 10 | 0 | 90 [&] | 10 | 0 | | - | |
| | SI9 | Pa | 81.9 | 0 | 18.1 | 85 | 0 | 15 | 13.3 | 13.8 | 21.8 |
| | SI29 | Pa | 0 | 87.5 | 12.5 | - | - | - | 13.1 | - | 69.3 |
| | PR16 | Pt | 68.8 | 24.9 | 6.3 | - | - | - | 11 | - | |
| South | KC33 | Pt | 0 | 100 | 0 | - | - | - | 13.5 | - | 47.4 |
| | CR17 | Pa | 82.5 | 12.5 | 5 | 75 | 12.5 | 12.5 | 14 | 14.2 | 61.9 |
| | BA20 | Pa | 87.5 | 12.5 | 0 | 0 | 100 | 0 | 6 | 3 | |
| | WH28 | Pt | 75 | 25 | 0 | 75 | 12.5 | 12.5 | 3 | 10 | |
| | YA22 | Pa | 87.5 | 0 | 12.5 | 87.5 | 12.5 | 0 | 12 | 10.3 | 92.3 |
| | AN21 | Pa | 87.5 | 12.5 | 0 | 62.5 | 25 | 12.5 | 7.2 | - | |
| | YR27 | Pa | 97.5 | 0 | 2.5 | 85 | 0 | 15 | 9.4 | 12.3 | 12.1 |
| | CH24 | Pa | 87.5 | 12.5 | 0 | 0 | 100 | 0 | 9.3 | 12.5 | 0 |
| | SW25 | Pa | 0 | 100 | 0 | 0 | 100 | 0 | 10 | 8 | 0 |
| Inland | BT18 | Pa | 75 | 25 | 0 | 12.5 | 87.5 | 0 | 13.8 | | |
| | CG19 | Pa | 0 | 100 | 0 | 37.5 | 25 | 37.5 | 14 | 10.8 | 14.3 |

The *P. bernaysii* plants in Myora Springs had a greater frequency of plants that setting more than 5 fruit per inflorescence (40%) compared to *P. australis* (25%), however this was not significant ($p$>0.05) when tested with a Chi-square test (Figure 2.2b). This suggests that *P. bernaysii* may have a reproductive advantage within the population, which could explain how a recessive albino allele is maintained in the population.

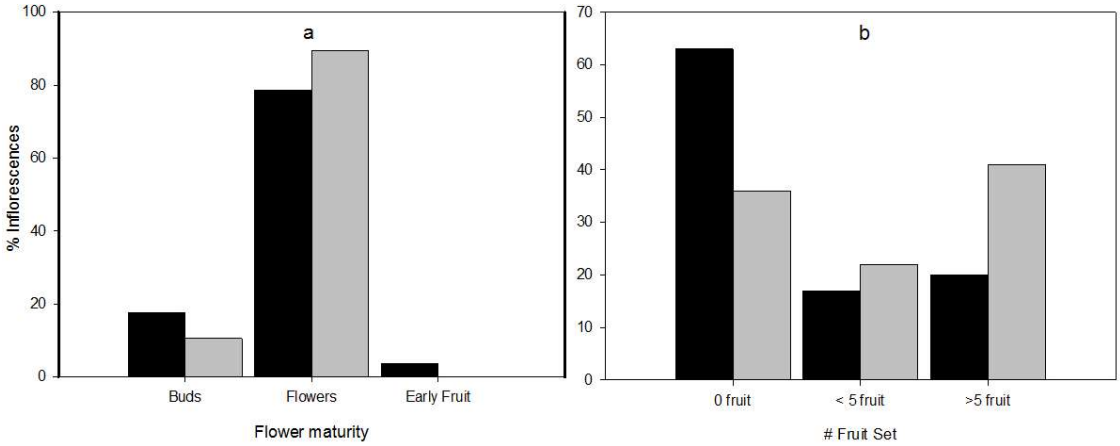


Figure 2.2. a) The proportion of *P. australis* (shown in black) and *P. bernaysii* (shown in grey) inflorescences in Myora with floral maturity (proportion buds, flowers, early fruit) and; b) the proportion of inflorescences with different fruit set abundance (0 fruit, < 5 fruit, >5 fruit).

Table 2.6. Variability of eight microsatellite loci in *Phaius australis*, *P. tancarvilleae* and *P. bernaysii* across all populations of the species. *A* = number of alleles per locus; Ae = number of effective alleles per locus; *He* = expected heterozygosity; *Ho* = observed heterozygosity, $n_x$ = number sampled, * indicates significant ($p$<0.01) departure from *HWE*.

| Locus | *P. australis* ($n_1$ = 617) | | | | *P. bernaysii* ($n_2$ = 9) | | | | *P. tancarvilleae* ($n_3$ = 1) |
|---|---|---|---|---|---|---|---|---|---|
| | *A* | *Ae* | *Ho* | *He* | *A* | *Ae* | *Ho* | *He* | *A* |
| *ml-Pa03* | 4 | 2.902 | 0.937 | 0.655* | 2 | 2.000 | 1.000 | 0.500* | 2.000 |
| *ml-Pa19* | 2 | 2.000 | 0.986 | 0.500* | 2 | 2.000 | 1.000 | 0.500* | 2.000 |
| *ml-Pa31* | 4 | 1.238 | 0.020 | 0.192* | 1 | 1.000 | mono | mono | 1.000 |
| *ml-Pa44* | 3 | 1.354 | 0.257 | 0.261* | 2 | 1.670 | 0.333 | 0.401 | 1.000 |
| *ml-Pa59* | 2 | 1.469 | 0.389 | 0.319* | 2 | 1.385 | 0.333 | 0.278 | 2.000 |
| *ml-Pa49* | 3 | 1.005 | 0.002 | 0.005* | 1 | 1.000 | mono | mono | 1.000 |
| *ml-Pa02* | 3 | 1.682 | 0.018 | 0.405* | 2 | 1.246 | 0.000 | 0.198* | 1.000 |
| *ml-Pa12* | 1 | 1.000 | mono | mono | 1 | 1.000 | mono | mono | 1.000 |
| *ml-Pa14* | 4 | 1.199 | 0.030 | 0.166* | 1 | 1.000 | mono | mono | 1.000 |
| *ml-Pa21* | 1 | 1.000 | mono | mono | 1 | 1.000 | mono | mono | 1.000 |
| *ml-Pa24* | 3 | 1.656 | 0.027 | 0.396* | 1 | 1.000 | mono | mono | 2.000 |
| *ml-Pa27* | 3 | 1.250 | 0.020 | 0.200* | 2 | 1.600 | 0.000 | 0.375* | 2.000 |
| *ml-Pa57* | 2 | 1.003 | 0.000 | 0.003* | 1 | 1.000 | mono | mono | mono |
| Means ± SD | 2.692 ± 0.286 | 1.443 ± 0.149 | 0.207 ± 0.099 | 0.239 ± 0.059 | 1.462 ± 0.144 | 1.300 ± 0.109 | 0.205 ± 0.104 | 0.173 ± 0.058 | 1.308 ± 0.175 |

*Microsatellite primer characterisation and cross amplification*

Twenty-five microsatellite loci were successfully amplified for 617 *P. australis* samples with 11 polymorphic loci obtained for the species (Table 2.6). The number of observed alleles per locus ranged from one to four, with an average of 2.692 per locus (Table 2.6). The observed and expected heterozygosity (*Ho* and *He*) ranged from 0.002 to 0.986 and 0.003 to 0.655 respectively (Table 2.6). All polymorphic loci for *P. australis* showed significant departure from HWE with excess heterozygosity for all loci other than *ml-Pa03*, *ml-Pa19* and *ml-Pa59* (Table 2.6). Null alleles in low frequency were detected for *P. australis* in 7 out of 13 loci (*ml-Pa31*, *ml-Pa49*, *ml-Pa02*, *ml-Pa14*, *ml-Pa24*, *ml-Pa27*, *ml-Pa57*). However, following a Bonferroni correction, significant linkage disequilibrium ($p$<0.00625) was detected in only seven out of 156 tests, with no consistent pairs of linked loci.

Thirteen loci successfully cross amplified to all nine *P. bernaysii samples* and 12 loci cross amplified to the one *P. tancarvilleae* sample indicating genetic similarity among the species (Table 2.6). The average number of alleles per locus was lower for *P. bernaysii* and *P. tancarvilleae* compared to *P. australis* (1.462 alleles, 1.308 alleles respectively; Table 2.6). There was a greater number of monomorphic loci for *P. bernaysii* and *P. tancarvilleae* than *P. australis*, which is not surprising given the smaller sample sizes (Table 2.6). The observed and expected heterozygosity (*Ho* and *He*) ranged from 0.000 to 1.000 and 0.198 to 0.500 respectively for *P. bernaysii* and loci Pa03, Pa19, Pa02 and Pa27 showed significant departures from *HWE* for *P. bernaysii* and null alleles were detected for loci *ml-Pa27*.

*Molecular differentiation between species*

There was no evidence of microsatellite allelic differences between *P. australis* and *P. bernaysii* with no alleles restricted to *P. bernaysii* indicating minimal genetic differences separating the species. Instead, there were repeated indications that *P. bernaysii* contains a subset of common *P. australis* alleles, which would be expected if the nine *P. bernaysii* individuals were a sub-sample or small population within *P. australis*. For example, *P. bernaysii* specimens were comprised of a subset of 2 of the four alleles found for *P. australis* samples at locus *ml-Pa03* (alleles 234, 240). All *P. bernaysii* samples were homozygote for allele 227 at locus *ml-Pa31* for which *P. australis* had four alleles occurring in varying frequencies. The lack of genetic differentiation supports the hypothesis that *P. bernaysii* is the phenotypic expression of a recessive allele that occurs in one, but potentially more populations.

In contrast and most significantly, there were four unique alleles found across four different loci in the single sample of *P. tancarvilleae* that were not found in the hundreds of *P*. *australis* or *P*. *bernaysii* samples (Table 2.7). *Phaius tancarvilleae* had a unique heterozygote composed of alleles 228 / 230 for locus *ml-Pa59* in comparison to alleles 232 and 230 found for *P. australis* and *P. bernaysii*. Again for locus *ml-Pa27*, *P. tancarvilleae* was a unique heterozygote with allele 209 found nowhere else and allele 211 which is common to *P*. *australis* and *P*. *bernaysii* (Table 2.7). Loci *ml-Pa12* and *ml-Pa21* were monomorphic for all species, with different homozygote forms detected for *P. tancarvilleae* specimen compared to the within *P. australis* and *P. bernaysii* (allele 317 in *ml-Pa12* and allele 156 in *ml-Pa21*; Table 2.7). Based on this allelic composition from one sample, *P. tancarvilleae* can be differentiated from all of the *P. australis* and *P. bernaysii* samples.

The results of the principle coordinates analysis (PCoA) of population genetic distance between current populations of *P. australis* show 21.5% of variation among populations is accounted for by the variation along coordinate 1, and 11.2% of variation along coordinate 2. The first three axes combined account for 42.5% of the genetic variation between the populations (Figure 2.3). While there is some genetic differentiation among populations this is not clustered by historic nomenclature (Figure 2.3). The PCoA shows a dense clustering of 15 populations and a dispersal of *P. australis* and *P. tancarvilleae* populations away from the main cluster (Figure 2.3). The *P. bernaysii* samples cluster within *P. australis* while the *P. tancarvilleae* 'control' sample is clearly separated from all other populations (Figure 2.3).

The output of the Bayesian Structure Analysis demonstrated that the substantial number of alleles in common between *P. australis*, *P. bernaysii* and *P. tancarvilleae* is causing admixture between all three (Figure 2.4). None of the three genetic clusters coincided definitively with the three separate species, despite the four alleles unique to *P. tancarvilleae*. Analogous to the morphological results and the PCoA, the UPGMA displaying Nei's population genetic distance clustered by historical population nomenclature indicated no clustering of historical *P. tancarvilleae* populations with the *P. tancarvilleae* sample (Figure 2.5). Such allelic and morphological overlaps are indicative of the southern range expansion and subsequent differentiation of *P. australis* in Australia.

Table 2.7. Allelic frequency table for 13 polymorphic loci for *P. australis*, *P. bernaysii* and *P. tancarvilleae*; alleles restricted to one species are in bold italics.

| **Locus** | **ml-Pa03** | | | | **ml-Pa19** | | | **ml-Pa31** | | | **ml-Pa44** | | | **ml-Pa59** | | | **ml-Pa49** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Allele/n** | **232** | **234** | **238** | **240** | **298** | **300** | **221** | **225** | **227** | **229** | **188** | **190** | **192** | **228** | **230** | **232** | **195** | **197** | **199** |
| **PA** | 0.189 | 0.398 | 0.026 | 0.387 | 0.502 | 0.498 | ***0.001*** | 0.045 | 0.896 | ***0.058*** | 0.140 | 0.848 | ***0.012*** | 0.000 | 0.801 | 0.200 | 0.001 | 0.998 | ***0.002*** |
| **PB** | 0.000 | 0.500 | 0.000 | 0.500 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 0.278 | 0.722 | 0.000 | 0.000 | 0.833 | 0.167 | 0.000 | 1.000 | 0.000 |
| **PT** | 0.500 | 0.000 | 0.500 | 0.000 | 0.500 | 0.500 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | ***0.500*** | 0.500 | 0.000 | ***1.000*** | 0.000 | 0.000 |

| **Locus** | **ml-Pa14** | | | | **ml-Pa12** | | | **ml-Pa27** | | | **ml-Pa24** | | | **ml-Pa02** | | | **ml-Pa21** | | **ml-Pa57** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Allele/n** | **276** | **286** | **288** | **290** | **315** | **317** | **209** | **211** | **213** | **215** | **235** | **238** | **241** | **198** | **200** | **202** | **156** | **158** | **157** | **159** |
| **PA** | ***0.002*** | 0.081 | 0.909 | ***0.008*** | 1.000 | 0.000 | 0.000 | 0.105 | 0.888 | ***0.007*** | ***0.004*** | 0.265 | 0.730 | 0.212 | 0.740 | 0.048 | 0.000 | 1.000 | 0.998 | ***0.002*** |
| **PB** | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.250 | 0.750 | 0.000 | 0.000 | 0.000 | 1.000 | 0.111 | 0.889 | 0.000 | 0.000 | 1.000 | 1.000 | 0.000 |
| **PT** | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | ***1.000*** | 0.500 | 0.500 | 0.000 | 0.000 | 0.000 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | ***1.000*** | 0.000 | 1.000 | 0.000 |

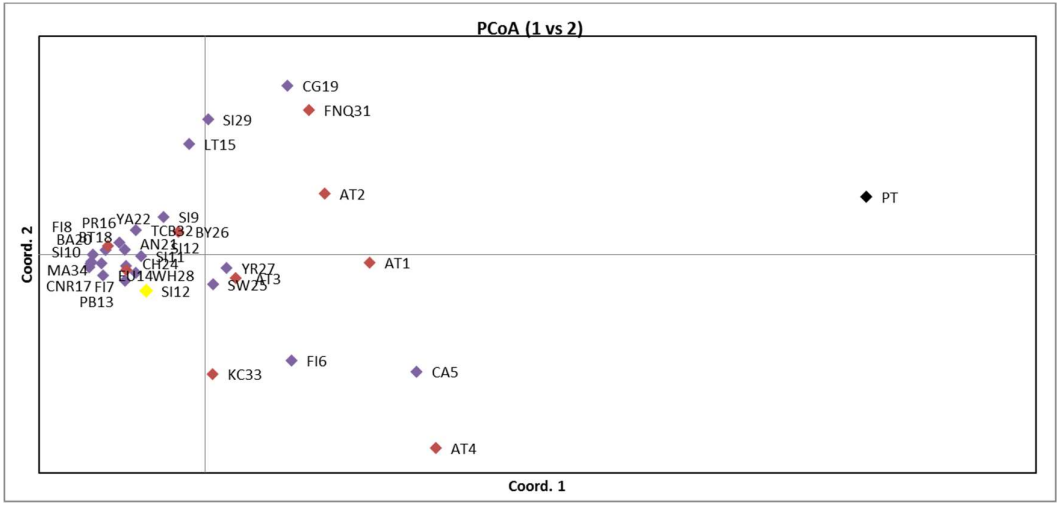


Figure 2.3. Results of principle coordinates analysis of genetic distance between 34 *P. australis* populations showing population identity. Symbols indicate historical nomenclature for populations; *P. australis* populations (n=23) purple diamonds, *P. bernaysii* population (n=1) yellow diamond, historic *P. tancarvilleae* populations (*n*=9) red diamond and 'control' *P. tancarvilleae* (n=1) black diamond.

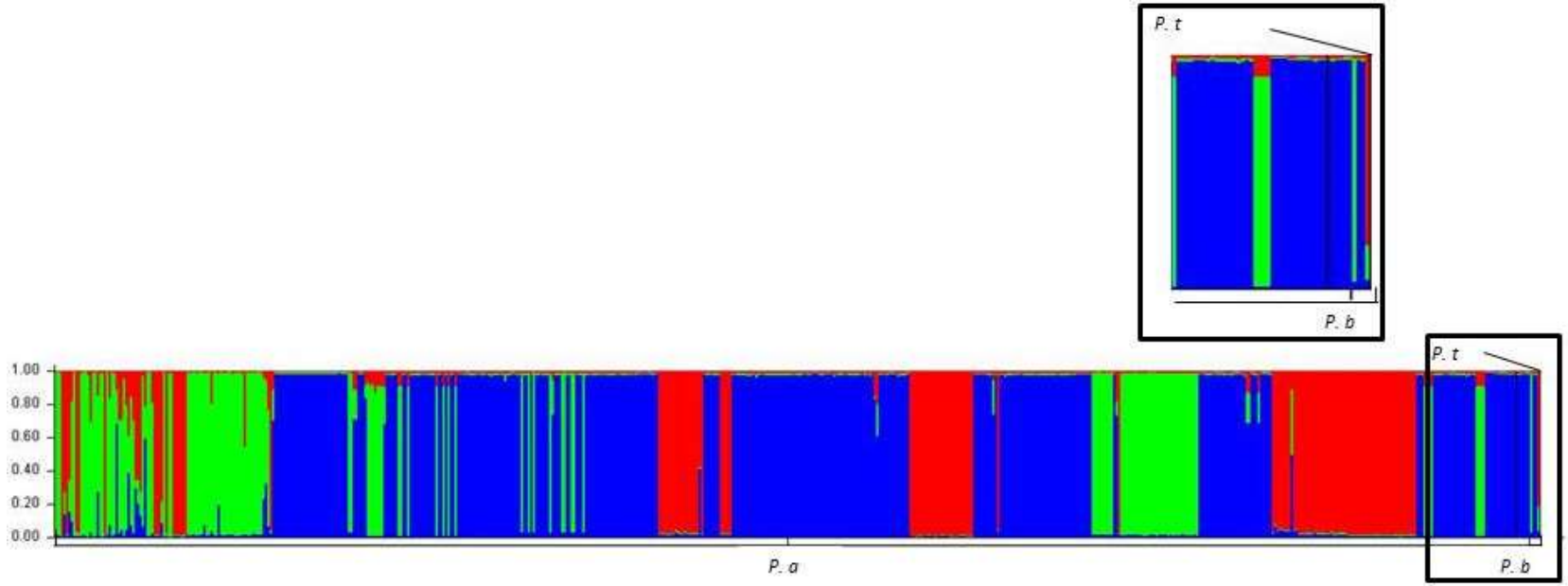


Figure 2.4. The bar chart of the structure Bayesian model-based clustering method for *P. australis* ($n_1$ = 617, indicated as *P.a* in diagram), *P. bernaysii* ($n_2$ = 9, indicated as *P. b* on right hand side of diagram) and *P. tancarvilleae* ($n_3$ = 1, indicated as *P. t* in diagram, last sample).

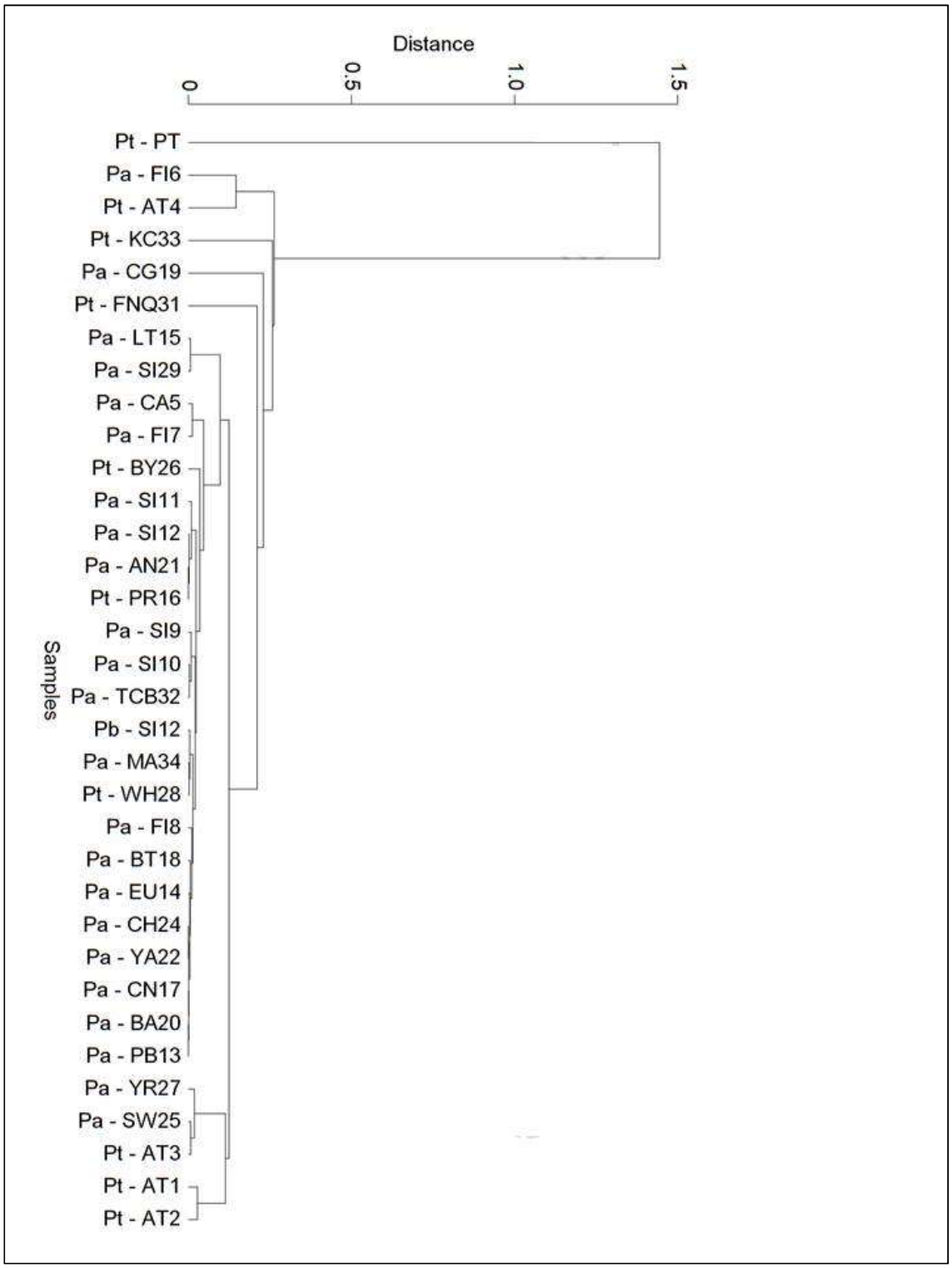


Figure 2.5. Unweighted pair group method with arithmetic averaging (UPGMA) dendrogram indicating genetic relationship (Nei 1978 distance) between historic *Phaius australis* (Pa), *P. tancarvilleae* (Pt) and *P. bernaysii* (P.b) populations across the species range.

## 2.4 Discussion

*How many populations of what size and are they in decline?*

Australian terrestrial orchids are often more abundant in coastal areas with the highest levels of land clearing for agriculture, urbanisation and forestry (Dixon et al. 2003, Vallee et al. 2004). The distribution of *Phaius australis* along the east coast of Australis is an area that has undergone considerable land-use change since European settlement (DE 2015a). While there was an overall decrease in the number of historic *P. australis* populations, there was an increase in number of populations in Queensland confirmed in 1994 (24) and on the EPBC threatened species listing (Benwell 1994, DE 2015a). The number of confirmed populations of *P. bernaysii* have reduced by two, as have the number of *P. australis* populations in New South Wales (south region, 10 confirmed; Benwell 1994, NPWS 1998, DE 2015a). While undertaking this work, there was ongoing discretion when describing population locations to protect wild plants from illegal collection, as previously experienced by other researchers (Benwell 1994). Whilst this is a legitimate concern, this did obstruct the confirmation of populations particularly in the south parts of the species range. Lodging specimens with relevant state and federal institutions such as herbaria is imperative to assist in enabling state and federal planning mechanisms to protect individuals and populations of *P. australis* from future human-impacts.

Estimations of population sizes (ramets) for *P. australis* have varied over the years (Benwell 1994, NPWS 1998, Freeman et al. 2011). Benwell (1994) recorded populations ranging in size from one individual to thousands of individuals, resulting in estimations of 1 500 plants in New South Wales and 5-10 000 plants in Queensland. This study has avoided calculating or estimating the total number of plants across the species range and has instead grouped population size into categories. As a result, the number of populations (14) with more than 100 individuals in Queensland roughly reflects that estimated by Benwell (1994). The presence of the two inland populations in Queensland in different ecological communities to the primary coastal distribution is significant for conservation. These populations have potentially been separated for hundreds of years and therefore have the potential to be genetically differentiated (Benwell 1994, Hampe and Petit 2005). In a similar fashion, the mountainous populations of *Cymbidium goeringii* were seen to be refugial repositories of diversity for lower altitude populations of the same species (Chung et al. 2014). Locating only two populations of the largest size category in the south region echoes the findings of Benwell (1994) and the National Parks and Wildlife Service (1998) in New South Wales in the past two decades.

The lack of large populations in the south region could be an indication that *P. australis* is reaching the southern limits of a suitable climatic range or that the species has reached the outer limits of its 'centre of abundance' (Sagarin and Gaines 2002). There may be flow on effects to the genetics of populations that are smaller and less dense at the edge of a range, potentially elevating the conservation concern for these locations (Ellstrand and Elam 1993, Leimu et al. 2006, Sagarin et al. 2006).

This study has documented that the direct loss of populations due to threats identified in the EPBC listing (DE 2015a), has remained an ongoing threat to the species across its range. Of concern is the loss of significant numbers of individuals in some populations between 2012 and 2015 through land development, road clearing and changed drainage regimes. Invasive plant species were recorded at all populations as previously observed for the species (Sparshott and Bostock 1993, Benwell 1994, Hartley 2008, DE 2015a), while browsing and trampling by feral pigs has been abated through fencing at CG19 and fencing to exclude livestock is being considered at the other population in this region (BT18). While the direct effects of sandmining on Stradbroke Island populations of *P. australis* may have decreased, similarly, the indirect effect of mining operations on the aquifer reserves that sustain the freshwater systems in 18 Mile Swamp on North Stradbroke Island is thought to be minimal (Freeman et al. 2011). This is supported by observations of tall, green, flowering and fruiting populations of *P. australis* in sections of 18 Mile Swamp adjacent to the current mining operations (pers. obs.).

Orchid enthusiasts have long regarded this species as one of the most desirable species for cut flower arrangements, propagation and cultivation, becoming first protected under the *Native Plants Protection Act of 1930* (Qld). The illegal collection of flower stalks and entire plants remains a great threat to the survival of *P. australis* and *P. bernaysii* in the wild. The removal of flower stalks may significantly interrupt recruitment of new plants. Finally, an individual *P. bernaysii* plant had been removed, presumably dug up, from North Stradbroke Island in 2014; a significant loss considering the small number of plants surveyed for this study and previous investigations (Sparshott and Bostock 1993). Iconic and novel species may not be under as great pressure from collecting from the wild as in centuries past, however an additional 144 nationally listed plants in Australia from the iconic families Orchidaceae, Zamiaceae and Cycadaceae have illegal collection from the wild identified as a current threat (EPBC Act; DE 2015a).

In 1994 it was estimated that 95% of pre-European settlement *P. australis* populations have been lost (Benwell 1994). This study reconfirmed the loss of some *P. australis* populations but also a decrease in population size since original identification, at over half of the populations in each region. This is particularly concerning for the populations in New South Wales (south region) where 80% of populations have decreased in size since original survey (NPWS 1998). Several other endangered species across south-east Queensland and northern New South Wales have experienced similar pressure. For example, increasing urban expansion resulted in the translocation of several endangered plant species, including *Acacia attenuata* and *Allocasuarina emuina* which occur in fragmented heathland adjacent to *P. australis* populations on the Sunshine Coast, Queensland (Brownlie et al. 2009, Lamont et al. 2012). In contrast, the Large Christmas Bell *Blandfordia grandifolia* which occurs in wet coastal heath of south east Queensland to southern New South Wales, is listed as endangered on the Queensland *Nature Conservation (Wildlife) Regulation* (2006) due to the effects of illegal collection, land use change and altered fire regimes, but has no threatened species listing in New South Wales (Conroy 2012).

*Characterisation of microsatellite loci for P. australis and cross-amplification*

Small populations for *P. australis* may have decreased genetic diversity lowering resilience to abiotic and biotic environmental change, and the ability to cope with pathogens and disease (Falk et al. 2006). Reintroduction or translocation of plants to enhance small populations should include the use of genetic data (Vallee et al. 2004, DE 2015a). Microsatellite markers are co-dominant markers of choice for intra- and inter-population variation studies in orchids, because they are highly polymorphic (Gustafsson 2000, Swarts et al. 2007, Bory et al. 2008). The optimisation of 11 polymorphic microsatellites markers for *P. australis* will be useful for revealing levels of genetic diversity, range expansion and the evolutionary relationships that may inform future assisted migrations or population enhancements (Gustafsson 2000, Bory et al. 2008, Hilbert et al. 2007, Kingsford and Watson 2011).

*Three, two or one taxa for the purpose of conservation?*

The diversity of the Orchidaceae family and the resultant frequency of taxonomic complexities of the family are well known (Dixon et al. 2003, Dressler 2005, Hopper 2009). Morphometric analysis combined with molecular techniques have been performed on many natural

populations to resolve taxonomically challenging species among many orchid genera, explain evolutionary history and elucidate morphological variation within a species (Goldman et al. 2004, Bateman et al. 2008, da Cruz et al. 2011, Tetsana et al. 2014).

The names *Phaius tancervilleae* and *P. wallichii* have been applied interchangeably to the populations now recognised as *P. australis* in Australia on the basis of differing floral morphology (Clements and Jones 2008, DE 2015a). It has been unsure if any the previously described floral morphologies hold true to the different species or if it is simply due to floral variation across the widespread *P. australis* (Benwell 1994, Bishop 2000). While this was not a strict morphometric study, field evaluations of floral characteristics found that there was no complete set of diagnostic traits for either *P. australis* or *P. tancarvilleae* at any population when investigated based on historical name of populations. There was considerable variability in floral morphology in *P. australis* across the latitude range, a phenomenon observed for other orchid species. Overall there was a greater resemblance to *P. australis* rather than *P. tancarvilleae*, supporting the current accepted taxonomy of *P. australis* (Clements and Jones 2008, CHAH 2010, DE 2015a).

Variation in floral morphology was described across the ecologically diverse range of *Liparis resupinata* as well as for *Cattleya elongata* in Brazil (da Cruz et al. 2011, Tetsana et al. 2014). Twenty-five microsatellite loci for *P. australis* were cross-amplified to *P. bernaysii* and *P. tancarvilleae* in this study; with unique alleles found in the Asian cultivated *P. tancarvilleae*. The lack of any of the P. *tancarvilleae* alleles in hundreds of *P. australis* samples supports the morphological evidence that none of the *P. australis* populations investigated in this study in Australia are *P. tancarvilleae*.

*Phaius bernaysii* is indistinguishable from *P. australis* differing only in the flower colour. This species was once suggested to be a colour morph *P. australis* var. *bernaysii* (Nicholls 1950, Sparshott and Bostock 1993, Jones 2006). Flowers of *P. bernaysii* that were critiqued possessed an equally high resemblance to *P. australis* morphology as flowers of *P. australis* across the species range. The intermixing of the two species and lack of geographic separation eliminates allopatric speciation such as that observed for Reunion Island polymorphic orchid *Calanthe sylvatica* (Juillet et al. 2010).

Cross-pollination of the two species in the natural population at Myora Springs was beyond the scope of this study. The known ability to cultivate hybrid crosses of *P. australis* x *P. bernaysii* (pers. com. B. Grey, P. March) points to a lack of reproductive separation (sympatric

speciation) between the two species. It was expected that there may be genetic distinction between *P. bernaysii* and *P. australis* in the microsatellite loci that may differentiate the two species as has been useful in differentiating ecotypes or phylogenies for other orchid species (Bory et al. 2008, Menz et al. 2015). Instead, *P. bernaysii* was found to have a subset of the alleles found for *P. australis*; while in contrast, only one *P. tancarvilleae* sample was required to identify distinct differences in allelic composition to *P. australis*. The microsatellite alleles in this study support the genetic findings of Harrison *et al.* (2005) that Australian *Phaius spp*. should be classified as a single species.

Polymorphism is commonly expressed in the flower of angiosperms, with forms occurring sympatrically creating mixed populations (Dressler 2005, Pellegrino et al. 2005, Hedrén and Nordström 2009). *Phaius bernaysii* was first described in 1873 as occurring among thousands of its congener, hence previous nomenclature in the mid-20$^{th}$ century of *P. australis* var. *bernaysii* (Hooker 1873, Nicholls 1950). In the early stages of this study it was not clear as to whether there were single populations of *P. bernaysii* in existence. Regardless of if there were separate populations, *P. bernaysii* is now only inter-mixed with *P. australis* with no different ecological requirements. Thus, based on the evidence, *P. bernaysii* is likely the expression of a recessive allele that might be present at a low frequency within the Myora Springs population. This may have resulted in the uncommon expression of the *P. bernaysii* phenotype amongst a greater proportion of heterozygote carriers of the more dominant *P. australis* allele. Pale morphs and white individuals within natural populations of a more common coloured-morph have been observed for many orchid species (Weiss 1995, Gigord et al. 2001, Pellegrino et al. 2005).

Polymorphs offer different colour or scents that may attract different or more abundant pollinators resulting in reproductive fitness equal to or greater than the more prevalent colour (Ackerman and Carromero 2005, Pellegrino et al. 2005, Chittka and Raine 2006, Streisfeld and Kohn 2007). The less frequent morph is maintained over evolutionary time through pollinator preference for the rare colour morph (Gigord et al. 2001, Ackerman and Carromero 2005). While the higher abundance of fruit set in *Phaius bernaysii* plants than *P. australis* in Myora Springs in 2013 could be explained by pollinator preference, *P. australis* has previously had no pollinators observed, with a propensity to autogamously self-pollinate (Sparshott and Bostock 1993, Benwell 1994, Bishop 2000). Given the similarity in floral morphology between the two species, it is assumed that the same reproductive biology applies for *P. bernaysii*.

The maintenance of *P. bernaysii* may be as a consequence of inbreeding. Self-fertilisation is likely to produce more homozygotes (Wright 1931, 1948), including more homozygotes of the white recessive allele (Clegg and Durbin 2000). Greater inbreeding rates through self-fertilisation or between two closely related individuals might thus maintain or produce higher frequencies of the pale-flowered individuals, perpetuating the recessive allele. Inbreeding is thought to partly explain the constancy of morphs within populations of *Dactylorhiza incarnata* (Hedrén and Nordström 2009) and the combination of self- or kin-fertilisation is thought to contribute to the maintenance of the white colour morphs of *Orchis mascula* in France (Dormont et al. 2010).

The consequence of *P. bernaysii* self-pollinating or mating between closely related individuals is inbreeding depression and the loss of fitness (vigour, seed set or viability) due to a build-up of deleterious alleles in the homozygous state (Young et al. 2000, Falk et al. 2006). Reduced seed production, germination, and female fitness was observed in small inbred populations compared to large outcrossed populations of Norwegian orchid *Gymnadenia conopsea* (Sletvold et al. 2012). On the other hand, there was no evidence of inbreeding depression for self-pollinating orchid *Neotinea maculata* (Duffy et al. 2009). The increased abundance of fruit set for *P. bernaysii* in comparison to *P. australis* may indicate increased fitness and selection for the species adding to the maintenance of the recessive albino allele in the population, in contrast to expectations for inbreeding depression. Further analysis via genetic and reproductive biology studies to examine the genetic relatedness between *P. australis* and *P. bernaysii* within the Myora population may assist in revealing the evolutionary driver of floral colour polymorphism in *P. australis*.

## 2.5 Conclusions

This study found that some populations of *Phaius australis* are no longer in existence, some populations were being negatively impacted and illegal collection of inflorescences and entire plants is also still occurring. Some populations have increased in size, while others have decreased since the original surveys or lodgement of specimens at herbaria. The higher frequency of small populations in the south of the range compared to the north potentially makes populations in New South Wales at greater risk of extinction. Eleven polymorphic microsatellite markers were successfully developed for *P. australis* and cross amplified to the putative yellow morph *P. bernaysii*, and congener *P. tancarvilleae*. Beyond taxonomic

determination, these microsatellite markers will be of use for investigating the genetic diversity of the species in Australia.

Allelic patterns from microsatellite amplification were used to determine taxonomic boundaries with one of the key findings that *P. bernaysii* appears to be a yellow morph of *P. australis*. Some species level differences can be identified between *P. australis* and *P. tancarvilleae*. With a larger sample size of *P. tancarvilleae*, further diagnostic alleles may identify true *P. tancarvilleae* populations. No wild populations displayed the full suite of floristic characteristics expected of *P. tancarvilleae* and therefore it is recommended that the current taxonomy for *Phaius australis* remain. The lack of geographic, reproductive and genetic distinction between *P. bernaysii* and *P. australis* points toward the latter species possessing a colour polymorphism and may result in classification changes to *P. bernaysii*. Rare alleles can be lost through genetic drift when the number of populations or the individuals within them are lost. Thus, there is an increased conservation importance of the extremely small number of plants of the *P. bernaysii* phenotype as well as any *P. australis* populations that may share the genotypes of the *P. bernaysii* individuals.

# Chapter 3: Genetic diversity of endangered orchid *Phaius australis* across a fragmented Australian landscape

## 3.1 Introduction

Past climatic changes causing periods of expansion and contraction of ecosystems are one of the main determinants of range shifts and distribution of plant species in Australia (Barlow 1981, Hill 1994; Hughes 2000, Williams et al 2003). As Australia was divided from the supercontinent Gondwana and moved away from the polar region, the Gondwanan flora on the Indo-Australian plate experienced changes in climate, including increased aridity (Ziegler et al. 2003). After millions of years of northwards drift, the wetter and cooler rainforest species in many places were replaced with species adapted to drier climates by the Early to Middle Miocene (<20 Ma; Byrne et al. 2009).

The major climatic oscillations throughout the Quaternary period (1-3 Ma) have had great influence on the assemblage of Australian flora. Land bridges between the Indo-Malayan and the Indo-Australian plate in glacial periods allowed the dispersal and colonisation of tropical flora in Australia (Barlow 1981, Hill 1994), while interglacial periods influenced evolution through isolation. At a high taxonomic level, Australia is composed of plant families that occur elsewhere in the world, while at lower taxonomic levels, many genera are only shared with previously connected continents and species within genera are restricted to the Australian continent (Barlow 1981, Byrne et al. 2011). This includes the Indo-Malayan elements that are represented in the fringing east coast tropics and subtropics zones in Australia (Hill 1994, Bowman et al. 2010, Byrne et al. 2011). The severe climatic fluctuations of the Last Glacial Maximum (LGM 21ka) caused expansion of species into new novel habitats then retraction to refugial areas of suitable climate and is one of the main determinants of the current distributions of plant species (Hewitt 2000; Mellick et al 2012). Temperature and moisture play critical roles in seed germination, survival, plant vigour and fecundity, therefore the distribution of species on medium spatial scales is a result of climatic conditions (Woodward and Williams 1987, Lawton 1993).

Historical colonisation processes such as climate induced range shifts can leave lasting imprints on population genetic diversity (Excoffier et al. 2009, Knowles and Alvarado-Serrano 2010; Mellick et al 2012; Rosetto et al 2012). Spatial population expansions that occur via a founder event are often accompanied by large demographic expansions without admixture with the broader genetic pool of the species; which are repeated along a pathway of colonisation (Currat and Excoffier 2005, Excoffier et al. 2009). Hence, as a species colonises a linear

pathway such as an environmental or latitude gradient, genetic diversity can steadily decline along the axis of colonisation with recently colonised populations possessing reduced genetic diversity with some level of genetic structure across a distribution (Wright 1948, Austerlitz et al. 1997; Mellick et al. 2012). Refugial, isolated or older populations may be more distinct because they are older and have been isolated longer than leading-edge populations (Hampe and Petit 2005; Mellick et al. 2012). Future climate change may result in the migration of species to new suitable habitat or retraction to refugia, perpetuating the historical evolutionary pattern of a species (Parmesan and Yohe 2003, Arenas et al. 2012, Bellard et al. 2012).

Other processes may complicate the evolutionary trajectory of a species. The population dynamics and demographics can also affect the evolutionary trajectory of a species. Population size tends to decline toward range margins, because in the first instance, newly founded populations are often small as they adjust to new conditions (Sexton et al. 2009). For orchids, the establishment and survival of populations is often limited by the presence of soil mycorrhizal fungi, required for seed germination and plant survival (McCormick et al. 2012). Organisms may persist in small populations at the edge of a geographic range even after generations of time because of the suboptimal conditions (abiotic and biotic) at the edge of the species range, causing lower density and population sizes and reduced genetic diversity at the edges of a species range, referred to as the 'abundant-centre' (Sagarin and Gaines 2002, Duffy et al. 2009).

Human-mediated fragmentation of natural habitats resulting in a reduced number of individuals, populations and population sizes can also complicate the evolutionary patterns of genetic diversity of a species and make a species more vulnerable to extinction (Young et al. 1996, Eckert et al. 2008). Such loss of populations and individuals decreases the gene flow between populations and increases detrimental ecological and demographic effects of small population size (Ellstrand and Elam 1993, Young and Clarke 2000, Broadhurst and Young 2007, Newman et al. 2013). The intrinsic nature of small fragmented populations means that they are have increased sensitivity to losing alleles through genetic drift, resulting in low genetic variation within populations (Wright 1948, Frankham 2005, Falk et al. 2006, Leimu et al. 2006).

The loss of genetic diversity may reduce the potential persistence in the face of abiotic and biotic environmental change, reducing the framework for adaptation to changing conditions (Davis and Shaw 2001, Frankham 2005, Jump et al. 2009). This diversity may be particularly important in the face of future climate change where some species may lack the ability or

speed to migrate to new suitable habitat (Parmesan 2006, Jump et al. 2009, Bellard et al. 2012, Corlett and Westcott 2013). An understanding of the differences between the biogeographic, demographic and the human-mediated effects on the amount and distribution of genetic variability is necessary for adequate and effective species conservation management particularly in the context of future climate change (Gaston 2000, Pillion et al. 2007, Eckert et al. 2008, Stone et al. 2012).

*Endangered swamp orchid Phaius australis*

The Orchidaceae (orchids) is the largest and most successful plant family found on all continents of the world, with greatest proliferation in the tropics (Koopowitz 2001, Dixon et al. 2003, Jones 2006). The genus *Phaius* is composed of 20 species, extending from India to China, Southward to Malaysia, Indonesia, Papua New Guinea, some Pacific Island, with three species recorded in Australia (Dockrill 1992, Jones 2006, WCSP 2015). Hence, it is expected that this genus has colonised Australia in past times of previous connectivity then expanded southwards under ideal climatic conditions to their current distribution. The family Orchidaceae is well known for the production of vast amounts of minute, dust-like seeds which enables rare long distance seed dispersal and the ability to colonise new favourable environments (Arditti and Ghani 2000, Murren 2003, Molnár et al. 2011). As a result, populations that are fragmented and separated by great distances may be connected by gene flow between populations, resulting in less genetic structuring within a species (Forrest et al. 2004, Bialozyt et al. 2006).

*Phaius australis* F.Muell. is Australia's tallest terrestrial orchid with inflorescences that can be over two metres tall, with four to twenty deep purple and cinnamon brown flowers produced in August-October (Jones 2006, Freeman et al. 2011). Flowers are 7-10 cm across but can be as large as 17 cm, opening one or two at a time (Dockrill 1992, Jones 2006) and lack of rostellum resulting in an ability to spontaneously self-pollinate in natural populations, offering reproductive security for colonisation of new habitats (Gandawidjaja and Arditti 1982, Benwell 1994, Jones 2006). *Phaius australis* occurs over a disjunct latitude gradient of 2 000km from tropical north Queensland to the southern limits of the sub-tropics in New South Wales on the east coast of Australia (Benwell 1994, Jones 2006; Chapter 2).

The species is closely related to *Phaius tancarvilleae*, a very similar terrestrial orchid that occurs in the warm tropics of Indonesia, Papua New Guinea, South East Asia and Pacific Islands

(Dockrill 1992, Jones 2006). Harrison *et al.* (2005) identified no molecular differences between *P. australis* and *P. tancarvilleae* using ITS markers. There was no morphological or genetic evidence that any current populations represent *P. tancarvilleae* in Australia consistent with current taxonomy (Chapter 2; Clements and Jones 2008, CHAH 2010). It is thought that the species has since diverged to what is now *P. australis* over periods of isolation after colonisation from the north and consequent range expansion under ideal climatic conditions across a latitude gradient to a southern boundary of its climatic niche (Dockrill 1992).

Habitat for *P. australis* is in the marginal coastal swampy wetlands and littoral rainforests of the east coast with several isolated inland populations (Chapter 2; Benwell 1994). However, the natural areas along the coast of Australia are now highly fragmented with present day *P. australis* population locations disjunct (Chapter 2; Benwell 1994, DE 2015a). *Phaius australis* was also once a highly desired species and heavily collected from the wild, resulting in an 'endangered' listing on the Australian federal *Environmental Protection and Biodiversity Conservation Act 1999* ('EPBC Act'; Benwell 1994, DE 2015a). Small population sizes have been documented across the range for *P. australis*, and the threat of illegal collection remains, particularly in the southern part of the species range (Chapter 2).

This study aims to investigate three conflicting hypotheses to explain the population genetic diversity of *P. australis*: biogeographical colonisation patterns, a demographic 'abundant centre' and fragmentation effects. First, what are the species and population levels of genetic diversity? Is genetic diversity distributed within or among populations? Is there evidence of gene flow between populations? How does genetic diversity and gene flow compare to other orchids. Then, does genetic diversity steadily decline southward along the latitudinal axis of range expansion southward from north Queensland to New South Wales? Consequently, are the older northern (north Queensland) populations and the younger southern (New South Wales) populations genetically differentiated? Additionally, does population size and density decline at the edges of the distribution, reducing genetic diversity according to the 'abundant centre' hypothesis? Does the genetic diversity of *P. australis* decrease with decreasing population size or density or with increasing isolation as a result of fragmentation? Are there differences in population genetic diversity between small and large populations or between populations that are more or less isolated?

## 3.2 Methods

Thirty-three populations were sampled across the entire distribution of *P. australis*, based on 36 known locations and site accessibility. Populations are scattered across a broad climatic range from Rossville, North Queensland to South West Rocks in New South Wales (1 800 km). A 'population' was defined as a group of plants separated by at least 300 m from another; populations were also defined if they were separated by an anthropogenic interference such as building infrastructure. A stratified sampling design was utilised to achieve even replication across biogeographic regions (north, mid, south, inland) covering the species distribution (Figure 3.1). Sampling design incorporated sampling of 17 large (greater than or equal to 50 individuals) and 16 small populations (less than 50 individuals) that were nested within each region to achieve replication within regions and investigate the effects of population size on genetic diversity. Replication was reduced for the Inland region as only two populations are known. A mixed population of *P. australis* and putative *P. bernaysii* plants was sampled at Myora on Stradbroke Island in the mid region. All plants at this location are considered as *P. australis* for the purpose of this study as there are no diagnostic differences between the species in the field without flowers and previous results (Chapter 2) indicated no differentiation in microsatellite markers between *P. australis* and *P. bernaysii*.

*Field Collection of Samples*

Populations were sampled on foot during the flowering and fruiting season between August 2012 and January 2013 to aid with identification of plants. Populations were located in the field utilizing a combination of GPS coordinates from historic records, maps, verbal descriptions or local guides. Searches were undertaken by systematically surveying the entire area of potential habitat in a series of contiguous parallel belt transects 10 m wide (Cropper 1993). All *Phaius* plants were documented across the geographic range of a site with geographic extent of populations marked with a GPS. At all locations, the starting location of each population/site was recorded using the GPS.

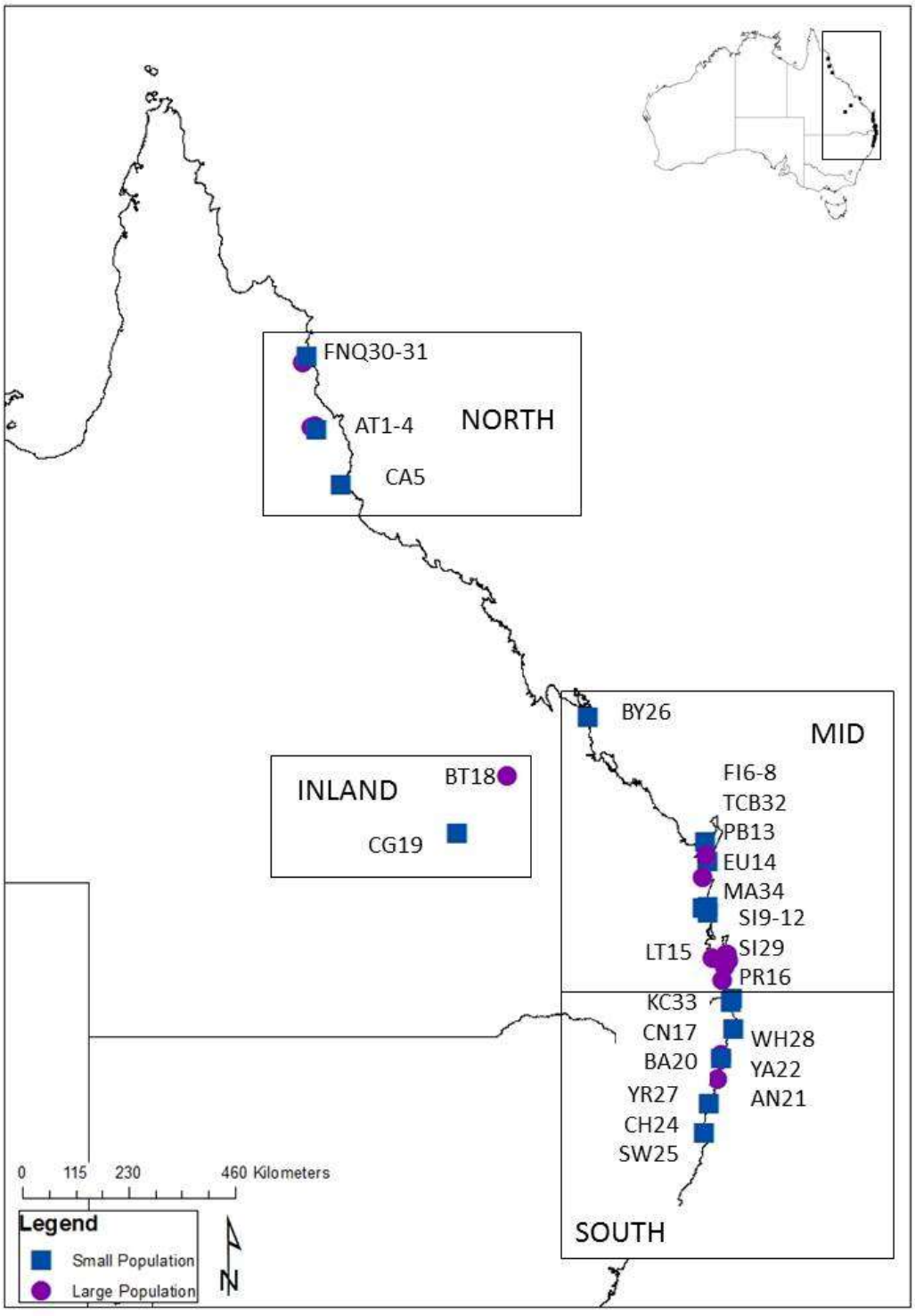


Figure 3.1. Map overview of the *Phaius australis* distribution on the east coast of Australia showing the locations and size class (small/large) of populations sampled for genetics across four regions which are shown (North, Mid, South and Inland).

At each site up to 90 individuals were recorded as representative of the population. Additional clumps of plants beyond 90 individuals were mapped with key features recorded at more extensive and/or dense populations. The relative location of each individual plant within the site was mapped using compass direction (degrees) and distance (metres) between clumps and plants measured with a LaserDisto measure (Leica geosystems). These were later converted using trigonometry to XY coordinates in metres based on start point.

Samples for genetic analysis were collected from 30 individuals per population. Sampling was spread evenly across the spatial occupancy to sample the random variation in the population approximately evenly distributed across all size classes including recruits, intermediates and sexually mature individuals. Where populations were smaller than 30 individuals, samples were collected from every individual. Genetics samples consisted of approximately 10 x 10 cm of green, healthy leaf material, placed in a sealed plastic bag labelled with the plant number. Surface mould, rust and foreign material was cleaned from the leaf, then sampled were placed in labelled plastic clip-seal bags with Grade 12, 28-200 mesh silica gel (Sigma-Aldrich). Samples were stored at room temperature away from light in the University of the Sunshine Coast Laboratory prior to DNA extraction.

*Laboratory genetics analysis*

Total genomic DNA was extracted from the leaf tissue of all samples using QIAGEN DNeasy® Plant Mini Kits (QIAGEN Valencia, CA, USA) following manufacturer's instructions as follows. Approximately 30-50 milligrams of plant tissue samples were frozen using liquid nitrogen and ground using a Retsch MM200 Tissue Lyser grinding mill (Qiagen) for 30 secs at 23 000 rpm. The manufacturer's protocol was followed for the extraction process (QIAGEN 2012). Genomic DNA was compared with known concentrations (5 ng/µL, 10 ng/µL, and 20 ng/µL) of Lambda EcoR1/HindIII digest molecular weight marker (Fisher-Biotech) to estimate yields using 1.5 % agarose gel electrophoresis; viewed under UV light using a Syngene gel documentation system and Syngene GeneSnap software.

DNA from 4 individuals was sent to Australian Genome Research Facility Ltd (AGRF, Brisbane, Australia; http://agrf.org.au) for microsatellite (SSR) marker development using Next-Generation 454 pyrosequencing and a random library was constructed using GS-FLX Titanium chemistry Illumina HiSeq (Roche Applied Science; Mannheim, Germany). Sequences were

identified and 61 unlabelled microsatellite primer pairs were trialled as detailed in Chapter 2. Final selection resulted in PCR amplification of 24 primer pairs for all individuals (Chapter 2).

PCR was performed using reaction volumes of 12 µl containing approximately 25 ng genomic DNA, 1x reaction buffer (67 mM Tris-HCl (pH 8.8), 16.6 mM $(NH_4)_2$ $SO_4$, 0.45% Triton X-100, 0.2mg/mL gelatine; Fisher Biotech), 200 µM of each dNTP, 2 mM MgCl2 (Fisher Biotech), 0.2 µM BSA (Fisher Biotech) 0.2 µM forward primer, 0.2 µM reverse primer and 0.5 U F1 Taq polymerase (Fisher Biotech). PCR was performed using a Mastercycler gradient thermocycler (Eppendorf) with the following cycling conditions: denaturation at 95 °C for 3 mins; 35 cycles of 94 °C for 30 secs, specific annealing temperature (Chapter 2) for 30 secs, 72 °C for 45 secs; final elongation step at 72 °C for 10 mins.

PCR products for all samples were multiplexed for the same individuals according to different dye sets and size ranges, and then separated by capillary electrophoresis on an AB3500 Genetic Analyser (Applied Biosystems). Fragment sizes were determined relative to internal lane standard (GS-600 LIZ; Applied Biosystems). GENEMAPPER v4.1 software (Applied Biosystems) was used to score fragments according to alleles and loci, double checked manually to ensure accuracy to expected banding size. Some loci (*ml-pa*05, *ml-pa* 40) did not yield consistent or easily scored results and these were eliminated from further analysis. As per Chapter 2, a resultant 22 loci were scored; yielding 13 polymorphic loci for final data analysis: (*ml-pa02*, *ml-pa03*, *ml-pa12*, *ml-pa14*, *ml-pa19*, *ml-pa21*, *ml-pa24*, *ml-pa27*, *ml-pa31*, *ml-pa44*, *ml-pa49*, *ml-pa57, ml-pa59*). The presence of null alleles, scoring errors, and large allele dropouts were checked for all loci using MICROCHECKER v2.2.3 (Van Oosterhout et al. 2004) and linkage disequilibrium was tested using Fisher's exact tests and a sequential Bonferroni correction implemented in GENEPOP v1.2 (Chapter 2).

*Data analysis*

GPS points of populations were mapped on ArcGIS (ESRI 10.2.2) in Easting and Northings (UTM) in metres and the number of individuals in each population (*N*) recorded. The area of occurrence (*Ar*) in $m^2$ was derived utilising boundary GPS coordinates for each population. Population density measurements (*D*) per hectare (Ha) were calculated utilising the following equation: *N*/*A* *10 000.

The GPS coordinates of populations were utilised to calculate pairwise-population distance-to-nearest neighbour (*pNN*) in kilometres (km) in ArcGIS (ESRI 10.2.2) and generate geographic

distance matrices. The relative isolation of *P. australis* populations was calculated via an isolation ranking (*IR*) incorporating *pNN* with the population size (*N*) of the nearest-neighbouring population. For this measure, each population was assigned an isolation ranking from 1-20 (with 20 being most isolated) based on a ranking system that combined a score of 1-10 for distance to nearest-neighbouring population (*pNN*) and a score of 1-10 for the nearest-neighbouring population size (Table 3.1).

Table 3.1. Classification system of the relative isolation (isolation rank, *IR*) of *P. australis* populations to give a combined score out of 20.

| **Distance to Nearest Neighbour (km)** | **Distance Rank** | **Nearest Neighbour Population Size** | **Nearest Neighbour Population Size Rank** |
|---|---|---|---|
| 0 to 1 | 1 | 1 to 10 | 1 |
| 1 to 2 | 2 | 10 to 20 | 2 |
| 2 to 5 | 3 | 20 to 40 | 3 |
| 5 to 10 | 4 | 40 to 60 | 4 |
| 10 to 15 | 5 | 60 to 80 | 5 |
| 15 to 25 | 6 | 80 to 100 | 6 |
| 25 to 50 | 7 | 100 to 200 | 7 |
| 50 to 100 | 8 | 200 to 300 | 8 |
| 100 to 200 | 9 | 300 to 500 | 9 |
| 200 + | 10 | 500 + | 10 |

*Population genetics*

Allelic frequencies and standard measures of genetic diversity, percentage polymorphic loci (*P*), number of alleles per locus (*A*), number of per polymorphic locus (*Ap*), the effective number of alleles per polymorphic locus (*Ae*), expected heterozygosity (*He*), the number of private alleles (*PA*), were calculated for the species and each population using GenAlEx 6.5 (Peakall and Smouse 2012). Measures of non-random mating and inbreeding are examined in a later chapter.

Distribution and partitioning of genetic diversity across individuals within the species ($F_{IT}$), individuals within populations ($F_{IS}$), and among populations ($F_{ST}$) using Wright's F statistics (Wright 1965) and mean average number of migrants per generation (*Nm*) were calculated for the species using GenAlEx 6.5 (Peakall and Smouse 2012). Analysis of molecular variance (AMOVA) was used to investigate partitioning of variation within and among regions using GenAlEx 6.5 (Peakall and Smouse 2012). Partitioning of genetic diversity was investigated between individuals within a population relative to all individuals within the species (*PhiPT*), among populations within a region (*PhiPR*) and among regions relative to all individuals within

the species (*PhiRT*; Excoffier et al. 1992) calculated in GenAlEx 6.5 (Peakall and Smouse 2012) using 9999 permutations. Pairwise population number of migrants per generation (*pNm*) was calculated in GenAlEx 6.5 (Peakall and Smouse 2012). A frequency-based population assignment test was conducted to designate a percentage 'self' or 'other' assignment by assigning samples to the site with the highest likelihood of genetic similarity using GenAlEx 6.5 (Peakall and Smouse 2012).

*The effects of range expansion on genetic diversity*

A Spearman's rank correlation test in SPSS v 19 (IBM 2010) was used to test if genetic diversity measures (*P*, *A*, *Ap*, *Ae*, *He*, *PA*) decline southwards with latitude (Lat) consistent with the expected biogeographic pattern of the species. Small populations were excluded in this analysis as it was expected that smaller populations may have minimal genetic variability and could therefore mask broader allelic patterns. One-way ANOVA with Tukey's post hoc tests were used to examine significant differences in population demographic parameters (*D*, *N*, *pNN*, *IR*) among regions (North, Mid, South, Inland) using SPSS v 19 (IBM 2010).

To examine if there is any genetic differentiation between the North and the South populations, Nei's genetic distance measures were calculated between all pairs of populations in GenAlEx 6.5 (Peakall and Smouse 2012). This was imported into PRIMER 6.1.5 (PRIMER-E Ltd 2006) and used to perform hierarchical cluster analysis (UPGMA - unweighted pair group method with arithmetic averaging) to investigate genetic relationships among populations. A one-way ANOVA with Tukey's post hoc tests was used to examine differences in population genetic measures (*P*, *A*, *Ap*, *Ae*, *He*, *PA*) among regions (North, Mid, South, Inland) using SPSS v 19 (IBM 2010). Principle Components Analysis was undertaken for all individuals, with 9999 bootstrap permutations in GenAlEx 6.5 (Peakall and Smouse 2012); plots presenting populations were grouped by region to further examine if there was evidence of genetic differentiation between the older north populations and the younger south populations.

To explore the possibility of the 'abundant centre hypothesis', the relationship between the climatic edge of the species distribution (*Lat*) and the population demographic parameters of plant density (*D*) and population size (*N*), were tested using Spearman's rank correlation tests in SPSS v 19 (IBM 2010).

*Effect of fragmentation on genetic diversity*

Spearman's rank correlation tests in SPSS v 19 (IBM 2010) were used to test if genetic diversity measures (*P*, *A*, *Ap*, *Ae*, *He*, *PA*) decline with population size (*N*) and plant density (*D*). An Independent T-Test was used to examine any differences in genetic diversity measures (*P*, *A*, *Ap*, *Ae*, *He*, *%P*) and small versus large population size groupings.

Populations were arranged in two sub-groups based on less isolation (isolation rank 1-10) or more isolation (isolation rank 11-20) and an Independent T-Test was used to examine any differences in genetic diversity measures (*P*, *A*, *Ap*, *Ae*, *He*, *%P*) between the more vs less isolated population sub-groups in SPSS v 19 (IBM 2010). A Mantel test was used to test for correlations between geographic and genetic distance matrices across the overall climatic distribution using 9999 permutations in GenAlEx 6.5 (Alexandersson and Ågren 2000, Peakall and Smouse 2012).

## 3.3 Results

Population sizes (*N*) ranged from two individuals at Moon Point (FI7) through to 696 at Flinders Beach (SI11), but the average population size was 138 individuals (Table 3.2). The average plant density was 924 plants per hectare but ranged enormously from very sparse populations of 1 to very dense populations of 5 500 plants per hectare (Byfield BY26 and Kingscliff KC33 respectively; Table 3.2). Populations were within 33 km of each other on average, with distance to the nearest population ranging from 0.3 km in North Queensland (AT1, AT2) to 209 km for Byfield (BY26; Table 3.2).

A total of 48 alleles across the 22 microsatellite loci were recorded for the 627 *P. australis* individuals assessed with 85% of the loci polymorphic ($\%P_S$; Appendix 1). The mean population percentage of polymorphic loci (*P*) was only 37.5% though there was considerable variation in the percentage of polymorphic loci across the populations (Table 3.3). There was a mean of 3.0 alleles per locus found for the species ($A_s$; Table 3.3). Results for polymorphic loci only are presented in the remaining analyses. Genetic diversity measures within populations were much lower, with an overall population mean of 1.5 alleles per locus (Table 3.3). Expected heterozygosity ($H_e$) was also quite low, with an overall population mean of 0.171 (Table 3.3).

Table 3.2. Population demographic parameters for 33 *P. australis* populations across the species range, overall average given;; *N*, total population size at time of sampling, *D*, density of plants per hectare (ha); *pNN*, population first nearest neighbour distance (km); *IR*, isolation ranking score where 20 is most isolated. Results of Kruskall-Wallis with significance indicated (*p<0.05, **p<0.01)

| Region | Population | Size (N) | D (plants/ha) | pNN (km) | IR |
|---|---|---|---|---|---|
| North | FNQ31 | 59 | 3919.48 | 152.77 | 9 |
| | AT1 | 252 | 583.41 | 0.30 | 10 |
| | AT2 | 338 | 459.42 | 0.30 | 9 |
| | AT3 | 591 | 547.31 | 6.49 | 12 |
| | AT4 | 8 | 8.423 | 8.26 | 12 |
| | CA5 | 4 | 25.97 | 128.92 | 10 |
| | **mean** | 209 | 924 | 49.41 | 10 |
| Mid | BY26 | 3 | 1.00 | 209.18 | 18 |
| | FI6 | 96 | 21.61 | 29.50 | 13 |
| | FI7 | 2 | 2.54 | 13.65 | 6 |
| | FI8 | 8 | 8.00 | 13.65 | 11 |
| | TCB32 | 151 | 275.63 | 36.56 | 8 |
| | PB13 | 5 | 109.58 | 11.08 | 7 |
| | EU14 | 17 | 5.00 | 11.08 | 6 |
| | MA34 | 12 | 870.22 | 13.33 | 6 |
| | LT15 | 224 | 343.52 | 23.82 | 13 |
| | SI9 | 330 | 213.03 | 2.68 | 13 |
| | SI10 | 286 | 150.64 | 2.68 | 11 |
| | SI11 | 696 | 185.81 | 7.44 | 14 |
| | SI12 | 113 | 51.34 | 9.33 | 11 |
| | SI29 | 110 | 752.04 | 15.65 | 15 |
| | PR16 | 136 | 287.34 | 28.18 | 14 |
| | **mean** | 146 | 218 | 28.52 | 11 |
| South | KC33 | 22 | 5500.00 | 7.45 | 6 |
| | CNR17 | 17 | 137.71 | 7.45 | 7 |
| | BA20 | 6 | 717.31 | 57.61 | 10 |
| | WH28 | 64 | 10.46 | 7.98 | 5 |
| | AN21 | 170 | 84.71 | 1.68 | 9 |
| | YA22 | 6 | 2400.00 | 1.68 | 3 |
| | YR27 | 369 | 997.60 | 42.38 | 14 |
| | CH24 | 12 | 1200.00 | 87.78 | 11 |
| | SW25 | 21 | 1033.97 | 87.78 | 10 |
| | **mean** | 76 | 1342 | 33.53 | 8 |
| Inland | BT18 | 219 | 1879.81 | 159.09 | 14 |
| | CG19 | 69 | 387.49 | 159.09 | 17 |
| | **mean** | 144 | 1133 | 159.09 | 16 |
| | **total mean** | 138 | 724.07 | 42.03 | 10 |
| | ***Kruskall Wallis (H)*** | 1.191 | 7.57 | 5.34 | 7.21 |

Table 3.3. Summary of *P. australis* genetic diversity measures across 33 populations from 13 polymorphic loci (Appendix 1). Where: *n*, number of samples; *P*, percentage polymorphic loci; *A*, mean number alleles per locus; *Ap*, mean number alleles per polymorphic locus; *Ae*, mean number of effective alleles per polymorphic locus; $H_e$, mean expected heterozygosity polymorphic loci. *H* = result of Kruskall-Wallis rank test between groups; Significance values *p <0.05, **p <0.01, ***p<0.001.

| Region | Population | *n* | *P* | *A* | *Ap* | *Ae* | *He* |
|---|---|---|---|---|---|---|---|
| North | FNQ31 | 29 | 30.77 | 1.227 | 1.455 | 1.340 | 0.156 |
| | AT1 | 28 | 76.92 | 1.500 | 2.000 | 1.667 | 0.349 |
| | AT2 | 28 | 69.23 | 1.500 | 2.000 | 1.555 | 0.320 |
| | AT3 | 27 | 61.54 | 1.545 | 2.091 | 1.339 | 0.179 |
| | AT4 | 5 | 30.77 | 1.182 | 1.364 | 1.291 | 0.154 |
| | CA5 | 4 | 30.77 | 1.182 | 1.364 | 1.327 | 0.172 |
| | mean | | 50.00 (21.62) | 1.42 (0.176) | 1.71 (0.220) | 1.422 (0.153) | 0.222 (0.088) |
| Mid | BY26 | 3 | 53.85 | 1.364 | 1.727 | 1.466 | 0.253 |
| | FI6 | 34 | 53.85 | 1.364 | 1.727 | 1.339 | 0.193 |
| | FI7 | 2 | 23.08 | 1.091 | 1.273 | 1.273 | 0.136 |
| | FI8 | 4 | 30.77 | 1.227 | 1.455 | 1.410 | 0.185 |
| | TCB32 | 29 | 53.85 | 1.364 | 1.727 | 1.313 | 0.186 |
| | PB13 | 4 | 23.08 | 1.136 | 1.273 | 1.262 | 0.134 |
| | EU14 | 4 | 23.08 | 1.182 | 1.364 | 1.333 | 0.148 |
| | MA34 | 9 | 30.77 | 1.182 | 1.364 | 1.303 | 0.163 |
| | LT15 | 31 | 38.45 | 1.273 | 1.545 | 1.405 | 0.194 |
| | SI9 | 32 | 46.15 | 1.273 | 1.545 | 1.358 | 0.193 |
| | SI10 | 31 | 46.15 | 1.318 | 1.636 | 1.258 | 0.154 |
| | SI11 | 30 | 38.46 | 1.273 | 1.545 | 1.357 | 0.192 |
| | SI12 | 31 | 46.15 | 1.273 | 1.545 | 1.287 | 0.167 |
| | SI29 | 32 | 30.77 | 1.227 | 1.455 | 1.323 | 0.149 |
| | PR16 | 33 | 38.46 | 1.227 | 1.455 | 1.293 | 0.159 |
| | mean | | 38.46 (11.26) | 1.25 (0.082) | 1.51 (0.153) | 1.33 (0.059) | 0.174 (0.031) |
| South | KC33 | 4 | 23.08 | 1.136 | 1.273 | 1.273 | 0.136 |
| | CNR17 | 12 | 23.08 | 1.182 | 1.364 | 1.289 | 0.140 |
| | BA20 | 6 | 23.08 | 1.136 | 1.273 | 1.268 | 0.135 |
| | WH28 | 31 | 38.46 | 1.273 | 1.545 | 1.290 | 0.157 |
| | AN21 | 37 | 38.46 | 1.227 | 1.455 | 1.294 | 0.157 |
| | YA22 | 4 | 23.08 | 1.136 | 1.273 | 1.273 | 0.136 |
| | YR27 | 33 | 30.77 | 1.227 | 1.455 | 1.299 | 0.147 |
| | CH24 | 3 | 23.08 | 1.136 | 1.273 | 1.273 | 0.136 |
| | SW25 | 9 | 23.08 | 1.182 | 1.364 | 1.250 | 0.127 |
| | mean | | 27.35 (6.78) | 1.18 (0.051) | 1.36 (0.102) | 1.27 (0.016) | 0.141 (0.010) |
| Inland | BT18 | 30 | 46.15 | 1.318 | 1.636 | 1.263 | 0.150 |
| | CG19 | 27 | 30.77 | 1.182 | 1.364 | 1.224 | 0.125 |
| | mean | | 38.46 (10.88) | 1.250 (0.096) | 1.50 (0.193) | 1.24 (0.028) | 0.137 (0.018) |
| Total / mean | | 626 | 37.50 | 1.264 | 1.506 | 1.328 | 0.171 |
| *H* | | | **7.899*** | **9.209*** | 6.994 | **13.489*** | **12.862*** |

*Biogeographic patterns of colonisation*

There was no evidence of significant differences ($p$>0.05; results not shown) in the density (*D*) and population nearest neighbour distance (*pNN*) between different regions that may indicate an abundant centre distribution. Similarly, there was no evidence of strong or significant correlations ($p$>0.05) that may indicate an abundant centre, between the demographic parameters of: density (*D*), population size (*N*), isolation ranking (*IR*) or population nearest neighbour distance (*pNN*) and distance south (*Lat*; Table 3.3).

Decreases across several genetic diversity measures were significantly ($p$<0.05) correlated with decreasing distance southwards as expected due to repeated founder effects as potential historical colonisation of *P. australis* from south east Asia occurred (*P*, *rho*=0.806; *A*, *rho*=0.928; *Ap*, *rho*=0.928; *He*, *rho*=0.584; Table 3.4). Sites located on the Atherton Tableland (north, AT1, AT2) had the highest measures of variation and diversity (*P, A, Ap, He*), while inland Carnarvon Gorge (CG19) and southern South West Rocks (SW25) consistently had the lowest measures of diversity (*P, A, Ap, He*; Table 3.4). Consequently, the north region had significantly ($p$<0.05) greater genetic diversity and variation measures compared to the south region (*He*; *H*=12.862; Table 3.3).

Table 3.4. Spearman's rank correlation tests for *P. australis*; where: *Lat*, latitude; *N*, population size; *D*, density; *pNN*, the population distance-to-nearest neighbour; *IR*, isolation ranking; *P*, percentage polymorphic loci; *A*, number of alleles per locus, *Ap*, number of alleles per polymorphic locus; *Ae*, the effective number of alleles per polymorphic locus; *He*, expected heterozygosity; *PA*, the number of private alleles; *Self*, percentage of population assigned to correct population in population assignment test. Significant correlation are indicated (*p<0.05, **p<0.01). Bold type indicates significant correlation after Bonferroni correction for multiple tests (α<0.001428).

| | *Lat* | | *D* | | *N* | | *pNN* | | *IR* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *rho* | *p* | *rho* | *p* | *rho* | *p* | *rho* | *p* | *rho* | *p* |
| *P* | **0.806** | 0.000** | -0.169 | 0.355 | **0.644** | 0.000** | -0.202 | 0.268 | 0.407 | 0.021* |
| *A* | **0.928** | 0.000** | -0.117 | 0.523 | **0.691** | 0.000** | -0.176 | 0.336 | 0.417 | 0.018* |
| *Ap* | **0.928** | 0.000** | -0.139 | 0.449 | **0.686** | 0.000** | -0.174 | 0.340 | 0.413 | 0.019* |
| *Ae* | 0.402 | 0.123 | -0.220 | 0.230 | 0.297 | 0.099 | -0.192 | 0.292 | 0.091 | 0.576 |
| *He* | **0.584** | 0.001* | -0.294 | 0.102 | 0.342 | 0.055 | -0.246 | 0.174 | 0.212 | 0.249 |
| *PA* | 0.295 | 0.267 | 0.116 | 0.527 | 0.116 | 0.528 | 0.006 | 0.976 | 0.277 | 0.124 |
| *Self* | 0.092 | 0.616 | 0.165 | 0.366 | -0.213 | 0.242 | -0.003 | 0.986 | -0.100 | 0.588 |

Genetic diversity at several measures was strongly correlated and declined significantly from north to south (*P*, *rho*=0.806; *A*, *rho*=0.928; *Ap*, *rho*=0.928; *He*, *rho*=0.584; $p<0.05$; Table 3.4). This is a pattern that is also seen in individual allelic frequencies across the species range. Fitting with the pattern of diversity lost from north to south, the frequency of individual alleles in six loci declined from north to south (*ml-Pa03*, *ml-Pa31*, *ml-Pa44*, *ml-Pa02*, *ml-Pa14*; *ml-Pa27*; Appendix 1). For example, allele 286 frequencies decreased southward for *ml-Pa14*, only found in moderate frequency in Atherton Tableland in the north and one Fraser Island population in the mid of the range (Figure 3.2a). This locus also had allele 290 that only occurred in Kingscliff in the south region and at inland region Blackdown Tableland, the latter a potential isolated refuge for rare alleles lost from more vulnerable coastal populations (Figure 3.2a, Appendix 1). There was a similar pattern for locus *ml-Pa31* with alleles restricted to the Atherton Tableland and a unique allele at Carnarvon Gorge another potential isolated refuge for rare alleles (Figure 3.2b; Appendix 1). This pattern was also the case for *ml-Pa03* with a decreasing loss of allele 232 in frequency from the north through the northern part of the mid region (Appendix 1). Likewise the presence of alleles for *ml-Pa27* and *ml-Pa24* became less common with southward distribution (Appendix 1).

The pattern of decreasing diversity southwards was also observed in the number of alleles that were restricted to individual populations. The north region had three alleles in three loci restricted to individual populations (*ml-Pa49*, *ml-Pa02*, *ml-Pa31*) as well as allele 229 for locus *ml-Pa31* restricted to the region (Appendix 1). The mid region had three alleles from three loci restricted to individual populations while there was only one for the south region (*ml-Pa57*, *ml-Pa49*, *ml-Pa14*, *ml-Pa27*; Appendix 1).

Despite differences among regions in genetic diversity, individuals did not form distinct clusters of similar allelic composition associated with climatic regions (Figures 3.3, 3.4). Similarly, there was no pattern of strong identity in the population assignment tests belonging to a particular region (Table 3.5). There were generally small genetic distances between many populations in the UPGMA (Figure 3.3). Coordinate 1 of the PCoA accounts for 24.62% of the variation in the data and coordinate 2 accounts for 13.52% of the variation in the data together all three coordinates account for 48.53% of variation in the data. The PCoA plot indicates some genetic clustering of individuals within regions, but consistent with the small genetic distances among populations and the UPGMA analysis, there is no large separation between regions (Figure 3.4a,b; Figure 3.2). The axis 1 vs axis 2 PCoA plot demonstrates the similarity between some of the more genetically diverse north populations, as well as that between the less genetically diverse south and inland populations.

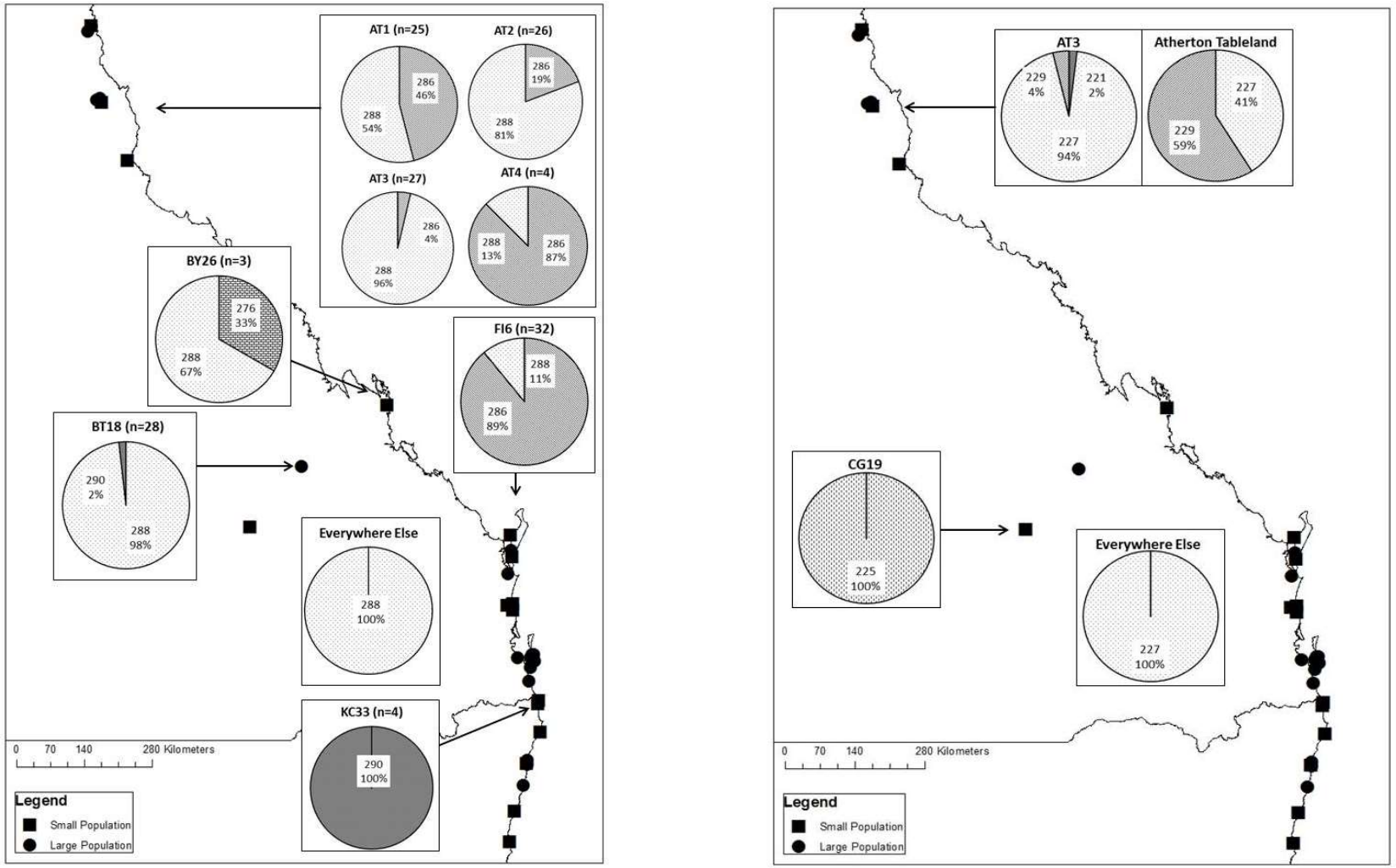


Figure 3.2. Map of allelic frequency pie charts showing the in individual allelic frequencies, from the north to the south across the range of *Phaius australis* with small and large populations indicated. a) for locus *ml-Pa14*; a) for locus *ml-Pa32*

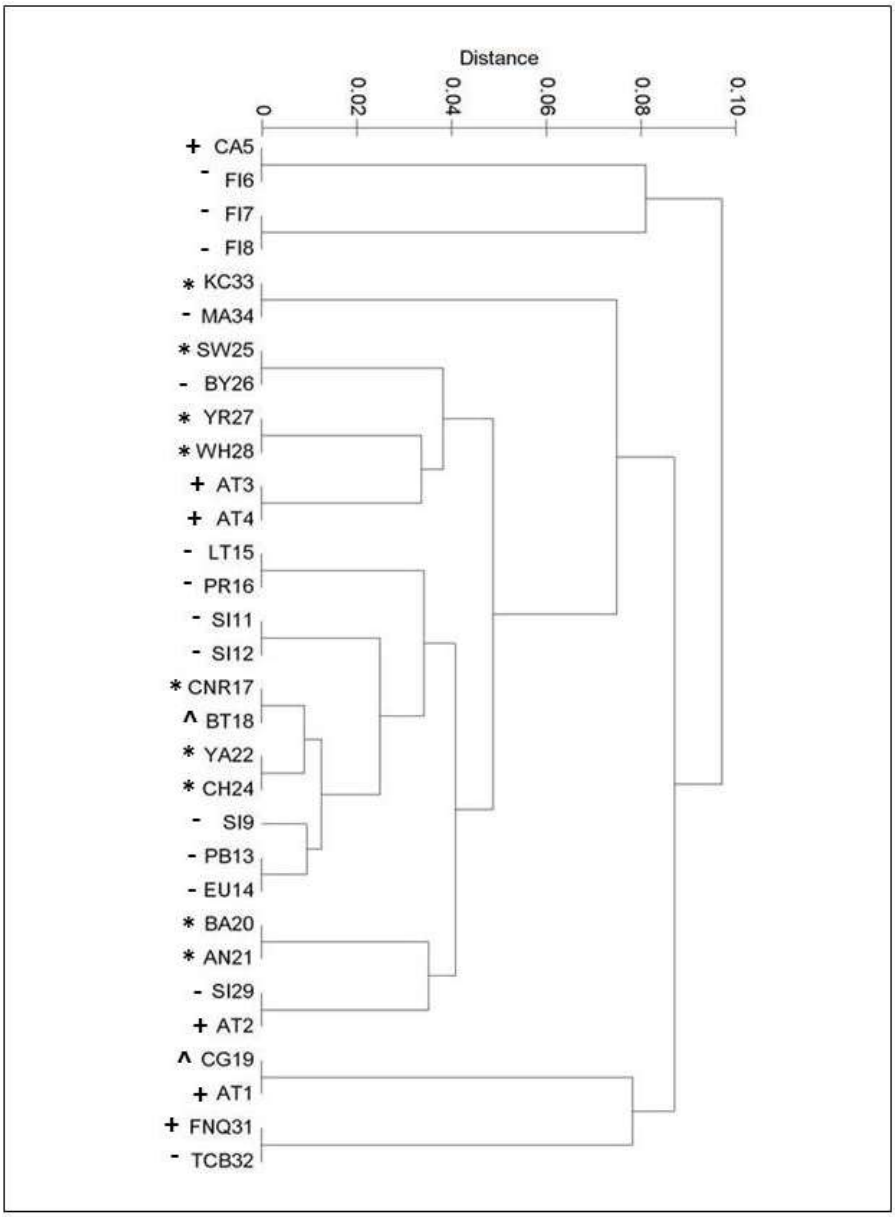


Figure 3.3. Unweighted pair group method with arithmetic averaging (UPGMA) dendrogram indicating genetic relationship (Nei 1978 distance) between *Phaius australis* populations across the species range. Symbols indicate regions + North Queensland, - South East Queensland, * New South Wales, ^ West Queensland.

For example, the populations from the north, more genetically diverse region (particularly AT1-3, FNQ 31) are spread across from the top of the plot and towards the middle (black squares, Figure 3.4a). The north region individuals (black squares) distributed across the centre then towards the bottom left and right side of the plot are from the north populations with lower genetic diversity (AT4 and CA5, Figure 3.4a, Table 3.4). Individuals from the less genetically diverse southern and inland region populations are scattered across the bottom of

the plot, while the mid region, moderately diverse populations comprise the bulk of individuals plotted towards the centre of the plot (Figure 3.4a).

The axis 2 vs. 3 PCoA plot shows a little clearer how individuals within several populations almost completely cluster together due to alleles restricted to these populations. Individuals from Shipton's Flat (FNQ31) in the north cluster together due to allele 202 at locus *ml-Pa02* that only occurs at this population (Figure 3.4b; Appendix 1). Likewise, all of the individuals from Carnarvon Gorge (CG19) cluster together on the bottom right side of the plot due to the restriction of allele 238 at locus *ml-Pa03* as well as homozygote form of allele 225 for locus *ml-Pa31* in every individual within this population (Figure 3.4b; Appendix 1).

The main cluster on the left side of the plot comprises individuals from populations from the mid region southwards (Fraser Island FI7-8, Sunshine Coast TCB32, EU14, PB13, MA34, Stradbroke Island SI9-12, PR16, Kinsgcliff KC33, Ballina BA20, Angourie AN21, Yamba YA20, Woody Heads WH28, Coffs Harbour CH24, South West Rocks SW25) and some individuals from the inland Blackdown Tableland population (BT18; Figure 3.4). The individuals within these populations are comprised of more common alleles or homozygote forms across several loci such as allele 197 for locus *ml-Pa49*, allele 200 for locus *ml-Pa02*, allele 288 for locus *ml-Pa14* and allele 241 for locus *ml-Pa24* (Appendix 1). As such, these loci would be contributing to the decreased diversity and variation southwards.

*Gene flow*

The common alleles and genotypes that are found in half of the loci coupled with the diagnostic alleles in the remaining loci influences the moderate genetic diversity amongst populations. There was considerable variation (40%) among populations ($F_{ST}$=0.329; *PhiPT*=0.506; Table 3.6). There was also some regional geographic genetic differentiation (11%; *PhiRT* =0.110; $F_{RT}$=0.072) with considerable variation among populations within the regions (40%; $F_{SR}$ =0.278; *PhiPR*=0.444; Table 3.6). This is most likely due to the possession of restricted alleles or other rare alleles at some populations in particular regions such as in the north, compared to populations composed of common alleles across many loci such as in the south region (Appendix 1). There was an average of 5.9 migrants per generation (*Nm*) between populations due to the low levels of overall differentiation among populations.

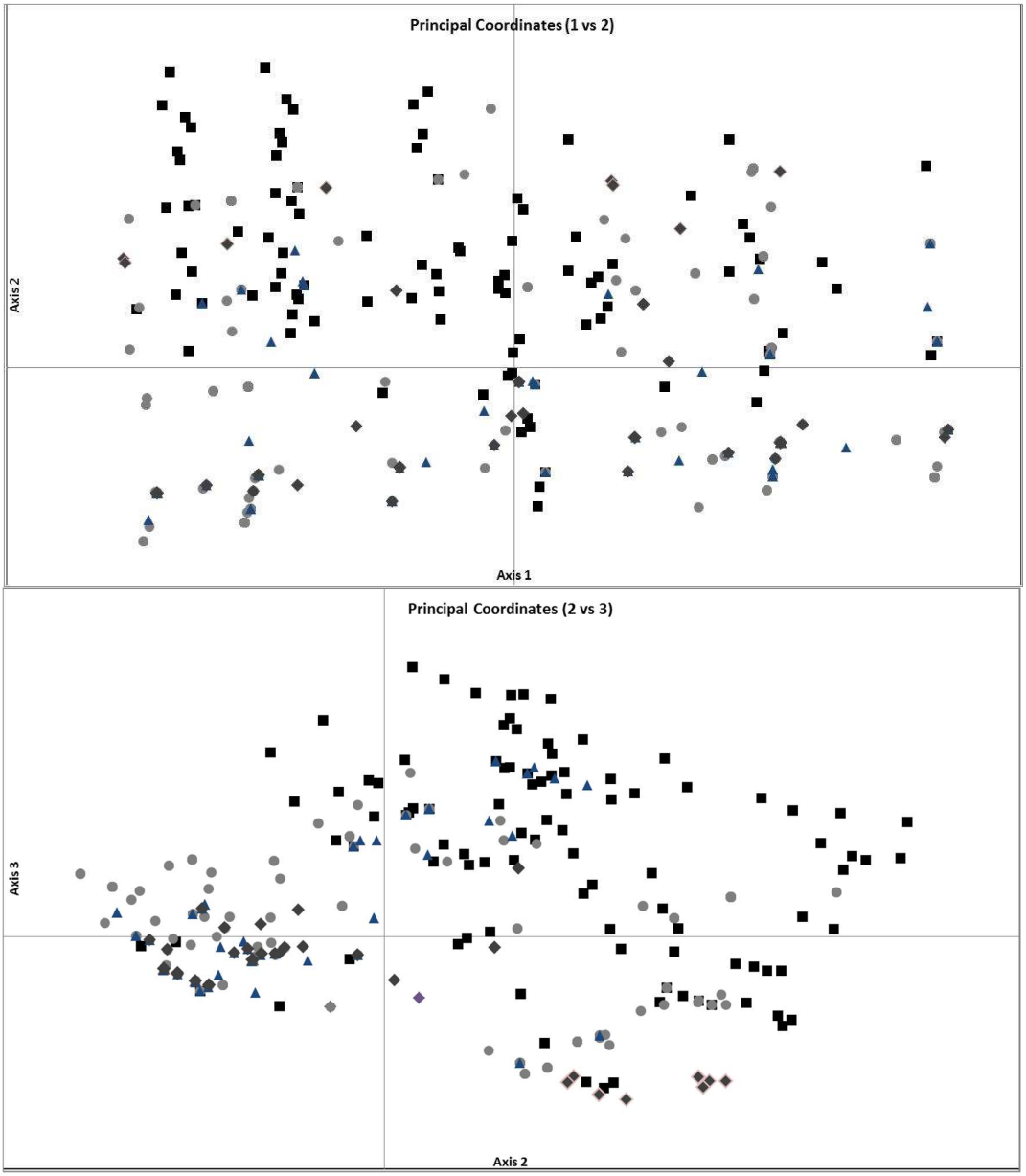


Figure 3.4. Principal coordinates analysis (PCoA) of all individuals across all populations, indicating the panmictic genetic relationship between the *P. australis* sites, coded by regions: North= Black squares, Mid = grey circles, South = triangles, Inland = grey diamonds. a) axis 1 vs. 2 The X-axis of the PCoA (coord. 1) accounts for 24.62% of the variation in the data, coord. 2 plotted on the Y-axis. Combined, these two axes account for 38.14 % of the variation in the data; and b) axis 2 vs 3.

Table 3.5. Frequency based population assignment analysis outcomes and the proportion of unique multilocus genotypes across 13 loci in 33 *P. australis* populations. *H* = result of Kruskall-Wallis rank test between groups. Significance values * $p<0.05$, ** $p<0.01$, ** $p<0.001$.

| Region | Population | Self (%) | Other (%) |
|---|---|---|---|
| North | FNQ31 | 100.0 | 0.0 |
| | AT1 | 27.6 | 72.4 |
| | AT2 | 67.9 | 32.1 |
| | AT3 | 18.5 | 81.5 |
| | AT4 | 80.0 | 20.0 |
| | CA5 | 50.0 | 50.0 |
| mean | | 57.3 [A] | |
| Mid | BY26 | 33.3 | 66.7 |
| | FI6 | 88.2 | 11.8 |
| | FI7 | 100.0 | 0.0 |
| | FI8 | 25.0 | 75.0 |
| | TCB32 | 0.0 | 100.0 |
| | PB13 | 0.0 | 100.0 |
| | EU14 | 25.0 | 75.0 |
| | MA34 | 33.3 | 66.7 |
| | LT15 | 3.2 | 96.8 |
| | SI9 | 34.4 | 65.6 |
| | SI10 | 51.6 | 48.4 |
| | SI11 | 0.0 | 100.0 |
| | SI12 | 3.2 | 96.8 |
| | SI29 | 93.8 | 6.3 |
| | PR16 | 12.1 | 87.9 |
| mean | | 33.5 [B] | |
| South | KC33 | 100.0 | 0.0 |
| | CNR17 | 0.0 | 100.0 |
| | BA20 | 0.0 | 100.0 |
| | WH28 | 6.5 | 93.5 |
| | AN21 | 73.0 | 27.0 |
| | YA22 | 100.0 | 0.0 |
| | CH24 | 0.0 | 100.0 |
| | YR27 | 84.8 | 15.2 |
| | SW25 | 88.9 | 11.1 |
| mean | | 50.4 [AB] | |
| Inland | BT18 | 0.0 | 100.0 |
| | CG19 | 100.0 | 0.0 |
| mean | | 50.0 [AB] | |
| Population Average Overall Species | | 42.7 | 57.3 |
| *H* | | **2.317*** | |

When pairwise estimates of *Nm* were investigated, as expected, the number of migrants per generation (*Nm*) significantly decreased with increasing geographic distance between population (*rho*=-0.304, *p*<0.05). Some neighbouring populations such as Flinders and Myora North Stradbroke Island (SI10, SI12) had very high levels of past gene flow (*Nm*=12.67, distance=9km). Likewise the two Yuruga populations (AT1, AT2) that are located within a kilometre of each other had high levels of past gene flow (*Nm*=3.778). Hence, across the entire species range, increasing pairwise population geographic distance was weakly significantly correlated (*rho*=0.386, *p*<0.05), with increased Nei's (1978) pairwise population genetic distance. This result was also reflected in the Mantel's Test for individual geographic and geographic distance matrices (*Rxy*=0.135, *p*<0.05) but there was no significant evidence of isolation by distance across the species range (Mantels *Rxy*=-0.036, *p*>0.05) when population genetic and geographic distance were analysed. Populations containing similar rare alleles were not necessarily located in close geographic proximity. There was no evidence of spatial genetic clustering or isolation by distance between populations within any region when tested with Mantel's Tests, supporting the *PhiPR* results of the AMOVA test.

*The effect of fragmentation on genetic diversity*

There were no significant differences (*p*>0.05) in density (*D, pNND*) between the small and large population sizes. Consistent with population genetic theory, genetic diversity (*P*, *A*, *Ap*, *He*) was positively correlated with population size (*p*<0.05; Table 3.4). Genetic diversity measures (*P*, *A*, *Ap*, *He*) were all significantly greater (*p*<0.05) for big populations compared to small (Table 3.7). Small populations in Northern New South Wales and South East Queensland (BA20, YA22, CH24, SW25, PB13, EU14) had the lowest measures of polymorphic loci (Table 3.3). There were no significant differences (*p*>0.05) between small and large populations in the proportion of populations assigned to 'self' in the frequency based assignment test (Table 3.5).

Some genetic diversity measures were weak but significantly correlated with increasing geographic isolation (*IR*; *P rho*=0.407; *A*, *rho*=0.417; *Ap*, *rho*=0.413; Table 3.4). However, populations were not significantly (*p*>0.05) less dense (*D*) or less genetically diverse (*Ap*, *Ae*, *He*, *PA*) between the two categories of more isolation, indicating a continuum rather than a distinction between more and less isolated groups (Table 3.7). Overall these results indicate that more isolated populations with moderate to large size neighbours may exist as an important reservoir of genetic diversity in a fragmented landscape compared to smaller populations that are relatively well connected with smaller neighbouring populations.

Table 3.6. Summary of the species level partitioning of genetic variation across 13 microsatellite loci and 33 *P. australis* populations; $F_{RT}$, diversity among regions within the total; $F_{SR}$, diversity among populations within a region; $F_{ST}$, diversity among subpopulations within the total; $F_{IS}$, diversity among individuals within subpopulations; $F_{IT}$, diversity among individuals within the total; *Nm*, mean number of migrants per generation. Results of AMOVA: *PhiRT*, differentiation among regions; *PhiPR*, differentiation among populations within regions and *PhiPT*, differentiation among populations. *P* values of significance given.

| **F-Statistics** | **Value** | **P** |
|---|---|---|
| $F_{RT}$ | 0.072 | 0.001 |
| $F_{SR}$ | 0.278 | 0.001 |
| $F_{ST}$ | 0.329 | 0.001 |
| $F_{IS}$ | -0.039 | 0.997 |
| $F_{IT}$ | 0.303 | 0.001 |
| *Nm* | 0.509 | |

| **Phi-Statistic** | **Value** | **P** |
|---|---|---|
| *PhiRT* | 0.110 | 0.000 |
| *PhiPR* | 0.444 | 0.000 |
| *PhiPT* | 0.506 | 0.000 |

| **AMOVA** | **Est. Var.** | **%** |
|---|---|---|
| Among Regions | 0.528 | 11% |
| Among Pops | 1.899 | 40% |
| Within Pops | 2.374 | 49% |
| Total | 4.801 | 100% |

Table 3.7. Summary of average genetic diversity measures for population size categories and isolation ranking categories; standard error follows in parentheses. Results of statistical analysis; U= result of Mann-Whitney U test; for all * p<0.05, ** p<0.01, ** p<0.001. Where: n, number of samples; *P*, percentage polymorphic loci within population; *A*, mean number alleles per locus; *Ap*, mean number alleles per polymorphic locus; *Ae*, mean number of effective alleles per polymorphic locus; *He*, mean expected heterozygosity polymorphic loci.

| | *n* | *P* | *A* | *Ap* | *Ae* | *He* |
|---|---|---|---|---|---|---|
| Small pop'n size | 16 | 27.93 | 1.1789 | 1.374 | 1.303 | 0.152 |
| Large pop'n size | 16 | 16.48 | 1.3495 | 1.648 | 1.352 | 0.190 |
| *U* | | **18.00**** | **16.00**** | **16.00**** | 81.00 | **47.00**** |
| Less Isolated (IR 1-10) | 16 | 21.59 | 1.244 | 1.489 | 1.336 | 0.176 |
| More Isolated (IR 11-20) | 16 | 22.73 | 1.284 | 1.523 | 1.320 | 0.167 |
| *U* | | 76.00 | 102.00 | 100.00 | 126.00 | 115.00 |

## 3.4 Discussion

*Biogeographical range expansion or 'abundant centre' patterns in genetic diversity and population differentiation*

The historic colonisation blended with contemporary habitat fragmentation and illegal collection of *P. australis* leads to challenges in the interpretation of present genetic patterns as it has for other perennial herbs (Hamilton and Eckert 2007). Congener *P. tancarvilleae* has a distribution that surrounds the island continent of Australia and is the likely ancestor to *P. australis* given the similarity of the two species (Dockrill 1992). Repeated founder effects can occur across the geographical distance that a species colonises as diversity is lost at each step of ideal habitat across the landscape (Austerlitz et al. 1997, Excoffier et al. 2009). As expected, a gradual decline in genetic diversity southwards following colonisation of *P. australis* southwards from the northern tropics to the southern sub-tropics was found. This decrease was observed through the loss of diversity in individual allelic frequencies within populations as well as in the number of alleles that were restricted to individual populations. A similar decline in the genetic diversity from long-term occupied populations towards the colonisation range limits in previously glaciated areas has been documented for other species (Hewitt 2004, Pierce et al. 2014, Sandoval-Castro et al. 2014). For example, Potter et al. (2015) found that for the Ponderosa pine (*Pinus ponderosa*) population allelic richness and populations with unique microsatellite alleles declined from the lower latitudes to higher latitudes. Two species of mangrove possess lower genetic diversity at their poleward range limits (Sandoval-Castro et al. 2014) and similar again to *P. australis*, genetic diversity was higher at the rear-edge of colonisation for North-African *Alnus glutinosa* (Lepais et al. 2013).

To complicate this, some species may occur as denser populations in the centre of their range and decline in both size and density towards their range margins according to an 'abundant-centre' distribution (Sagarin and Gaines 2002). Therefore, decreases in genetic variation at the limits of a species range may be attributed to an abundant centre, resulting in lower genetic diversity at the periphery of a species range due to smaller population sizes and density at the edge compared to the centre of the range (Leimu et al. 2006, Eckert et al. 2008). Initial population surveys revealed fewer large *P. australis* populations in the south region compared to the mid and north regions (Chapter 2). However, density (*D*) was not significantly correlated with the distance south (*Lat*), indicating a lack of support for an 'abundant-centre' distribution for *P. australis*. Plants with a wide, disjunct distribution or with fragmented populations are thought to possess genetic differentiation among populations because gene flow may

decrease across a distribution (Wallace and Case 2000, Lowe et al. 2004). This is particularly true for a species that expands its range across a linear gradient, losing genetic diversity through recurrent founder effects without admixture (Currat and Excoffier 2005). Such a process would result in differentiation between the 'leading' and 'trailing' edges. For example, Chung et al. (2014) found significant differentiation between populations of terrestrial orchid *Cymbidium goeringii* at the top and the bottom of the species' mountainous distribution in South Korea. Populations of *Cypripedium parviflorum* were also strongly differentiated across a physical distance (Wallace 2002).

Given the wide, fragmented distribution of *P. australis*, differentiation among populations was to be expected in this study. While 11% of genetic differentiation in the AMOVA was among regions, both the PCoA and the UPGMA analyses lacked structure between regions. In the AMOVA, almost half of the variation in genetic diversity for *P. australis* was found between populations (49%), with considerable variation (40%) found among populations within the regions. It was unexpected that there was no evidence for genetic isolation across a geographic distance, given the approximate 2 000 km distribution of the species and the hundreds of kilometres that separate several populations.

There are three possibilities that may explain the patterns of genetic diversity in and the differentiation between *P. australis* populations and regions. The first are those populations with distinctive restricted alleles such as Shipton's Flat (FNQ31), Carnarvon Gorge (CG 19) and Yuruga, Atherton Tableland (AT1). Refugial or isolated populations may be more distinct because they are older and have been isolated longer than leading-edge or younger populations (Hewitt 2004, Hampe and Petit 2005). The Shipton's Flat and Carnarvon Gorge populations occur in small spring fed creek ecotones isolated from other populations by greater than 150 km and thus, possibly retaining distinctive alleles due to isolation. Populations on the Atherton Tableland that neighbour the diverse Yuruga (AT1) show indications of a founder effect through the gradual loss of diversity from less than a kilometre (AT2), several kilometres (AT3) to 10 km away (AT4). A correlation between genetic diversity and spatial distance within this region is affected by the large distances between populations CA5 and FNQ31 and the Atherton Tableland. The Yuruga population (AT1) is a potential repository of diversity in a local context (Maurer 1994, Hanski and Gilpin 1997). In a similar colonisation situation, the higher mountainous populations were seen to be the founder population of diversity for the lower populations of *Cymbidium goeringii* (Chung et al. 2014).

The second possibility to explain genetic diversity patterns may be where genetic diversity in a population has been lost due to sudden decreases in population size ('bottlenecking'; Wright 1931, 1948, Ellstrand and Elam 1993, Young et al. 1996). Examples of such *P. australis* populations include Fraser Island (FI7) and Yamba (YA22). The recent loss of all individuals at KC33 highlights the vulnerability of some populations of *P. australis* in the wild and how quickly genetic diversity can be lost from the wild through human impacts alone.

Finally a there is a third possibility to explain genetic diversity patterns. Some populations may cluster in the genetic analysis because they contain none of the distinctive alleles that separate other populations. Several large populations in the middle of the species range (TCB32, LT15, SI11, SI12) lack identity because they contain common alleles at average frequencies. As discussed by Eckert et al. (2008) and Excoffier et al. (2009), gradual establishment of *P. australis* populations along the north-south axis of colonisation followed by rapid and uninterrupted population growth may have maintained the more common alleles received upon establishment and hence, maintain the similarity of populations.

*The effect of fragmentation on genetic diversity and differentiation*

An equal number of small and large populations was surveyed across each region in this study to assess the relative contributions of biogeographical range expansion and habitat fragmentation on the genetic diversity of *P. australis*. If large populations in the southern part of the species range had relatively high genetic diversity, it could be concluded that genetic processes of habitat fragmentation and small population size prevail over the post-colonization population in the genetic legacy of the species. This was reported for the north-American orchid species *Isotria medeoloides* in which contemporary patterns were maintaining population diversity at the edges of the species range (Stone et al. 2012). Conversely, for *P. australis*, the large populations in the south region were found to be genetically depauperate (*Ap*, *He*) compared to the north and mid regions, suggesting that bottlenecks at range expansion dictate population genetic diversity, with a secondary effect on genetic diversity from a reduction in population size due to habitat fragmentation. A similar poleward decline in genetic diversity due to colonisation process was identified for the herbaceous perennial *Silene nutans*, as the census size of populations is uncorrelated with latitude (Van Rossum and Prentice 2004).

Plant population sizes are often reduced when habitat patch size is reduced through habitat fragmentation, because population size is commonly correlated with patch area (Hanski and Gilpin 1997, Krebs 2009). Two of the most crucial factors influencing the decline of plant populations are the size of the remnant populations and the degree of their isolation (Saunders et al. 1991, Young et al. 1996). These factors in turn affect the genetic diversity within populations, either through the direct loss of individuals possessing unique alleles or by increasing barriers to gene flow which in turn increases likelihood of random genetic drift (Wright 1931, 1948, Ellstrand and Elam 1993, Young et al. 1996, Lowe et al. 2004). Orchids are susceptible to habitat fragmentation due to their specific interactions with symbionts and often specific habitat requirements. There was a weak indication that *P. australis* population size (*N*) and population genetic diversity (*P*, *A*, *Ap*) were negatively affected by population isolation (*IR*). Other genetic studies have reported that small fragmented populations exhibit less genetic diversity than well-preserved, continuously distributed populations (Van Rossum and Prentice 2004, Honnay and Jacquemyn 2007, Geiger et al. 2014).

Ouberg et al. (2006) suggested that population isolation and population size should be addressed separately when examining the effects of fragmentation on population genetic diversity. Reduced population size is commonly described to have negative effects on genetic variation through genetic drift, manifesting as a reduction in the average number of alleles per locus (Leimu et al. 2006, Ouborg et al. 2006). Smaller *P. australis* populations consistently had lower allelic diversity and lower presence of rare alleles resulting in significantly lower diversity measures in such populations (*P*, *A*, *Ap*, *He*). Allelic richness was greater in continuous populations of *Cymbidium goeringii* than fragmented populations in South Korea (Chung et al. 2014). Thus, similar to a perennial, herb *Geum triflorum* in mid-western Canada and the USA, the genetic diversity patterns for *P. australis* support both predictions for geographically disjunct populations: a finger print of range expansion colonisation as well as fragmentation effects (Hamilton and Eckert 2007).

The two notable exceptions to the genetic diversity in small populations were the presence of private alleles in BY26 and KC33, both small populations. Both populations have undergone a dramatic loss of population size since original surveys (Chapter 2). The population BY26 is greatly isolated from other *P. australis* populations and may have been for a long time, given there were no historical records within hundreds of kilometres of this population. However, several historical populations in the area of KC33 were unable to be located in this study, thought to be due to illegal collection (Chapter 2). The presence of some genetic diversity in

small populations of terrestrial orchid *Calanthe tsoongiana* is thought to be due to a recent decline in population size and number from a much wider historical distribution, as could have been the case for population KC33 (Qian et al. 2013). Sadly, in the case of population KC33, all plants in this population have reportedly been illegally collected in 2015, highlighting the vulnerability of small populations to the loss of diversity through thievery and local extinction.

Leimu et al. (2006) suggests that species that are the most prone to the negative effects of small population size are those species that are self-incompatible. Conversely it could be said that those species that are least affected by a loss of diversity in small populations may be self-pollinating species, the reproductive strategy that *P. australis* is widely thought to possess (Harden 1990, Jones 2006). The genetic diversity of the self-compatible, autogamous terrestrial orchid *Neotinea maculata* was not found to be adversely affected, despite a self-pollinating system believed to be an evolutionary 'dead-end'' (Takebayashi and Morrell 2001, Duffy et al. 2009). Inbreeding within populations and the reproductive mechanisms behind this are investigated in Chapter 4.

*Genetic diversity and population differentiation compared to other orchids*

Hamrick and Godt (1996) and Forrest et al. (2004) have argued that the Orchidaceae family is one of the most genetically diverse families. Levels of population genetic diversity were low for *P. australis* with an average proportion of polymorphic loci equal to 37.5%, a mean of 1.5 alleles per locus (*Ap*) and the expected heterozygosity (*He*) being 0.171 on average within populations. This is lower than that found for other terrestrial orchids. *Cymbidium goeringii* in Korea has higher genetic diversity across all measures (*P%*=64.3%, *Ap*=2.53; *He*=0.268; Chung et al. 2014) than *P. australis*. Rare and highly fragmented *Calanthe tsoongiana* in China has higher average proportion of polymorphic loci (50%) and expected heterozygosity (*He*=0.183) than *P. australis* (Qian et al. 2013). Genetic diversity was also greater for *Caladenia huegelii* (6.73 alleles per locus, *He*=0.690; (Swarts et al. 2010) and for *Drakaea elastica* (*Ae*=5.632, *He*=0.746; Menz et al. 2015) in Western Australia as well as for three endangered Korean *Calanthe* species (Chung et al. 2013). On the other hand, *P. australis* had greater population genetic diversity (*P%*=22.3%, *A*=1.48, *He* 0.093) than populations of the long-lived Eurasian orchid *Platanthera bifolia* in north east Poland (Brzosko et al. 2009) as well as *Cypripedium reginae* in North America (*P%*=10.0%, *A*=1.11, *He*=0.051; Kennedy and Walker 2007). Genetic variation and diversity enable a species to cope with pathogens, disease, survival and selection

in changing abiotic and biotic conditions. Species can be at risk of extinction when diversity is lost (Reed and Frankham 2003, Frankham 2005, He 2013).

Low population differentiation is generally associated with taxa that have substantial dispersal abilities. Orchids with their often species-specific pollinators and minute wind-borne seed that enables long distance dispersal and gene flow, have often been reported to have low population differentiation (Arditti 1992, Hamrick and Godt 1996, Forrest et al. 2004; Jacquemyn et al. 2007). For example, Chung *et al.* (2005) found very weak differentiation for terrestrial orchid *Orchis cyclochila* in Korea ($F_{ST}$=0.030). Low differentiation and low variation was also found among populations of *Caladenia huegelii* in Western Australia ($F_{ST}$=0.047; (Swarts et al. 2009). The overall estimate of population differentiation for *P. australis* is relatively high ($F_{ST}$=0.399) surpassing both examples as well as the mean $F_{ST}$=0.146 (±0.134 SD) calculated for orchids by Phillips et al. (2012).

Past gene flow estimates were high for many population pairs, therefore the following scenario is proposed for population differentiation in *P. australis*. Self-fertilisation in plant species is a method for reproductive assurance that can also aid in rapidly colonising new habitats through the production of sexually reproduced clones (Sakai et al. 2001, Ramula et al. 2008). *Phaius australis* is thought to possess some levels of autogamy, resulting in high fruit set, the production of potentially thousands of minute seeds and reported clusters of seedlings (Dockrill 1992, Sparshott and Bostock 1993, Arditti and Ghani 2000, Freeman et al. 2011). Some populations of congener *P. tancarvilleae* are recognised as being autogamous and cleistogamous, which may have promoted the species naturalisation in Florida, the West Indies, Central and South America and Hawaii (Gandawidjaja and Arditti 1982, Robinson et al. 2011). In this study, *P. australis* was found to have has less genetic structure and identity than other self-fertilising orchids (Duffy et al. 2009, Stone et al. 2012). There was no pattern of population or regional identity in the population assignment tests, or isolation by distance across the tropical north to subtropical south. This could be explained by long distance dispersal events resulting in a patchy but a wide distribution of allele frequencies (Bialozyt et al. 2006). When long distance dispersal is followed by exponential population growth in newly colonized sites, the genetic diversity, particularly the common alleles but also rare alleles of the founding individuals may be retained, particularly for self-pollinating species (Hamrick and Godt 1996, Excoffier et al. 2009).

## 3.5 Conclusions

The gradual loss of diversity across the poleward latitude gradient is an insight into the biogeography of the species. The migration of species to new suitable habitat or retraction to refugia as the climate changes in the future could perpetuate the historical evolutionary pattern of a species (Parmesan and Yohe 2003, Arenas et al. 2012, Bellard et al. 2012). Therefore, it is important to conserve genetic diversity for *P. australis* across the species range. While moderately low, the genetic diversity of *P. australis* falls in the range of that identified for other terrestrial orchids globally (Swarts et al. 2009, Chung et al. 2013, Qian et al. 2013, Chung et al. 2014, Menz et al. 2015). Of high conservation importance are the more diverse *P. australis* populations in the north region as well as the additional populations that possess unique alleles (FNQ31, AT1, CG19) which have distinctive allelic composition, as these represent repositories of diversity for the species. Given the current threats to the species as described in Chapter 2 (urban development and illegal collection of flower stalks, plants and entire populations), the loss of genetic diversity due to decreasing population sizes is an ongoing threat to *P. australis* into the future. These threats are possibly of greater concern in the south region (New South Wales; Chapter 2), coinciding with less genetic diversity. Thus, the southern populations also are of great conservation priority, particularly the populations that harbour higher genetic diversity. The loss of one population in recent years (KC33) serves as an extreme example of how quickly genetic diversity can be lost from wild populations.

The conservation of genetic diversity in wild populations is particularly important for reducing the extinction risk of species that may lack the ability and speed to track climate or altogether suitable habitat as the climate changes (Parmesan 2006, Jump et al. 2009, Bellard et al. 2012, Corlett and Westcott 2013). While it is of particular importance to conserve larger populations across the species range because of the higher genetic diversity, ex-situ collections of all populations regardless of size should be maintained, especially those populations possessing unique alleles.

Assisted colonization to recreate populations in suitable habitat within or outside of the historic range is a 'last resort' option to conserve species in the wild under future climate change (Hoegh-Guldberg et al. 2008, Vitt et al. 2010, Thomas 2011, Dalrymple et al. 2012). The augmentation of small populations of *P. australis* may increase the resilience to the stochastic loss of small populations. However, as with any translocation, the ability arises to determine the population's evolutionary potential by choosing the founding individuals to be introduced to create a genetically enriched population that may be capable of persisting in environmental

change (Rice and Emery 2003, Weeks et al. 2011, Neale 2012). While there is some genetic structuring between populations across the *P. australis* populations this is mostly due to the presence of rare alleles in low abundance within populations, rather than differentiation between populations. The high pairwise *Nm* values among some populations, both adjacent or remote within a region indicates that mixing between populations may not be overall detrimental in terms of evolutionary relationships. Thus, sourcing propagules from any small *P. australis* populations within the highly fragmented and disjunct distribution should be avoided as small populations in this study, as well as numerous others, have been shown to have low levels of genetic variation that may influence viability of receiver populations (Field et al. 2008, Swarts et al. 2009, Weeks et al. 2011).

Source populations would ideally be from a combination of many large more genetically diverse source populations ('composite-provenancing') from within the same region, which could promote the genetic adaptive potential and persistence of the populations, whilst maintaining any adaptation to regional environmental conditions (Vallee et al. 2004, Broadhurst et al. 2008). Careful consideration should be given to the composite-provenancing from beyond regional or climatic provenance under the premise that individuals from a current climatic region that may resemble what is predicted for a different region in the future and hence may influence the evolutionary potential and adaptation to a new climate (Hoegh-Guldberg et al. 2008, Weeks et al. 2011). Investigations into the levels of inbreeding within populations and the breeding system would contribute to the assessment of inbreeding and outbreeding potential associated with translocations. In addition, the assessment of climatically related demographic or reproductive characteristics of *P. australis* may assist in identifying those populations with more demographically resilient individuals.

# Chapter 4: Within-population genetics and the reproductive strategy of the endangered orchid *Phaius australis*

## 4.1 Introduction

Orchids are well known for their interesting and diverse pollination mechanisms, including autogamy and self-fertilisation (Dixon et al. 2003, Gaskett 2011). Reversion to self-fertilisation may be an advantage for species living at the margins of their range where environmental conditions are extreme, where flowering is asynchronous, or an absence of pollinators may limit options for outcrossing (Baker 1955, Moeller and Geber 2005, Caradonna and Ackerman 2010). Self-pollination is also a cheap and fast mechanism for reliable reproduction where climatic or environmental conditions are ephemeral or variable, or for rapidly establishing in new habitats (Sakai et al. 2001, Kalisz et al. 2004, Ramula et al. 2008, Willmer 2011, Suetsugu 2013). However, the disadvantage of self-fertilisation is inbreeding, the fixation of genotype frequencies and leading to a reduction in genetic variation and the formation of family structures within populations (Wright 1931, 1948, Leimu et al. 2006, Honnay and Jacquemyn 2007).

In small populations, genetic diversity can be lower than large populations to begin with, amplifying the effects of random genetic drift, further reducing allelic diversity, leading to fixed homozygous and cloned genotypes within a population (Ellstrand and Elam 1993, Young et al. 1996, Leimu et al. 2006, Ouborg et al. 2006). Clones of a particular genotype may dominate a population in a particular location. While the possession of a genotype selected for a narrow set of environmental conditions can be of survival benefit to current conditions, it can be a potentially lethal disadvantage decreasing tolerance to future environmental variation, reducing the framework on which natural selection can occur (Bradshaw and McNeilly 1991, Frankel et al. 1995, Bijlsma and Loeschcke 2005).

Allelic fixation can also lead to inbreeding depression. The negative effects of inbreeding depression or the lost ability to mask disadvantageous alleles can be observed in reduced reproductive output through low or no flower output, low seed viability or set, affecting the recruitment and overall growth and persistence of populations (Agren 1996, Young et al. 1996, Young et al. 2000, Pullin 2002, Ramula et al. 2007). Autogamy has led to inbreeding depression and reduced seed set or offspring vigour in some orchids, deeming plant species with a self-pollinating system termed an evolutionary ‘dead-end’’ (Barrett and Harder 1996, Takebayashi and Morrell 2001). On the other hand 100% autogamy in a European orchid did not negatively affect fruit and seed production (Duffy et al. 2009).

Cross-pollination is of topmost benefit for maintaining genetic diversity and gene flow within and between populations. However, self-fertilisation is still a sexual event, with independent chromosomes at meiosis and recombination events that produce some possible genetic variability, albeit from a small genetic pool (Willmer 2011). Breeding between closely related individuals or self-fertilisation can also perpetuate genotypes carrying advantageous alleles or traits (Dickson and Petit 2006, Jersáková et al. 2006, Hedrén and Nordström 2009). When the fitness of a self-fertilising morph exceeds that of an outcrossing morph, self-fertilisation becomes a selective reproductive strategy that replaces outcrossing individuals over time (Asmussen et al. 1998). Self-fertilisation has the advantage of passing on two sets of genes to offspring (the 'automatic selection hypothesis') compared to the one set of genes in outcrossing (Fisher 1941, Schoen et al. 1996, Busch and Delph 2012). If selection favours a self-fertilising individual with a heterozygous genotype, a corresponding selection against homozygotes occurs, reducing the homozygote excess that is often associated with inbreeding in fragmented systems with small population sizes (Raijmann et al. 1994, Kang et al. 2005, Honnay and Jacquemyn 2007). Thus, the effects of genetic drift are overcome and diversity is retained in a population through the fixation of heterozygous genotypes that are autogamous (Fisher 1941). Because reproduction is a vital contribution to recruitment and persistence of populations, information on the reproductive strategy combined with genetic information such as levels of inbreeding is essential for designing comprehensive plans for rare plant conservation (Woodward and Williams 1987, Falk and Holsinger 1991, Hamrick and Godt 1996, Winkler et al. 2009, Walck et al. 2011).

The swamp orchid *Phaius australis* F.Muell. is found in the margins of coastal freshwater swamps, littoral rainforests, in woodland and wet heath ecotones and in isolated inland ephemeral freshwater springs across a 2000 km latitude gradient from North Queensland to New South Wales in Australia (Sparshott and Bostock 1993, Benwell 1994; Chapter 3). The plant produces numerous (4-20), large (7-10 cm across but up to 17 cm), showy deep purple to cinnamon brown flowers on inflorescences that can be over two meters tall making it the tallest terrestrial orchid in Australia (Dockrill 1992, Jones 2006, Stephens and Sharp 2009, Freeman et al. 2011). The coastal swamps and inland ephemeral spring habitat of *P. australis* are particularly vulnerable to the effects of future climate change such as sea level rise, changes in rainfall, temperature and increased extreme weather events (DECCW 2010, Kingsford and Watson 2011).

The attractive flowers made the swamp orchids once one of the most highly desired and illegally collected orchids in Australia (DE 2015a). The east coast of Australia has also been extensively developed into agricultural land and urban areas with natural ecosystems now small and isolated fragments of their pre-European settlement size (Groves 1994, Hobbs and Yates 2003, Lindenmayer and Fischer 2006, NSWG 2006). *Phaius australis* is listed as 'Endangered' under the Australian federal *Environment Protection and Biodiversity Conservation Act 1999* due to loss of individuals and small population sizes by illegal collection from the wild, the loss of available habitat and precarious geographic distribution in fragmented locations (Benwell 1994, DE 2015a).

Autogamy and cleistogamy (pollination and fruit set occurring in flowers that fail to open) has been reported for some populations of *Phaius tancarvilleae* in Indonesia, a species found in South-east Asia and the Pacific Islands that is closely related to *P. australis* (Gandawidjaja and Arditti 1982). Some *P. australis* populations in Australia were formerly applied the name *P. tancarvilleae* as the observed defining feature of *P. australis* was higher fruit set compared to *P. tancarvilleae* (Harden 1990, Dockrill 1992, Sparshott and Bostock 1993, Benwell 1994). This was presumed to occur due to autogamy with an absent rostellum to separate the pollinia from the stigma in *P. australis* (Harden 1990, Dockrill 1992, Sparshott and Bostock 1993, Benwell 1994). Natural autogamy in wild *P. australis* populations may assist in explaining the low genetic diversity found in the species (Chapter 3), but may affect levels of inbreeding within populations leading to deleterious effects of inbreeding depression over time.

The aim of this part of the study is to investigate levels of inbreeding within populations of *Phaius australis* across the species range on the east coast of Australia. This study specifically asks if populations are inbred as a result of decreased population sizes and if smaller more isolated populations are more inbred than larger connected populations. Is there evidence of clonal spread or particular genotypes and family structures that may lead to genetic structure within populations? Does clonal spread or genetic structure within populations differ between populations of different density, size and isolation? How fecund are populations and is there evidence of inbreeding depression? Do population inbreeding levels, unique genotypes and inbreeding depression vary among regions and what are the conservation implications? What is the breeding system of *P. australis* and can it spontaneously self-pollinate in a natural population?

## 4.2 Methods

As part of a large species wide study, thirty-three *P. australis* populations were sampled from across the species distribution on the east coast of Australia ranging from Rossville, North Queensland to South West Rocks in New South Wales (1 800 km; Figure 4.1). Sites were selected in a stratified manner to achieve replication of populations across four biogeographic regions with replications within regions of small (less than 50 individuals) or large (greater than or equal to 50 individuals) categories. Sampling was thus undertaken at six sites in North Queensland (north), eight sites on mainland South East Queensland, seven sites on islands in South East Queensland to comprise 15 total in the middle of the range (mid), nine sites between the Tweed and Hastings Rivers in New South Wales (south) and two inland sites (Figure 4.1). Replication was reduced for the Inland region as only two populations are known. One population, Myora, Stradbroke Island, was a mixture of *P. australis* and *P. bernaysii* growing together. Results from Chapter 2 indicated that there were no diagnostic differences between *P. australis* and *P. bernaysii* in this population based on the microsatellite markers tested; therefore all plants at this location were considered as *P. australis*.

*Field collection of genetic samples*

To aid with identification of plants, populations were sampled on foot during the flowering and fruiting season between August 2012 and January 2013. Populations were located in the field utilising a combination of GPS coordinates from historic records, maps, verbal descriptions or local guides. Searches were undertaken by systematically surveying the entire area of potential habitat in a series of continuous parallel belt transects 10 m wide (Cropper 1993). All *Phaius* plants were documented across the geographic range of a site with geographic extent of populations marked with a GPS. At all locations, the starting location of each population/site was recorded using the GPS. The relative location of each individual plant within the site was mapped using compass direction (degrees) and distance (metres) between clumps and plants measured with a LaserDisto measure (Leica geosystems). These were later converted using Cosine and Sine trigonometry to XY coordinates in metres based on start point for later spatial distribution analyses.

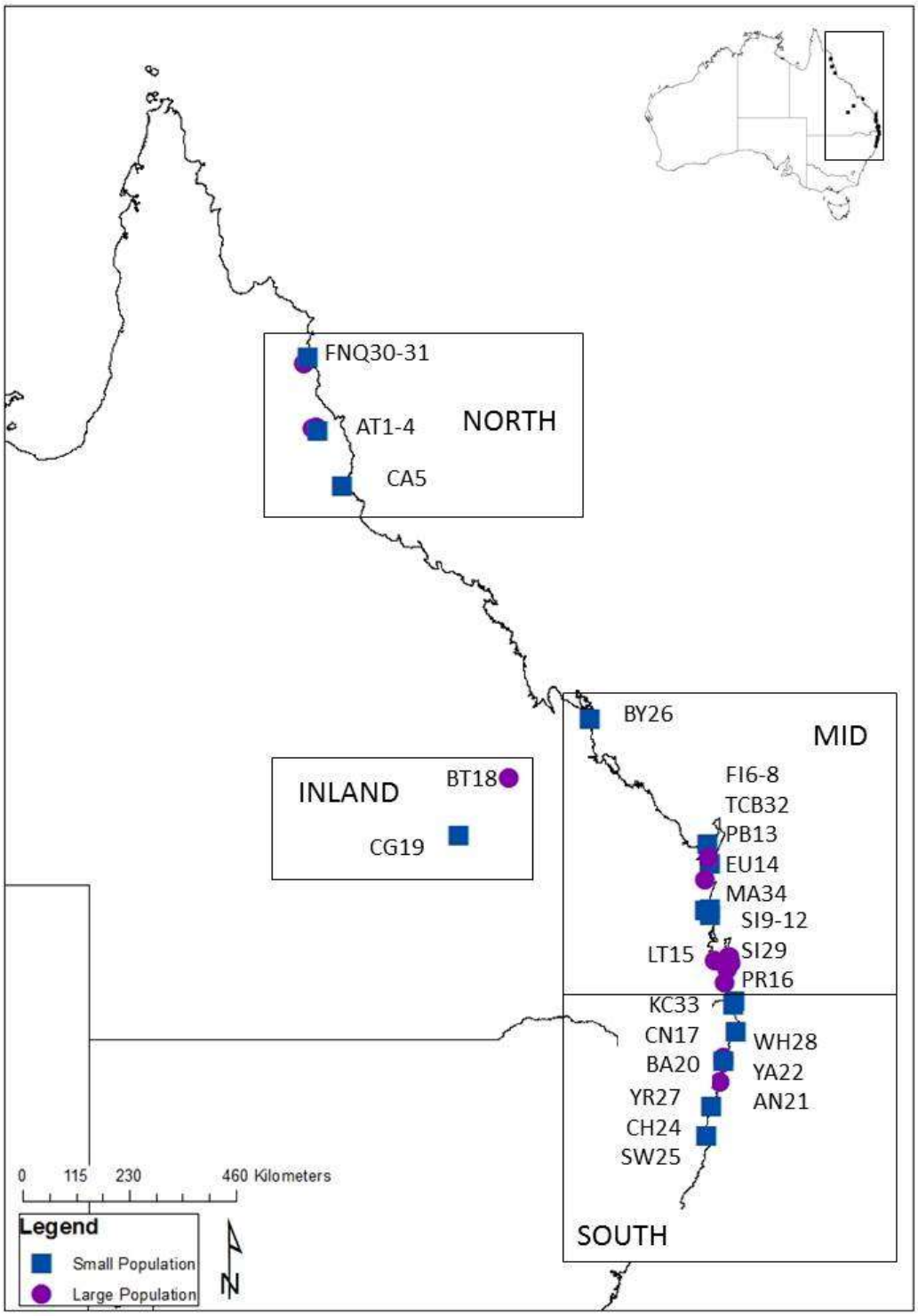


Figure 4.1. Map overview of the *Phaius australis* distribution on the east coast of Australia showing the locations and size class (small/large) of populations sampled for genetics across four regions which are shown (North, Mid, South and Inland).

All *Phaius* plants were assessed across the geographic range of a site. In sparse, smaller populations, the location of each individual was marked with a GPS. For more extensive and/or dense populations, the each individual was recorded along meandering surveys through the best representation of the population for 90 individuals. Beyond this, additional clumps of plants were mapped and key features recorded.

Samples for genetic analysis were collected from 30 individuals per population. Sampling was spread evenly across the spatial occupancy to sample the random variation in the population approximately evenly distributed across all size classes including recruits, intermediates and sexually mature individuals. Where populations were smaller than 30 individuals, samples were collected from every individual. Genetics samples consisted of approximately 10 x 10 cm of green, healthy leaf material, placed in a sealed plastic bag labelled with the plant number. Surface mould, rust and foreign material was cleaned from the leaf, then sampled were stored in labelled plastic clip-seal bags with Grade 12, 28-200 mesh silica gel (Sigma-Aldrich). Samples were stored at room temperature away from light in the University of the Sunshine Coast Laboratory prior to DNA extraction.

*Field fecundity surveys*

Inbreeding depression was measured through reproduction observed at the time of surveys. At all sites, each individual plant within the same population sampled for genetics was classified as either too small for reproduction (no pseudobulb formed <0.4 m, and one active pseudobulb formed, no sign of previous flowering), or of reproductive age (>1 inactive pseudobulb, >1 active pseudobulb, sign of previous flowering). Floral output was recorded as the number of flowers per inflorescence. Populations were revisited 3 months after initial surveys and fruit set was recorded for each inflorescence within the population. The number of flower scars and the number of fruiting bodies (pods) were recorded for each inflorescence, graded as healthy, unhealthy, split or dead. Healthy pods were swollen, green and full looking, unhealthy pods were those appearing wrinkled, shrivelled, smaller in size, light green to yellow and dead pods were shrivelled brown/black pods not of mature size. A sub-sample of 17 populations was then revisited the following flowering season and flower output of the same plants was reassessed using the same methods to account for annual differences in reproduction.

*Laboratory genetics analysis*

Total genomic DNA was extracted from the leaf tissue of all samples using QIAGEN DNeasy® Plant Mini Kits (QIAGEN Valencia, CA, USA) following manufacturer's instructions as follows. Approximately 30-50 mg of finely cut plant tissue was placed inside Eppendorf 2.0 mL Safe-Lock tubes. A 3 mm tungsten bead was placed in each tube and samples were frozen using liquid nitrogen for 30 secs and ground using a Retsch MM200 Tissue Lyser grinding mill (Qiagen) for 41 secs at 23 000 rpm. The process was repeated several times, with immersion in liquid nitrogen for at least 30 secs between each cycle. The manufacturer's protocol was followed for the rest of the extraction process (QIAGEN 2012) with some alterations: 500 µL of AP1 162.5 µL of buffer AP2 were used. Genomic DNA was eluted in a final volume of 200 µL AE buffer and compared with known concentrations (5 ng/µL, 10 ng/µL, and 20 ng/µL) of Lambda EcoR1/HindIII digest molecular weight marker (Fisher-Biotech) to estimate yields using 1.5% agarose gel electrophoresis; viewed under UV light using a Syngene gel documentation system and Syngene GeneSnap software.

DNA was purified and sent at ambient temperature to Australian Genome Research Facility Ltd (AGRF, Brisbane) for microsatellite (SSR) marker development using Next-Generation 454 pyrosequencing. Sequences were identified in MsatCommander and 61 unlabelled microsatellite primer pairs were trailed as detailed in Chapter 2. Final selection resulted in PCR amplification of 24 primer pairs for all individuals with each pair being end-labelled directly with one of four fluorescent dyes (VIC, NED, PET, Applied Biosystems, FAM, Geneworks, Chapter 2) to enable multiplexing in fragment analysis.

PCR was performed using reaction volumes of 12 µl containing approximately 25 ng genomic DNA, 1 x reaction buffer (67 mM Tris-HCl (pH 8.8), 16.6 mM $(NH_4)_2$ $SO_4$, 0.45% Triton X-100, 0.2 mg/mL gelatine; Fisher Biotech), 200 µM of each dNTP, 2 mM MgCl2 (Fisher Biotech), 0.2 µM BSA (Fisher Biotech) 0.2 µM forward primer, 0.2 µM reverse primer and 0.5 U F1 Taq polymerase (Fisher Biotech). PCR was performed using a Mastercycler gradient thermocycler (Eppendorf) with the following cycling conditions: denaturation at 95 °C for 3 mins; 35 cycles of 94 °C for 30 secs, specific annealing temperature (Chapter 2) for 30 secs, 72 °C for 45 secs; final elongation step at 72 °C for 10 mins.

PCR products for all samples were multiplexed for the same individuals according to different dye sets and size ranges, and then separated by capillary electrophoresis on an AB3500 Genetic Analyser (Applied Biosystems). Fragment sizes were determined relative to internal lane standard (GS-600 LIZ; Applied Biosystems) using GENEMAPPER v4.1 software (Applied

Biosystems) and double checked manually accuracy with expected banding size. Some loci (*ml-pa05*, *ml-pa 40*) did not yield consistent or easily scored results and these were eliminated from further analysis. A resultant 22 loci were scored; yielding 14 polymorphic loci for final data analysis: (*ml-pa02*, *ml-pa03*, *ml-pa12*, *ml-pa14*, *ml-pa19*, *ml-pa21*, *ml-pa24*, *ml-pa27*, *ml-pa31*, *ml-pa40*, *ml-pa44*, *ml-pa49*, *ml-pa57, ml-pa59*). The presence of null alleles, scoring errors, and large allele dropouts were checked for all loci using MICROCHECKER v2.2.3 (Van Oosterhout et al. 2004).

*Reproductive biology experiment*

Plant breeding systems for *Phaius australis* were investigated at one population (SI10) on in Flinders Swamp, North Stradbroke Island in the year following preliminary surveys (2013). This population was selected because it is a large population (over 200 individuals), with little signs of decline or immediate threats, has plants of all demographic classes including small recruits and plants of reproductive age and is unlikely to be affected by the removal of seeds . This population covers an area of approximately 1.9 ha in a line along the northern edge of Flinders Swamp. The population is one of the largest populations recorded for the species, with several smaller populations within a few kilometres.

Twenty-nine (29) plants within the Flinders Swamp population that had previously been sampled for genetic analysis were selected for the detailed pollination study. Each plant studied had an inflorescence with a large cluster of flowers (15-20) and was situated at least 1 metre from nearest neighbour to avoid sampling from a genetically identical plant in the cross-pollination experiment. Plants were tagged using surveyors tape and a Jewellers tag tied to the base of the inflorescence stalk to enable them to be revisited over the entire duration of the study.

All flowers on the selected plants were examined every second day over a period of a month to assess mean floral lifespan. Seven plants were allocated to each of four treatments across the study. Plants were randomly assigned to each treatment. The four treatments were: (1) un-bagged, no manipulation (open/control), (2) bagged, no manipulation (spontaneous self), (3) bagged, within flower pollen transfer (manipulated self, pollinaria removed and used to hand pollinate the same flower before re-bagging) and (4) bagged, pollen added from another individual >4m away (cross, pollinaria removed and pollinaria from another individual >4m away to hand pollinate before re-bagging). Inflorescences were cleaned of insects such as ants,

spiders and thrips and debris such as leaves and cobwebs, and then the entire inflorescence was bagged with a fine mesh bag (nylon, 2mm mesh size). Any flowers that had already opened were removed at the start of the experiment.

*Phaius australis* flowers opened sequentially from the base to the top of the flower spike over 4 weeks. The first opening flower on the inflorescence was labelled and the pollination treatment applied to each sequential flower upon opening for each day of stigma receptivity. The treatments were applied to flowers every 2 days for a period of 4 weeks with a minimum of 6 flowers and a maximum of ten flowers treated on each plant over this period.

Each flower was inspected on each treatment day to record the flowering pattern for each plant. Buds were defined as developing flowers with no sepals opening, either upward or downward facing. Open flowers had completely unfurled sepals and labellum that were brightly coloured with reproductive parts displayed. Half-open flowers were recorded for some *P. australis* plants where sepals do not open to the widest possible extent at maturity. Receptivity was recorded from visual observations of stigma appearing sticky, with a light green to blue tinge and individual pollinia turning dark yellow when investigate, hanging slightly (anthesis) at which point pollination was undertaken. Old flowers had reduced vivid colouration of the sepals and labellum with some drooping/wilting of the flower. Dead flowers were those drying out and turning black. Successful pollination was recorded when there was swelling of the gynostemia, swelling of the ovary, a rippled labellum and drooping of the sepals and petals. Fruit set were recorded in the following categories, 'early developing' with successful pollination features as well as elongation and swelling of the ovary, and drying of floral parts with ovary remaining within the first month post pollination and developed 'fruit' as pods within the size range described by Jones (2006).

Additional casual observations such as perceived floral scent, health of the flower, insect visitors on open plants were recorded. Three days after the final treatment was applied, the bags were removed and any remaining unopened flowers were removed. Plants were then assessed for 'fruit' set at one, two and three months after the final treatment date.

Three months after the final treatment, plants were assessed and all fruit was harvested. Each fruit was weighed in grams (g), and then measured in millimetres (mm) at a) the length of the longest part of the fruit, b) diameter of at top of fruit (D1) and c) diameter of bottom end of fruit (D2). Average diameter was calculated using the average diameter of the fruit and volume ($cm^3$) calculated using standard scientific formula. The measurement of fruit weight, volume

and length to width ratio were utilised as a proxy for seed counts as some fruit had begun splitting via hairline cracks at the time of harvest with some seed dispersal already commencing. Because orchid seed are so small, seed counts were avoided for consistency across all treatments and plants. Fruit with smaller volume and lesser weight were assumed to have lower seed production than fruit with a large volume and greater weight.

*Data analysis*

*Population demographics and fecundity*

Populations GPS points were mapped on ArcGIS (ESRI, v. 10.2), with latitude and longitude in UTM extracted for later analyses. The area of occurrence (*A*) in $m^2$ was derived utilising boundary GPS coordinates for each population and the number of individuals in each population (*N*) recorded. Population distance-to-nearest neighbour (*pNN*) in kilometres (km) and population density measurements (*D*) per hectare (Ha) were extracted from GPS coordinates of populations following the methods detailed in Chapter 3. A population isolation ranking (*IR*) incorporating *pNN* with the population size (*N*) of the nearest-neighbouring population was derived following the methods detailed in Chapter 2, where each population was assigned an isolation ranking from 1-20 (with 20 being most isolated) based on a ranking system that combined a score of 1-10 for distance to nearest-neighbouring population (*pNN*) and a score of 1-10 for the nearest-neighbouring population size (Chapter 3, Table 3.1). Within population distance to nearest neighbour (*NNi*) in metres (m) was calculated in GenAlEx 6.5 (Peakall and Smouse 2012).

For each population, the proportion of the reproductive aged plants that were flower flowering (*%F*), the number of inflorescences per plant and the number of flowers per inflorescence were calculated for each year and averaged across both years. Flower scars and fruiting bodies (pods) were converted to the proportion of fruit set per inflorescence and the average proportion of fruit set per inflorescence *(%S*) per population was calculated. Population averages were calculated for the number of the number of flowers per inflorescence (*F/Inf*) over both years.

The possible relationship between climatic distribution southwards (*Lat*) and the population demographic and fecundity measures of within population distance to nearest neighbour (*NNi*), the proportion of the reproductive aged plants that were flowering (*%F*) and the average flowers per inflorescence (*F/Inf*) and the proportion of fruit set per inflorescence *(%S*)

were tested using Spearman's rank correlation tests in SPSS v 19 (IBM 2010). Relationships between the between plant density (*D*), population size (*N*), the population distance-to-nearest neighbour (*pNN*) isolation ranking (*IR*) and the within population demographic and fecundity measures (*%F*, *F/Inf*, *%S*) were tested using Spearman's rank correlation tests in SPSS v. 19 (IBM 2010). One-Way analysis of variance (ANOVA) with Tukey's post hoc tests were used to examine differences in population demographic parameters (*NNi*, *%F*, *F/Inf*, *%S*) among regions (North, Mid, South, Inland) using SPSS v 19 (IBM 2010).

*Population genetics*

Allelic frequencies were used to determine observed heterozygosity (*Ho*), expected heterozygosity (*He*), fixation index (*F*) and each populations was tested for deviation from Hardy-Weinberg equilibrium (HWE) using chi-square goodness-of-fit tests using GenAlEx 6.5 (Peakall and Smouse 2012). To test for recent decreases in effective population sizes (bottlenecks) within population and regions, differences across loci between the HWE and the equilibrium heterozygosity (Heq) expected from the number of alleles assuming mutation-drift equilibrium were evaluated. These differences were evaluated using a sign test and a Wilcoxon sign-rank test conducted under infinite allele model (IAM) and stepwise mutation model (SMM) using the program BOTTLENECK (Cornuet and Luikart 1996, Piry et al. 1999).

Each population and individual was examined for the occurrence of putative multi-locus clones using GenAlEx 6.5 (Peakall and Smouse 2012). These data were then converted to the number of unique genotypes per population and the proportion of each population with shared genotypes ($G_s$; clones). Allelic frequency tables were examined for populations with high proportions of clones to identify loci most influential to heterozygosity. One-Way ANOVA with Tukey's post hoc tests were used to examine difference in proportion of each population with shared genotypes ($G_s$) among regions (north, mid, south, inland) using SPSS v. 19 (IBM 2010).

Pairwise genetic distance and geographic distance matrices were constructed for each population and Mantel tests were used to conduct correlation analysis geographic and genetic distance matrices for each population using 9999 permutations in GenAlEx 6.5 (Peakall and Smouse 2012). Spatial autocorrelation analyses were used to test for non-random spatial genetic clustering within sites typical of family structure (Alexandersson and Ågren 2000). The analysis was run several times utilising a variety of different annulus distances classes based on

estimates of orchid seed dispersal distances (1 m, 5 m, 10 m; (Alexandersson and Ågren 2000, Arditti and Ghani 2000, Chung et al. 2005, Jacquemyn et al. 2009).

*The effect of isolation, differences between regions and tests for inbreeding depression*

The relationship between population isolation (*IR*) or population size (*N*), and population genetic parameters of fixation index (*F*), number of loci deviating from Hardy-Weinberg equilibrium (*HWE*) and proportion of each population with unique genotypes (clonality $G_U$) were tested using Spearman's rank correlation tests in SPSS v 19 (IBM 2010). A Spearman's rank correlation test in SPSS v 19 (IBM 2010) was also used to test the relationship between within population density (*D*) and within population nearest neighbour (*NNi*) and the population genetic parameters (*F*, *HWE*, $G_U$). To test for evidence of inbreeding depression in populations, the relationship between the population genetic parameters (*F*, *HWE*, $G_U$) and the demographic and fecundity measures (*N*, *D*, *NNi*, *%F*, *F/Inf*, *%S*) were tested using Spearman's rank correlation tests in SPSS v 19 (IBM 2010).

To test the effect of isolation on inbreeding and fecundity, populations were arranged in two sub-groups based on less isolation (isolation rank 1-10) or more isolation (isolation rank 11-20). An independent t-test was used to examine any differences in genetic measures of non-random mating and clonality (*F*, *HWE*, $G_S$) and demographic and fecundity measures (*NNi*, *%F*, *F/Inf*, *%S*) between the more vs less isolated population sub-groups in SPSS v. 19 (IBM 2010). The effect that population size (N) may have on genetic measures of non-random mating and clonality (*F*, *HWE*, $G_S$), demographic and fecundity measures (*NNi*, *%R*, *%F*, *F/Inf*, *%S*) were tested between the two population size groups using an independent t-test in SPSS v. 19 (IBM 2010). Differences in the population genetic parameters related to non-random mating and clonality (*F*, *HWE*, $G_S$) between regions were tested utilising one-way ANOVA with Tukey's post hoc tests in SPSS v 19 (IBM 2010). Small populations were removed from that test to exclude the bias of extreme allelic fixation in small populations.

*Breeding system*

To investigate floral patterns in Flinders Swamp, the proportion of all flowers across all treatments at different maturity stages were plotted against each day of the pollination treatments and floral lifespan for each plant was calculated as the average number of days

flowers were open from first show of sepal colour to senescence. The average proportion of all treated flowers that set fruit (early fruit set, fruit and dead flowers) was calculated for the end of the treatment period and for each monthly assessment until harvest. Differences in fruit set between treatments were tested ANOVA with Tukey's post hoc analyses. Average fruit weight and dimensions were calculated for each plant and ANOVA with fruit nested within treatments and Tukey's post hoc analyses were used to compare treatments.

## 4.3 Results

*Population demographics and fecundity*

Plants were generally scattered within populations with 13.76 m the average distance to nearest neighbour within the populations (*NNi*) when all plants were included. When outliers were excluded the *NNi* was considerably smaller (1.28 m; Table 4.1). This varied between populations across the species range with the smallest and greatest average within population distance to nearest neighbour (*NNi*) were both recorded on Fraser Island, ranging 0.07 m at Dilli Village (FI8) to 388 m at Moon Point (FI7). Population sizes (*N*) ranged from two individuals at Moon Point (FI7) through to 696 at Flinders Beach (SI11) with plant density ranging from 1 to 5 500 plants per hectare (Byfield BY26 and Kingscliff KC33 respectively; Table 4.1).

Across the entire species range 48% of the reproductive aged individuals had active reproduction evident at the time of surveys (*%F*). The proportion of the reproductive aged plants that were flowering (*%F*) averaged 40.6% across all populations, ranging from 0 at Barney Springs on the Atherton Tableland (AT4) to 100% at small populations in Eumundi and Kingscliff (EU14, KC33; Table 4.1). Across all populations plants that were flowering produced an average of 10.6 flowers on each inflorescence (*F/Inf*; Table 4.1). Average fruit set *(%S*) was 49.2% but highly variable and ranged from 0% at Coffs Harbour and South West Rocks to 69% at Canaipa Passage (SI29) and 92% at Yamba (YA22; Table 4.1).

There was minimal evidence to support the hypothesis that smaller or more isolated populations had lower fecundity with no significant ($p$>0.05) correlations between any reproductive measures (*%F*, *F/Inf*, *%S*) population size (*N*), or isolation rankings (*IR*; Table 4.2). There was also no significant difference in reproductive measures (*%F*, *F/Inf*, *%S*), between small and large population sizes or isolation rankings (Table 4.1 and Table 4.3). Within population factors also had minimal effect on reproduction.

Table 4.1. Population demographic parameters for 33 *P. australis* populations across the species range, overall average given with standard deviation in parentheses; *Ar*, population area of occupancy (ha); *N*, total population size at time of sampling, *D*, density of plants per hectare (ha); *pNN*, population first nearest neighbour distance (km); *IR*, isolation ranking, *NNi*, within population distance to nearest neighbour in metres (m), *%F*, the proportion of the reproductive aged plants that were flowering, , *F/Inf*, number of flowers per inflorescence, *%S*¸ proportion of fruit set per inflorescence.

| Region | Population | *Ar* (ha) | N | *D* (plants/ha) | *pNN* (km) | *IR* | *NNi* (m) | *%F* | *F/Inf* | *%S* |
|---|---|---|---|---|---|---|---|---|---|---|
| North | FNQ31 | 0.015 | 59 | 3919.48 | 152.77 | 9 | 0.27 | 40.9 | 17.6 | 53.0 |
| | AT1 | 0.432 | 252 | 583.41 | 0.30 | 10 | 0.36 | 78.6 | 14.5 | 24.0 |
| | AT2 | 0.735 | 338 | 459.42 | 0.30 | 9 | 1.19 | - | 13.9 | 10.0 |
| | AT3 | 1.081 | 591 | 547.31 | 6.49 | 12 | 0.49 | - | 8.9 | 11.3 |
| | AT4 | 0.009 | 8 | 8.423 | 8.26 | 12 | 0.6 | 0 | - | - |
| | CA5 | 0.154 | 4 | 25.97 | 128.92 | 10 | 1.31 | 66.7 | 3.5 | - |
| **North mean** | | 0.40 (0.43) | 209 (232) | 924 (1489.4) | 49.41 (29.08) | 10 (1) | 0.7 (0.18) | 31.0 (36.1) | 9.7 (2.8) | 24.6 (20.0) |
| Mid | BY26 | 0.000 | 3 | 1.00 | 209.18 | 18 | | 100.00 | 12.0 | |
| | FI6 | 4.442 | 96 | 21.61 | 29.50 | 13 | 2.63 | 5.2 | 10.3 | 24.0 |
| | FI7 | 0.787 | 2 | 2.54 | 13.65 | 6 | 388 | 100.0 | 7.5 | 44.0 |
| | FI8 | 0.003 | 8 | 8.00 | 13.65 | 11 | 0.07 | 12.5 | 3.5 | 59.0 |
| | TCB32 | 0.546 | 151 | 275.63 | 36.56 | 8 | 0.78 | 64.4 | 22.9 | 67.0 |
| | PB13 | 0.046 | 5 | 109.58 | 11.08 | 7 | 4.84 | 75.0 | 16.8 | 53.0 |
| | EU14 | 0.001 | 17 | 5.00 | 11.08 | 6 | 0.76 | 100.0 | 12.5 | .0 |
| | MA34 | 0.014 | 12 | 870.22 | 13.33 | 6 | 1.01 | 10.0 | 11.25 | 63.0 |
| | LT15 | 0.653 | 224 | 343.52 | 23.82 | 13 | 1.23 | 74.1 | 11.3 | 67.0 |
| | SI9 | 1.549 | 330 | 213.03 | 2.68 | 13 | 0.35 | 3.9 | 13.6 | 22.0 |
| | SI10 | 1.899 | 286 | 150.64 | 2.68 | 11 | 0.23 | 26.9 | 11.3 | 11.0 |
| | SI11 | 3.743 | 696 | 185.81 | 7.44 | 14 | 1.09 | - | 15.4 | - |
| | SI12 | 2.197 | 113 | 51.34 | 9.33 | 11 | 1.09 | - | 4.2 | - |
| | SI29 | 0.146 | 110 | 752.04 | 15.65 | 15 | 2.7 | 83.5 | 13.1 | 69.0 |
| | PR16 | 0.473 | 136 | 287.34 | 28.18 | 14 | 7.25 | - | 11.0 | - |
| **Mid mean** | | 1.10 (1.41) | 146 (186) | 218 (267.41) | 28.52 (50.93) | 11 (4) | 1.8 (0.58) | 37.0 (40.3) | 10.3 (1.6) | 39.9 (27.0) |

| Region | Population | *Ar* (ha) | N | *D* (plants/ha) | *pNN* (km) | *IR* | *NNi* (m) | *%F* | *F/Inf* | *%S* |
|---|---|---|---|---|---|---|---|---|---|---|
| South | KC33 | 0.004 | 22 | 5500.00 | 7.45 | 6 | 0.35 | 100 | 13.5 | 47.0 |
| | CNR17 | 0.123 | 17 | 137.71 | 7.45 | 7 | 0.81 | 85.7 | 14.1 | 62.0 |
| | BA20 | 0.008 | 6 | 717.31 | 57.61 | 10 | 1.57 | 30.0 | 4.5 | - |
| | WH28 | 6.118 | 64 | 10.46 | 7.98 | 5 | 2.34 | 15.6 | 6.5 | - |
| | AN21 | 2.007 | 170 | 84.71 | 1.68 | 9 | 2.54 | 19.2 | 7.2 | - |
| | YA22 | 0.003 | 6 | 2400.00 | 1.68 | 3 | 0.18 | 66.7 | 11.2 | 92.0 |
| | YR27 | 0.369 | 369 | 997.60 | 42.38 | 14 | 0.55 | 67.9 | 10.9 | 12.0 |
| | CH24 | 0.010 | 12 | 1200.00 | 87.78 | 11 | 0.13 | 55.6 | 10.9 | 0 |
| | SW25 | 0.020 | 21 | 1033.97 | 87.78 | 10 | 0.19 | 42.9 | 9.0 | 0.0 |
| **South mean** | | 0.96 (2.04) | 76 (122) | 1342 (1726.13) | 33.53 (36.38) | 8 (3) | 0.9 (0.95) | 50.4 (33.7) | 9.8 (1.1) | 30.4 (36.9) |
| Inland | BT18 | 0.116 | 219 | 1879.81 | 159.09 | 14 | 0.62 | 60.3 | 13.8 | - |
| | CG19 | 0.178 | 69 | 387.49 | 159.09 | 17 | 1.03 | 42.3 | 10.8 | 14 |
| **Inland mean** | | 0.15 (0.04) | 144 (106) | 1133 (1055.23) | 159.09 (0) | 16 (2) | 0.8 (0.29) | 51.3 (12.7) | 12.3 (1.5) | 14.0 |
| **Overall mean** | | 0.87 (1.45) | 138 (31) | 724.07 (212.73) | 42.03 (10.21) | 10 (1) | 13.7(68.47) | 40.6 (36.0) | 10.9 (0.8) | 33.5 (28.5) |

Table 4.2. Spearman's rank correlation tests for *P. australis* population location, density and genetics attributes: *Lat*, latitude; *N*, population size; *NNi*, within population distance to nearest neighbour in metres (m); *IR*, isolation ranking; *%F*, proportion of the reproductive aged plants flowering; *F/Inf*, mean number of flowers per inflorescence; *%S*, mean proportion of fruit set per inflorescence; *Ho*, observed heterozygosity; *He*, expected heterozygosity; *F*, fixation index; *HWE*, number loci significantly deviating from Hardy-Weinberg equilibrium; *Gs*, proportion of shared genotypes. Significant correlation are indicated (*- p<0.05, **p<0.01). Bold type indicates significant correlation after Bonferroni correction for multiple tests (α<0.00625).

| | *Lat* | | *N* | | *NNi* | | *%F* | | *F/Inf* | | *%S* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *rho* | *p* | *rho* | *p* | *rho* | *p* | *rho* | *p* | *rho* | *p* | *rho* | *p* |
| *%F* | -0.218 | 0.519 | -0.088 | 0.670 | 0.409 | 0.212 | | | | | | |
| *F/Inf* | 0.174 | 0.342 | 0.421* | 0.016 | -0.100 | 0.080 | 0.164 | 0.630 | | | | |
| *%S* | -0.339 | 0.337 | -0.038 | 0.860 | 0.555 | 0.096 | 0.602 | 0.114 | 0.348 | 0.325 | | |
| *Ho* | 0.274 | 0.305 | -0.140 | 0.444 | 0.370 | 0.159 | 0.319 | 0.339 | 0.031 | 0.865 | -0.110 | 0.763 |
| *He* | **0.660**** | 0.005 | 0.426* | 0.011 | -0.121 | 0.656 | -0.471 | 0.143 | 0.152 | 0.407 | -0.220 | 0.541 |
| *F* | **0.727*** | 0.001 | **0.603**** | 0.002 | **-0.500*** | 0.049 | **-0.636*** | 0.035 | 0.183 | 0.316 | -0.323 | 0.362 |
| *HWE* | **0.835**** | 0.000 | **0.745**** | 0.000 | -0.333 | 0.208 | -0.445 | 0.170 | 0.237 | 0.191 | -0.529 | 0.116 |
| $G_s$ | -0.265 | 0.612 | 0.248 | 0.011 | 0.116 | 0.827 | -0.316 | 0.683 | 0.000 | 0.999 | 0.400 | 0.600 |

There were no significant ($p$>0.05) correlations between any reproductive measures (*%F*, *F/Inf, %S*), with measures of density (*D*) or within population distance to nearest neighbour (*NNi*). There was a significant difference ($H$=13.09, $p$<0.05 in the average number of flowers per inflorescence between regions with SEQ having greater than Nth Qld and NSW (Table 4.1). There were no other significant differences in reproductive measures between regions (Table 4.1). There was a moderate correlation between the area occupied by populations (*Ar*) and the proportion of reproductive aged plants flowering (*%F*; *rho*=-0.582). As area occupied by populations increased, there were decreases in the proportion of reproductive aged plants in flower at the time of the surveys, however this correlation was not significant ($p$=0.06; Table 4.2).

*Is there inbreeding in isolated populations and does it differ between populations?*

In contrast to expectations, populations trended more towards outbreeding rather than being inbred with a mean fixation index (*F*) of -0.274 (Table 4.3). This varied across populations. Two populations in the north region had low levels of inbreeding with $F$=0.273 and $F$=0.113 at both populations on the Yuruga Nursery in Walkamin (AT1, AT2; Table 4.4). The Flinders Beach population on Stradbroke Island (SI10) in the mid region also had low levels of inbreeding with $F$=0.122 while five other populations in the mid region had values close to that expected for populations in Hardy-Weinberg equilibrium for random mating (SI9, $F$=-0.021; BY26, $F$=0.038; FI6, $F$=0.029; TCB32, $F$=0.041) including the Myora population containing the yellow variety (SI12, $F$=-0.067; Table 4.4). On

the other hand, several populations exhibited high levels of heterozygote excess (*F*>-0.5), including North populations at Shipton's Flat (FNQ31) and Cardwell (CA5), small populations in the middle of the range at Eumundi (EU14) and Peregian Beach (PB13) and in the south region, small populations at Cudgen (CNR17), Ballina (BA20), South West Rocks (SW25) and a large population at Yuraygir National Park (YR27; Table 4.4). Several extremely small populations had fixation indices toward complete outbreeding (FI7, 2 individuals, KC33, 4 individuals, YA22, 4 individuals and CH24, 3 individuals). Overall the north and mid regions were significantly ($p$<0.05) less outbred (*F*=-0.260, *F*=-0.280 respectively) compared to the South region (*F*=-0.704; Table 4.4); a result reflected in the significant increase in outbreeding with increasing distance south (*Lat*; *rho*=0.727; Table 4.3).

Table 4.3. Summary of reproductive measures, population size, isolation, genetic measures of non-random mating and clonality measures for *P. australis* by regions, population size categories and isolation ranking categories; standard error follows in parentheses. Results of statistical analysis of differences between genetic diversity, variation and structuring measures (polymorphic loci only) between different climatic regions; *H* = result of Kruskall-Wallis rank test between groups, differences indicated in superscript; *U* = result of Mann-Whitney U test; for all * $p$<0.05, ** $p$<0.01, ** $p$<0.001. Where: *NNi*, within population distance to nearest neighbour in metres (m); *%F*, proportion of the reproductive aged plants flowering; *F/Inf*, mean number of flowers per inflorescence; *%S*, mean proportion of fruit set per inflorescence; *Ho*, observed heterozygosity; *He*, expected heterozygosity; *F*, fixation index; *HWE*, number loci significantly deviating from Hardy-Weinberg equilibrium; $G_S$, proportion of shared genotypes. Significant correlations are indicated.

| | *n* | *NNi* | *%F* | *F/Inf* | *%S* | *F* | $G_S$ |
|---|---|---|---|---|---|---|---|
| North | 121 (6) | 0.7 (0.18) | 31.0 (36.1) | 9.7 (2.8) | 24.6 (20.0) | -0.260 $^{A}$ (0.170) | 62.47 $^{A}$ (14.63) |
| Mid | 309 (15) | 1.8 (0.58) | 37.0 (40.3) | 11.8 (1.2) | 39.9 (27.0) | -0.284 $^{A}$ (0.087) | 73.89 $^{AB}$ (6.23) |
| South | 139 (9) | 0.9 (0.95) | 50.4 (33.7) | 9.8 (1.1) | 30.4 (36.9) | -0.704 $^{B}$ (0.106) | 92.16 $^{B}$ (3.25) |
| Inland | 57 (2) | 0.8 (0.29) | 51.3 (12.7) | 12.3 (1.5) | 14.0 | -0.311 $^{AB}$ (0.151) | 83.75 $^{AB}$ (4.37) |
| *H* | | 2.89 | 1.85 | 1.524 | 1.50 | **7.95*** | 6.45 |
| Small pop'n size | 16 | 26.74 (9.95) | 49.9 (37.1) | 9.9 (1.2) | 34.8 (31.3) | -0.668 (0.076) | 80.44 (6.52) |
| Large pop'n size | 16 | 1.59 (1.74) | 31.2 (33.5) | 11.8 (1.1) | 31.7 (25.4) | -0.131 (0.057) | 74.56 (5.93) |
| *U* | | 87.00 | 163.50 | **18.0*** | 63.00 | **23.00**** | 96.00 |
| Less Isolated (IR 1-10) | 16 | 1.32 (1.25) | 48.2 (35.9) | 9.9 (1.0) | 38.1 (30.3) | -0.466 (0.106) | 76.31 (6.52) |
| More Isolated (IR 11-20) | 16 | 27.03 (99.88) | 32.9 (35.7) | 11.8 (1.3) | 28.1 (26.5) | -0.334 (0.082) | 78.69 (6.01) |
| *U* | | 110.00 | 91.00 | 153.00 | 61.50 | 101.00 | 127.00 |

There were very few populations without at least one loci deviating from Hardy-Weinberg equilibrium (*HWE*; Table 4.3). The number of loci deviating from Hardy-Weinberg equilibrium significantly ($p$<0.05) increased with distance south (*Lat*; *rho*=0.835; Table 4.2). Three populations in the north region had greater than half of the loci exhibiting while populations in the other regions predominantly had less than 3 loci deviating from Hardy-Weinberg equilibrium (*HWE*; Table 4.3).

There was no significant correlation with population nearest neighbour (*pNN*) and fixation index (*F*). However, there was a significant ($p$<0.05) correlation between fixation index (*F*), and population size (*N*) but not in the direction expected. Populations became significantly ($p$<0.05) more inbred with increasing population size (*rho*=0.527; Table 4.2). Mann-Whitney tests also established that with smaller populations were significantly ($p$<0.05) more outbred than large populations (*F*=-0.668, *F*=-0.131, respectively; Table 4.3). Populations also became more outbred with increasing population isolation (*IR*; *rho*=0.381, $p$<0.05).

Historic loss of populations or the loss of individuals within populations can 'bottleneck' a population resulting in a temporary heterozygosity excess under the Wilcoxin sign test ($p$<0.05) indicating a greater loss of rare alleles relative to the loss of alleles occurring at intermediate frequencies. The entire north region had results indicating significant historic bottlenecks (Table 4.5). Large populations in the north region that were moderately inbred showed evidence of significant bottlenecks in the BOTTLENECK tests (AT1, AT2) as well as the extremely small population at Cardwell (CA5; Table 4.5). Several small and large populations in the middle of the species range also exhibited evidence of significant bottlenecks in the BOTTLENECK tests (FI7, FI8, MA34 LT15, SI9, SI10, SI11 and PR16; Table 4.5). While the south region as a whole exhibited signs of historic bottlenecking, only the Woody Heads population individually exhibited signs of BOTTLENECK (Table 4.5). Across the species range, particularly in the north and south regions, there is strong evidence that *P. australis* plants and flowers were harvested from the wild (Chapter 2).

*Clonal spread and the heterozygosity of genotypes*

The excess heterozygosity within smaller populations could be due to the clonal spread of the species. A total of 81.63% of all individuals across the species range were cloned genotypes ($G_S$). This ranged from 0% clonal genotypes in an extremely small population at Byfield (BY26) and 14% and 21% clonal genotypes in two Atherton Tableland populations (AT1, AT2). There

were greater than 90% clonal genotypes at several large populations (SI10, SI11) and 100% clones at small populations at Fraser Island (FI7) and Yamba (YA22) and at the large Yuraygir population (YR27; Table 4.4). The percentage of clonal genotypes ($G_S$) increased significantly ($p$<0.05) with increasing distance southwards (*rho*=-0.749; Table 4.2). There was a low but significant ($p$<0.05) correlation between population size ($N$) and clonal genotypes ($G_S$), as populations size increased, the percentage of cloned genotypes ($G_S$) increased (*rho*=0.248; Table 4.2) an indication of clonal spread within populations. It is possible that the population expansion via clonal genets in the larger populations has decreased the ability to detect heterozygosity in larger populations, resulting in $F$ values trending towards inbreeding.

In most cases, those populations that had high levels of outbreeding had high proportions of cloned genotypes with populations ($F$, $G_S$ *rho*=-0.640, $p$<0.01). Exceptions were populations at Tin Can Bay (TCB32) and Myora Springs (SI12) which had greater than 75% clonal genotypes within the population and fixation index close to that for random mating (Table 4.4). Chapter 3 discusses the variation and diversity within the individual loci for *P. australis* that have been utilised in this study; there are six loci in total that are highly variable, however only four of these vary considerably between individuals within the populations (*ml-Pa03*, *ml-Pa19*, *ml-Pa44*, *ml-Pa59*). When examined individually within populations, those that were cloned genotypes had a greater number of individuals that were entirely heterozygous for each of the highly variable loci. For example, cloned individuals were only entirely heterozygous for one locus at the Walkamin population (AT3) that has minimal deviation from random mating. However, populations with moderate to high levels of outbreeding, greater percentages of populations made up by cloned genotypes (AT4, CA5, FI7, FI8, PB13, EU14, LT15, KC33, BA20, YA22, YR27, CH24) had entirely heterozygous individuals for at least 3 loci and in some cases, the majority of the fourth locus (generally *ml-Pa44* or *ml-Pa59*).

Thus, the number of loci that were completely heterozygous for all clonal individuals was significantly ($p$<0.01) correlated with increasing outbreeding and the proportion of individuals that had shared genotypes within a population ($F$, *rho*=-0.622; $G_S$, *rho*=0.382).

Table 4.4. Summary of allelic fixation for *P. australis* across 33 populations from 13 polymorphic loci (*ml-Pa03*, *ml-Pa31*, *ml-Pa19*, *ml-Pa44*, *ml-Pa59*, *ml-Pa49*, *mlPa-02*, *ml-Pa12*, *ml-Pa14*, *ml-Pa21*, *ml-Pa24*, *ml-Pa27*, *ml-Pa57*). Where: *n*, number of samples within population; *Ho*, observed heterozygosity; *He*, expected heterozygosity; *F*, fixation index; *HWE*, number loci significantly deviating from Hardy-Weinberg equilibrium; $G_S$, proportion of shared genotypes. Significance values **p* <0.05, ***p* <0.01, ****p*<0.001.

| *Region* | *Population* | *n* | *Ho* | *He* | *F* | *HWE* | $G_S$ |
|---|---|---|---|---|---|---|---|
| North | FNQ31 | 29 | 0.270(0.133) | 0.156 (0.075) | -0.603 (0.121) | 3* | 100 |
| | AT1 | 28 | 0.286(0.102) | 0.349 (0.062) | 0.273 (0.189) | 7* | 20.7 |
| | AT2 | 28 | 0.290 (0.086) | 0.320 (0.055) | 0.113 (0.161) | 7* | 21.4 |
| | AT3 | 27 | 0.233 (0.105) | 0.179 (0.066) | -0.093 (0.134) | 10* | 57.7 |
| | AT4 | 5 | 0.241 (0.114) | 0.154 (0.068) | -0.502 (0.123) | 1* | 75.0 |
| | CA5 | 4 | 0.303 (0.131) | 0.172 (0.072) | -0.750 (0.087) | 0 | 100.0 |
| mean | | | 0.270 | 0.222 | -0.260 | | 62.47 |
| Mid | BY26 | 3 | 0.242 (0.101) | 0.253 (0.067) | 0.038 (0.205) | 1* | 0.0 |
| | FI6 | 34 | 0.270 (0.128) | 0.193 (0.063) | 0.029 (0.273) | 6* | 64.7 |
| | FI7 | 2 | 0.273 (0.141) | 0.136 (0.070) | -1.000 (0.000) | 0 | 100.0 |
| | FI8 | 4 | 0.250 (0.131) | 0.185 (0.080) | -0.286 (0.286) | 4* | 75.0 |
| | TCB32 | 29 | 0.211 (0.117) | 0.186 (0.060) | 0.041 (0.246) | 5* | 75.9 |
| | PB13 | 4 | 0.250 (0.131) | 0.134 (0.069) | -0.867 (0.070) | 2* | 75.0 |
| | EU14 | 4 | 0.227 (0.124) | 0.148 (0.077) | -0.600 (0.209) | 2* | 50.0 |
| | MA34 | 9 | 0.222 (0.123) | 0.163 (0.069) | -0.321 (0.284) | 3* | 77.8 |
| | LT15 | 31 | 0.261 (0.126) | 0.194 (0.076) | -0.227 (0.229) | 4* | 90.0 |
| | SI9 | 32 | 0.202 (0.120) | 0.193 (0.070) | -0.021 (0.270) | 4* | 75.0 |
| | SI10 | 31 | 0.203 (0.120) | 0.154 (0.057) | 0.122 (0.290) | 5* | 90.3 |
| | SI11 | 30 | 0.266 (0.125) | 0.192 (0.071) | -0.317 (0.242) | 4* | 70.0 |
| | SI12 | 31 | 0.246 (0.118) | 0.167 (0.062) | -0.067 (0.254) | 4* | 77.4 |
| | SI29 | 32 | 0.233 (0.119) | 0.149 (0.073) | -0.448 (0.122) | 2* | 93.8 |
| | PR16 | 33 | 0.270 (0.127) | 0.159 (0.067) | -0.336 (0.248) | 4* | 93.9 |
| mean | | | 0.242 | 0.173 | -0.284 | | 73.89 |
| South | KC33 | 4 | 0.273 (0.141) | 0.136 (0.070) | -1.000 (0.000) | 3* | 100.0 |
| | CNR17 | 12 | 0.240 (0.127) | 0.140 (0.072) | -0.725 (0.144) | 3* | 75.0 |
| | BA20 | 6 | 0.258 (0.134) | 0.135 (0.070) | -0.905 (0.050) | 2* | 100.0 |
| | WH28 | 31 | 0.202 (0.117) | 0.157 (0.066) | -0.106 (0.217) | 3* | 93.5 |
| | AN21 | 37 | 0.268 (0.127) | 0.157 (0.067) | -0.326 (0.247) | 4* | 89.2 |
| | YA22 | 4 | 0.273 (0.141) | 0.136 (0.070) | -1.000 (0.000) | 3* | 100.0 |
| | YR27 | 33 | 0.253 (0.127) | 0.147 (0.071) | -0.579 (0.120) | 3* | 93.9 |
| | CH24 | 3 | 0.273 (0.141) | 0.136 (0.070) | -1.000 (0.000) | 0* | 100.0 |
| | SW25 | 9 | 0.222 (0.123) | 0.127 (0.067) | -0.702 (0.112) | 2* | 77.8 |
| mean | | | 0.251 | 0.142 | -0.705 | | 92.16 |
| Inland | BT18 | 30 | 0.215 (0.108) | 0.150 (0.062) | -0.160 (0.176) | 4* | 86.0 |
| | CG19 | 27 | 0.202 (0.093) | 0.125 (0.061) | -0.462 (0.128) | 2* | 81.5 |
| mean | | | 0.209 | 0.137 | -0.311 | | 83.75 |
| Total / mean | | 626 | 0.248 | 0.178 | -0.274 | 3.25 | 77.50 (24.71) |

Table 4.5. Results of the bottleneck test for *P. australis*. Expected (Exp.) and observed (Obs.) values for the number of loci showing higher than expected heterozygosity under H-W than drift mutation equilibrium for the infinite allele model (IAM) and the stepwise mutation model (SMM). Wilcoxon test comparing the observed and expected values P values and number of loci used in analysis is given (Loci).

| | | IAM | | SMM | |
|---|---|---|---|---|---|
| | **Loci** | **Sign** | **Wilcoxon** | **Sign** | **Wilcoxon** |
| **North** | *10* | *0.003** | *0.007** | 0.197 | 0.116 |
| **FNQ31** | 4 | 0.247 | 0.062 | 0.325 | 0.063 |
| **AT1** | *10* | *0.021** | *0.005** | 0.053 | 0.122 |
| **AT2** | *9* | *0.007** | *0.003** | 0.267 | 0.164 |
| **AT3** | 8 | 0.421 | 0.680 | 0.095 | 0.902 |
| **AT4** | 4 | 0.399 | 0.033 | 0.398 | 0.094 |
| **CA5** | *4* | *0.023** | *0.032** | 0.060 | 0.031 |
| **Mid** | 10 | 0.177 | 0.246 | 0.385 | 0.813 |
| **BY26** | 7 | 0.436 | 0.594 | 0.633 | 0.766 |
| **FI6** | 7 | 0.341 | 0.234 | 0.527 | 0.469 |
| **FI7** | *3* | *0.016** | 0.063 | 0.033 | 0.063 |
| **FI8** | 4 | 0.103 | *0.031* | 0.147 | 0.031 |
| **TCB32** | 7 | 0.353 | 0.289 | 0.048 | 0.406 |
| **PB13** | 3 | 0.158 | 0.063 | 0.257 | 0.063 |
| **EU14** | 3 | 0.215 | 0.063 | 0.173 | 0.063 |
| **MA34** | 4 | 0.070 | *0.031** | 0.057 | *0.031** |
| **LT15** | 5 | 0.124 | *0.031** | 0.185 | *0.031** |
| **SI9** | 6 | 0.196 | *0.039* | 0.293 | 0.078 |
| **SI10** | *6* | *0.047** | 0.500 | 0.350 | 0.656 |
| **SI11** | 5 | 0.120 | *0.031* | 0.203 | 0.312 |
| **SI12** | 6 | 0.199 | 0.055 | 0.289 | 0.219 |
| **SI29** | 4 | 0.231 | 0.063 | 0.330 | 0.063 |
| **PR16** | 5 | 0.105 | *0.047* | 0.453 | 0.078 |
| **South** | 7 | 0.098 | *0.039** | 0.452 | 0.188 |
| **KC33** | 3 | 0.156 | 0.063 | 0.254 | 0.063 |
| **CNR17** | 3 | 0.098 | 0.063 | 0.184 | 0.063 |
| **BA20** | 3 | 0.079 | 0.063 | 0.162 | 0.063 |
| **WH28** | 5 | 0.115 | *0.047** | 0.184 | 0.109 |
| **AN21** | 5 | 0.348 | 0.078 | *0.045** | 0.078 |
| **YA22** | 3 | 0.169 | 0.063 | 0.269 | 0.063 |
| **YR27** | 4 | 0.228 | 0.063 | 0.317 | 0.094 |
| **CH24** | 3 | 0.091 | 0.063 | 0.177 | 0.063 |
| **SW25** | 3 | 0.124 | 0.063 | 0.156 | 0.063 |
| **Inland** | 6 | 0.211 | 0.125 | 0.635 | 0.156 |
| **BT18** | 6 | 0.549 | 0.422 | 0.350 | 0.500 |
| **CG19** | 4 | 0.204 | 0.094 | 0.283 | 0.094 |

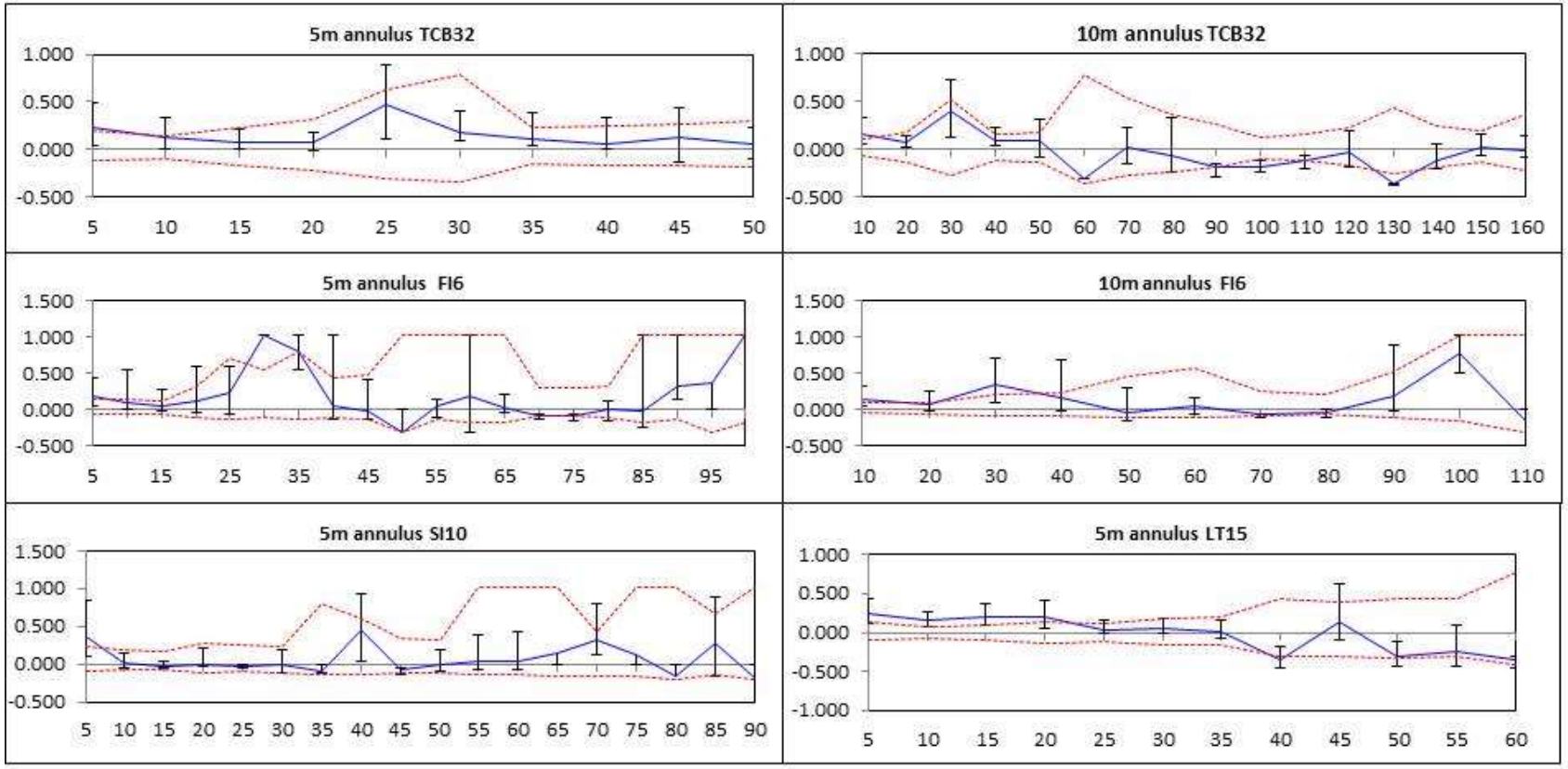


Figure 4.2 The results of the significant ($p$<0.05) autocorrelation tests within *P. australis* populations for 5 m and 10 m annulus distances; only populations yielding significant spatial autocorrelations are displayed. All populations displayed are from the mid region; TCB32, Tin Can Bay; FI6, Pile Valley, Fraser Island; SI10, Flinders Swamp, Stradbroke Island; LT15, Lota.

*Are there family genetic structures within populations?*

Some populations had weak but significant spatial autocorrelations found over a variety of different annulus distance classes (1 m, 5 m, 10 m). For example, there was a significant spatial autocorrelation (bootstrap $r$= 0.239, $p$<0.05) at Tin Can Bay (TCB32) for 5 m distance classes from 0 m with an intercept at 10 m; and for a 10 m distance class (bootstrap $r$=0.364 $p$<0.05) at 120-130 m (Figure 4.2). This may reflect small scale seed dispersal and a local clonal spread. There were also significant autocorrelations (bootstrap $r$= 0.827, $p$<0.05) at the Pile Valley population (FI6) for 5 m distance classes from 25-35 m with an intercept at 42.9 m; and for a 10 m distance class (bootstrap $r$= 0.349 $p$<0.05) at 20-30 m with an intercept at 48 m (Figure 4.2). This population is distributed in small clumps scattered across a large area (4.42 ha). Population SI10 in Flinders Swamp has significant spatial autocorrelations (bootstrap $r$=0.415, $p$<0.05) for 5 m distance classes at 0-5 m with an intercept at 12 m indicating localised seed spread, similar to significant spatial autocorrelations (bootstrap $r$= 0.258, $p$<0.05) for 5 m distance classes from 0 m to 20 m with an intercept at 35 m were observed for LT15 (Figure 4.2). The 18 Mile Swamp population (SI9) had significant spatial autocorrelations for 10 m distance classes at 150-160 m (bootstrap $r$=0.0.566, $p$<0.05) and 170-180 m (bootstrap $r$= 0.0.825, $p$<0.05) with an intercept at 57.86 m.

*Does P. australis spontaneously self-pollinate?*

The flowering period for the experimental *P. australis* plants in Flinders Swamp lasted 24 days from August 30 to September 23 2013 (Figure 4.3). By the end of the flowering period many plants had produced up to 14 flowers that matured from buds and were pollinated depending on experimental treatment undertaken (Figure 4.3). A peak in the number of receptive flowers occurred in the middle of September with the number of flowers opening and maturing lessening until the end of the month indicating some level of synchronicity in flowering allowing outcrossing potential (Figure 4.3). Flowers opening at the start or the end of the season would have reduced synchronicity with other plants, with some level of self-pollination required in order for successful fruit set. The first evidence of early fruit formation began on 19 days into the study and 38% of flowers had formed fruit at the end of the experimental treatments (Figure 4.4).

All plants selected for the pollination study were clones within the population exhibiting genotypes with 10 of 13 loci homozygous, with the remaining three heterozygous (SI10, $G_S$=90%, $F$=0.122; Table 4.4); the genotype for the father/pollen donor for the cross-pollination

treatment was unknown. One month after final treatments, there was significantly ($p$<0.05) less fruit set in the open pollination treatment compared to the cross pollination treatment (28.21%, 52.63% respectively; $f$=156.67; Figure 4.5A) indicating some success towards manipulated outcrossing between different plants. Proportions of fruit set on the bagged, self-pollinated and cross pollination treatments were similar at this point in time (Figure 4.5A). There was some fruit drop for all treatment types in the three months following the experiment (Figure 4.6). While there were no significant differences ($p$>0.05) in the proportions of fruit remaining on the experimental plants between the treatments in January, there were more fruit remaining on the cross-pollinated inflorescences indicating greater success and survival as a result of outcrossing (Figure 4.5B).

The lower fruit set on the open pollination treatment compared to all manipulated treatments indicates that *Phaius australis* may be pollinator limited in Flinders Swamp. Correspondingly, the similarity in the proportions of fruit set at both the early and late fruiting season between the bagged (spontaneous self) treatment and the manipulated self-pollination treatment indicates that the species is capable of self-pollination in the absence of pollinators (autogamy). The absence of the rostellum to separate the pollinia from growing into the stigma is the potential reason this can happen.

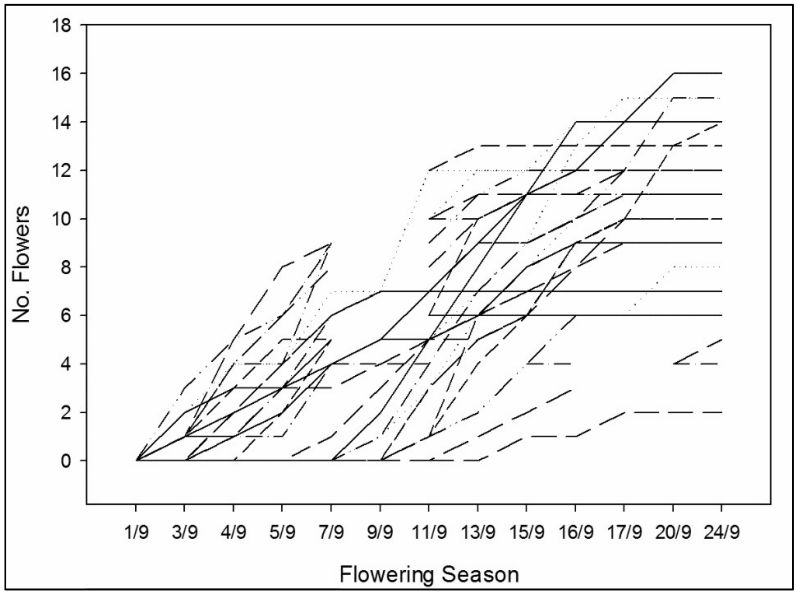


Figure 4.3. The cumulative number of flowers open for 29 *P. australis* experimental plants at Flinders Swamp for the month of September 2013. Each line indicates a different plant.

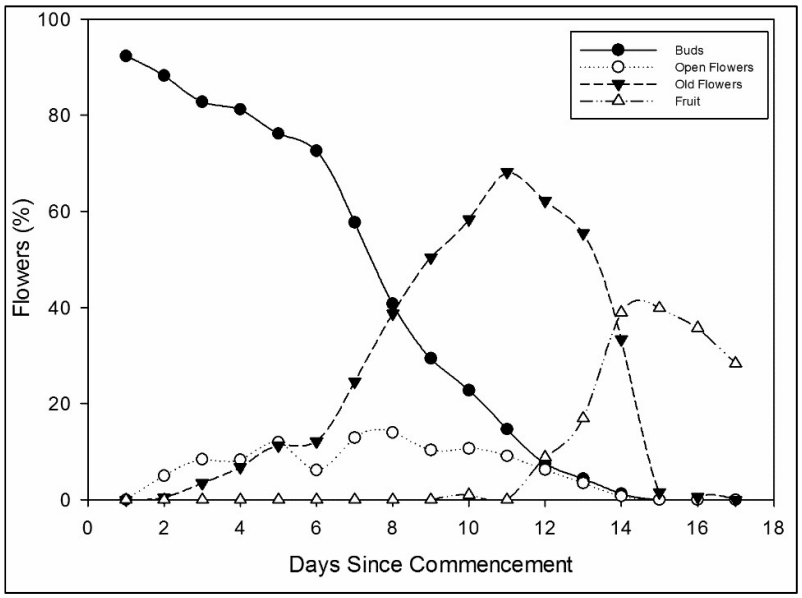

Figure 4.4. Flowering pattern for 29 *P. australis* experimental plants at Flinders Swamp over the duration of the study from September to January. Lines indicate average proportion (%) of flowers per inflorescence at the different flowering stages.

There were no significant differences in the proportion of fruit set between populations in any region (Table 4.1-3) and given that the same flower structure is observed across the species range (Chapter 2), it can be assumed that the reproductive strategy identified in Flinders Swamp is analogous to all populations. While the Flinders Swamp population is more inbred compared to the majority of populations, the same reproductive success would hold true for more heterozygous populations. Given the potential success for *P. australis* to self-pollinate, the resulting heterozygote excess is surprising, unless the plants are exhibiting some level of hybrid (heterozygote) vigour.

One hundred and three fruit were collected from 24 *P. australis* experimental plants after 130 days of maturation. While fruit yield was not significantly different between treatments ($f$=1.746, $p$>0.05), the fruit of the open and spontaneous self-pollination treatments were smaller and lighter (Figure 4.7A-D). There was some evidence to suggest evidence of outcrossing fruit vigour, with outcrossed fruit larger as well as more abundant than open and spontaneous self-pollinated fruit. Fruit from the cross pollination treatment was significantly ($p$<0.05) heavier than the open and spontaneous self-pollination treatments but similar in weight to the manipulated self-pollination treatment ($f$=6.952; Figure 4.7C). The diameter of fruit from the open and spontaneous self-pollination treatments was significantly smaller ($p$<0.05) than the fruit from the cross and manipulated self-pollination treatments ($f$=8.365; Figure 4.7B). Similarly the length to width ratio was significantly ($p$<0.05) smaller for the

manipulated self-pollination treatment compared to the spontaneous self-pollination and open pollinated treatments with the length to width ratio of the cross pollination fruit less than the open pollination treatment ($f$=11.860; Figure 4.7D). As a result, the volume ($cm^3$) of the cross pollinated fruit was significantly ($p$<0.05) larger than the spontaneously self-pollinated fruit, with the volume of the open pollinated fruit significantly ($p$<0.05) smaller than the manipulated self-pollination treatment (6.9 $cm^3$ Figure 4.7A).

For all fruit dimensions there were no significant differences ($p$>0.05) between plants within treatments. The larger yield and larger fruit size of the cross pollinated flowers is potential evidence of outcrossing fruit vigour. Lower fruit set (yield) and smaller fruit sizes of the open pollinated treatments compared to the manipulated self-pollination treatment supports that there may be pollinator limitations or interference in the natural populations resulting in reduced fruit output compared to if plants outcrossed.

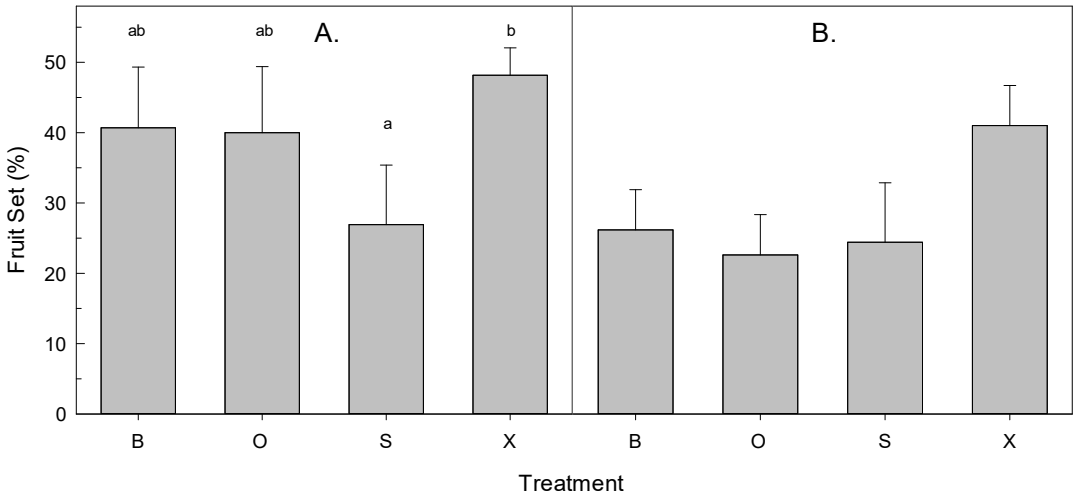


Figure 4.5. Mean proportion of fruit set per treatment at A) early fruit set, 29/10/2014 and B) fruit retention, 07/01/2014; where for *B*, Bagged (no manipulation); *O*, Open pollination (control);*S*, Self-pollination (manipulated self-pollination); *X*, Cross-pollination (manipulated cross-pollination) treatments. Standard error bars shown and lower case letters show Tukey's post hoc analysis homogeneous groups.

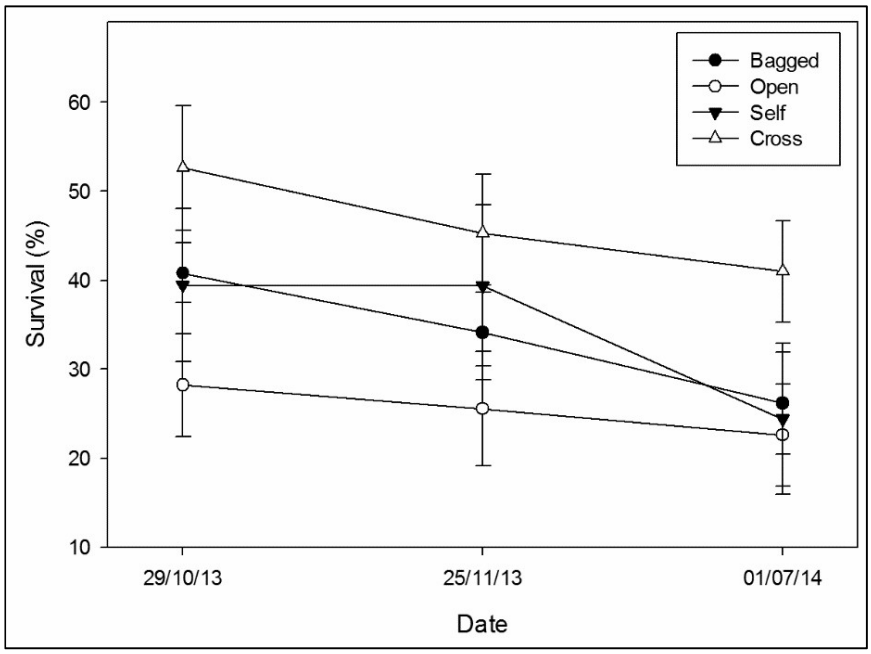


Figure 4.6. The monthly survival of fruit set on *Phaius australis* experimental plants at Flinders Swamp from 1 month after final pollination day (29/10; early fruit set), to the end of the study (07/01; late fruit set). Lines indicate average proportion of fruit set per inflorescence for different treatments, standard error bars shown.

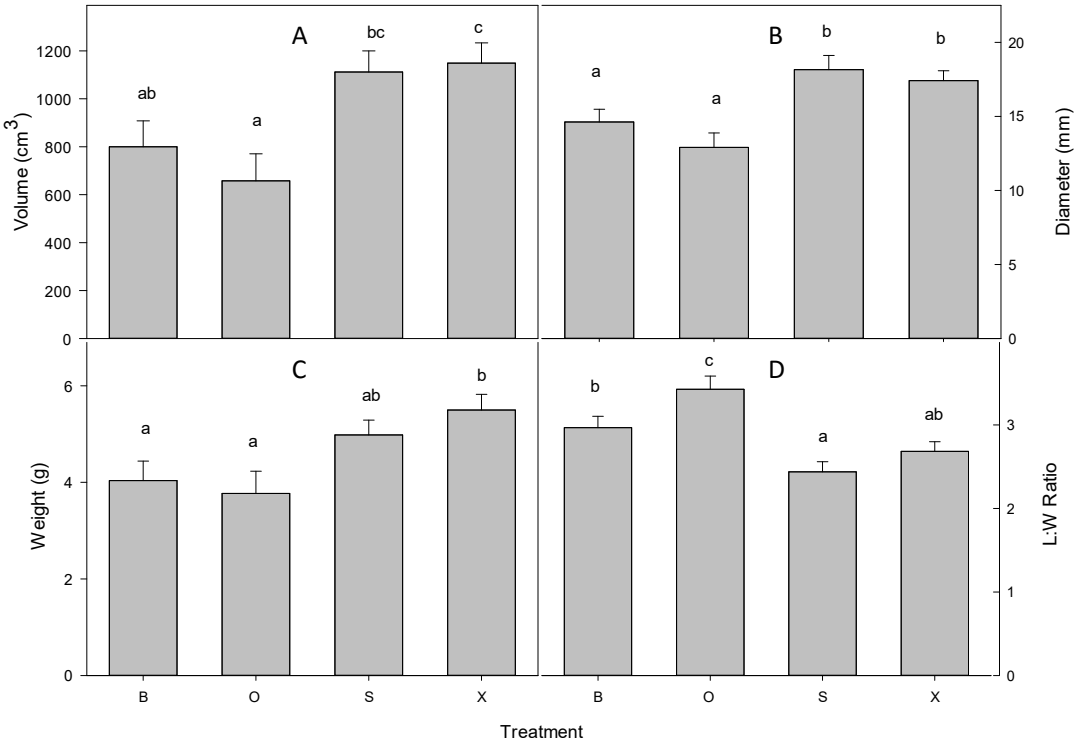


Figure 4.7. Fruit measures by treatment A) volume ($cm^3$), B) average diameter (mm), C) weight of fruit in grams (g) and D) length to width ratio; for *B*, Bagged (no manipulation), *O*, Open pollination (control); *S*, Self-pollination (manipulated self-pollination); and *X*, Cross-pollination (manipulated cross-pollination) treatments. Standard error bars given lower case letters indicate significant differences between treatments.

*Evidence of heterozygote vigour*

There was some additional evidence of possible heterozygote advantage with populations containing individuals of greater excess heterozygosity having greater fruit set. There were increases in the proportion of fruit set (*%S*; *rho*=-0.529, *p*=0.116) with increasing deviation from Hardy-Weinberg Equilibrium (*HWE*) and increased heterozygosity (*F*, rho=-0.323, *p*=0.362) though correlations were not significant (Table 4.3). Populations with high heterozygosity had more reproduction and higher fruit set than populations tending to homozygosity, possibly indicating some level of heterozygote advantage within populations containing individuals with greater excess of heterozygosity more reproductively active as well as more likely to set fruit.

## 4.4 Discussion

*Are small, isolated populations inbred?*

Theory predicts that populations that have become fragmented and lost individuals within populations and lost connectivity with neighbouring populations lose genetic diversity through bottlenecks, random genetic drift and a direct loss of alleles per locus (Wright 1931, 1948, Nei et al. 1975, Young et al. 1996). This can lead to an increase in the occurrence of fixed and cloned genotypes in a population. Some orchids, including the critically endangered Australian ground orchid *Caladenia huegelii* (Swarts et al. 2009) have been found to be inbred with reduced heterozygosity in small populations (Gustafsson and Sjögren-gulve 2002, Chung et al. 2012). In contrast to expectations, *P. australis* populations trended on average towards outbreeding rather than inbreeding. Inbreeding is not necessarily the rule for all plants according to meta-analyses of species subjected to effects of fragmentation (Honnay and Jacquemyn 2007, Aguilar et al. 2008, Vranckx et al. 2012). For example, Chung et al. (2014) found no evidence of increased inbreeding with decreasing population sizes of fragmented *Cymbidium goeringii*.

The loss of populations or the loss of individuals within populations can result in a 'bottleneck', with genotypes deviating from those expected under Hardy-Weinberg equilibrium for non-random mating (Nei et al. 1975, Cornuet and Luikart 1996). Meta-analyses suggest that the effects of inbreeding and deviation from Hardy-Weinberg equilibrium increases with the number of generations elapsed since the bottlenecking event (Aguilar et al. 2008). Several of

the larger populations on Atherton Tableland, thought to be older populations from early colonisation of *P. australis* in Australia, as well as a large population in the mid region exhibited evidence of bottlenecking and increase in homozygotes in line with an increase in inbreeding.

Orchids in particular are known for their elaborate mechanisms that promote cross-pollination and are therefore expected to have high out-crossing rates (Tremblay et al. 2005). Inbreeding is expected to increase in small isolated populations through a heightened probability of close relatives mating or mating among related individuals (Ellstrand and Elam 1993, Honnay et al. 2005, Honnay and Jacquemyn 2007, Frankham 2010a). Allelic fixation varied between inbreeding and outbreeding for *P. australis* populations. A study of *Plantanthera bifolia* in Poland found similar variance in inbreeding and outbreeding across different populations (Brzosko et al. 2009). However, there was a correlation between fixation index, population size, isolation between populations and the isolation within the population, but it was not in the direction expected. Populations became more inbred with increased population size and with increased population isolation ranking and several extremely small populations had fixation indices toward complete outbreeding, indicating clonal expansion of small populations after colonisation. Absence of homozygous rare alleles can be associated with decreased levels of inbreeding (autogamy) in fragmented systems (Raijmann et al. 1994, Kang et al. 2005, Honnay and Jacquemyn 2007). For *P. australis*, there was an increase in outbreeding with increasing distance south, with north and mid regions were less outbred compared to the South region. This follows the expansion of the species as it has colonised southwards from the tropics (Chapter 3).

An increase in inbreeding in small, fragmented, isolated populations may result in inbreeding depression and reduce population fitness (Ellstrand and Elam 1993, Young et al. 1996). Inbreeding depression can be observed as a lack of vigour in plants and recruits, reduced flowering or reduced output of flowers or reduced fruit set or fruit size and is thought to allow inbred populations to purge harmful recessive alleles (Charlesworth and Charlesworth 1990, Husband and Schemske 1996, Tremblay et al. 2005, Smithson 2006, Juillet et al. 2007). Sletvold *et al.* (2012) quantified strong negative effects of inbreeding in populations of *Gymnadenia conopsea*, a Norwegian Orchid. Reduced fitness in the size of the fruit, the seed production and recruitment of young orchids were observed in inbred (self-pollinated) individuals (Sletvold et al. 2012). This study delivers minimal evidence that smaller, more isolated populations would exhibit lower fecundity (*%F*, *F/Inf*, *%S*) possibly because small populations of *P. australis* have excess heterozygosity and an abundance of cloning rather than

homozygosity in contrast to theories for small population sizes (Wright 1931, 1948, Ellstrand and Elam 1993). Consistent with predictions for inbreeding rather than population size, there was evidence of reduced fitness in more inbred populations regardless of the size of the population. This study found evidence that populations composed of individuals with greater excess heterozygosity were more likely to set fruit.

*Clonal spread, the advantage of autogamy and ongoing genetic outcomes*

The spatial arrangement of individuals within a population can influence the potential for inbreeding, particularly if the plants do not flower synchronously reducing potential mates even further (Falk and Holsinger 1991, Chung et al. 2011). For example, an isolated flowering event of *Xanthorrhoea johnsonii* created temporary isolation within a larger population of the species resulting in progeny sampled to occur as a result of some level of bi-parental inbreeding and self-fertilisation (King and Zalucki 2012). This resulted in some levels of family group structuring within a 10 m area. *Phaius australis* was observed to have a strongly synchronous flowering period in the study population in 2013; while this would allow some level of local breeding, this also increases the chance of pollen interchange between plants across a whole population. There were minimal indications of within population clustering of genetically similar individuals. Such weak indications of localised family structure indicate that the excess heterozygosity is not the result of progeny of between family crosses located within close proximity of each other. In contrast, fine-scale genetic families were observed in populations of terrestrial orchid *Cymbidium goeringii* (Chung et al. 2011) as well as for *Caephalanthera longibratata* (Chung et al. 2004).

A plant that autonomously self-fertilises has previously been termed an evolutionary 'dead-end' because this system is likely to produce more homozygotes or build up deleterious alleles within a population (Wright 1931, 1948, Takebayashi and Morrell 2001, Frankham 2005). Autogamous or mixed mating reproductive systems have been well described for many orchids (Takebayashi and Morrell 2001, Chung et al. 2004, Gale 2007, Duffy et al. 2009, Hedrén and Nordström 2009, Dormont et al. 2010, Gale 2010, Suetsugu 2013). The evidence from the reproductive biology experiment indicates that *P. australis* may be pollinator limited in Flinders Swamp. Despite a strongly synchronous flowering period, fewer fruit were set and retained to full maturity in the open pollination treatments compared to the treatments where either self or outcrossed pollen was applied. The lack of differentiation in the proportions of early fruit set and the retention of fruit between the bagged, self-pollinated and cross pollinated

treatments is indicative that *P. australis* is fully self-compatible and autogamous. The results of this study support previous observations that large amounts of fruit set on *P. australis* could be an indication of autogamy (Sparshott and Bostock 1993, Benwell 1994, Tremblay 2005, Jones 2006). Autogamy is enabled in *P. australis* as the flowers lack a rostellum that separates the pollinia from the stigma (Sparshott and Bostock 1993, Benwell 1994, Jones 2006). Therefore, it appears that *P. australis* in Flinders Swamp possesses a mixed mating system with autogamy as reproductive assurance. Additional pollination studies across several populations would add to the strength of these results.

Autogamy has the advantage of being 'cheaper' by reducing the investment in colour, size or number of flowers on display to attract pollinators (Kalisz et al. 2004, Willmer 2011, Suetsugu 2013). However, *P. australis* provides a large investment into the production of very tall (up to 2m tall) inflorescences that can sometimes have a diameter of several centimetres (Freeman et al. 2011). On average across all populations *P. australis* plants produced over 10 large (15 cm) flowers per inflorescence, with some populations producing more than 20 flowers per inflorescence; hence *P. australis* is certainly a plant that is investing in floral output for cross-pollination.

Autogamy can reduce a dependency on pollinators (Kalisz et al. 2004, Morgan and Wilson 2005, Willmer 2011). *Xylocopa* bees (Hymenoptera, Carpenter Bees) are responsible for pollination of some natural populations of *Phaius tancarvilleae* which is a close relative to *P. australis* that occurs in South-east Asia and the Pacific (Gandawidjaja and Arditti 1982). The genus *Xylocopa* occurs in Australia (QM 2007) and while one of these bees was possibly observed in Flinders Swamp during the pollination experiments, no pollination event was witnessed. It could be said that a pollinator could be limited in Flinders Swamp due to the location of population on an island with pollinators moving less among small, sparse and non-continuous patches (Grindeland et al. 2005). However, Flinders Swamp occurs on a section of Stradbroke Island that is connected to large continuous stretches of native forest. There is also little distance separating North and South Stradbroke Islands with the mainland. Hence the possibility of island isolation restricting pollinators is low. Observations of pollinators for *P. australis* in Australia have similarly been lacking in the literature (Benwell 1994, Jones 2006).

Strong negative effects of inbreeding can lead to an observed reduction in fitness, in turn indicating a predominantly outcrossing system for a plant (Husband and Schemske 1996). Reduced seed and fruit set, germination and vigour across many stages of the plants life-cycle, would act as a mechanism to prevent the exposure of future generations to harmful alleles

(Charlesworth and Charlesworth 1990, Charlesworth and Willis 2009). For example, reduced seed mass, number of seeds, germination and fitness of self-pollinated adults in orchid *Gymnadenia conopsea* highlighted the strong preference towards an outcrossing strategy for the species (Sletvold et al. 2012). While the measures of reproductive output and fruit weight were crude and additional quantitative measurements such as seed viability or germination vigour were beyond the scope of this pollination experiment, the lack of a large difference in the number of fruit produced between the manipulated self-fertilised and the cross-fertilised flowers may indicate a mixed-mating strategy for the species, with autogamy accounting for at least some of the fruit set in natural populations. The proportion of successful fruit set (embryo formation) in both the self-fertilised treatment group in the pollination experiment as well as in natural fruit set observed across all *P. australis* populations is similar to that of autogamous orchids as reviewed by Tremblay et al. (2005). Fruit set (*%S*) was no different between regions, nor correlated with demographic factors or population size; thus the reproductive strategy employed at Flinders Swamp is potentially the same strategy across all populations of *P. australis*.

A plant with a mixed mating system utilising autogamy is likely to build up deleterious alleles within a population and show physical signs of inbreeding depression (Wright 1931, 1948, Tremblay et al. 2005, Smithson 2006, Juillet et al. 2007, Charlesworth and Willis 2009). For example, Smithson (2006) found lower seed mass for fruit and lower vigour of protocorms from manipulated self-pollination of three orchids in Southern France. In contrast, an autogamous terrestrial orchid *Neotinea maculata* was not found to be adversely affected by this reproductive strategy (Duffy et al. 2009). Although floral biology and experimental pollinations showed that *P. australis* is capable of autonomous self-fertilisation and fruit yield was not significantly different between treatments, the fruit of the spontaneous self-pollination treatments were smaller and lighter than that from the manipulated cross-pollination treatment. As the cross-pollinated *P. australis* fruit were larger and more abundant, there could be some level of inbreeding depression. Inbreeding over generations may be reduced in populations by purging deleterious alleles by selection (Charlesworth and Charlesworth 1987). However, this process requires relatively large population size and experimental evidence for such purging in natural populations is mixed (Lande and Schemske 1985, Charlesworth and Charlesworth 1987, Crnokrak and Barrett 2002). Instead, interference from the environment or by performing the self-pollination may have resulted in somewhat reduced vigour in the fruit of the *P. australis* autogamous experimental group.

While self-pollination is a sexual event, this mating strategy can result in a build-up of homozygotes in a population as a consequence of genetic inheritance (Wright 1931, 1948, Lande and Schemske 1985, Tremblay et al. 2005). The offspring of self-pollinated plants perpetuate the genotypes of the parents, with an expected increase homozygous genotypes. There were extremely high proportions (average 77%) of shared genotypes within all populations except for two populations in North Queensland. In contrast to expectations for self-pollinating plants, when the multi-locus shared *P. australis* genotypes were examined, genotypes were almost entirely heterozygous at each highly variable locus. Such dominance of heterozygote multi-locus genotypes could indicate some level of heterozygous advantage or selection. Decreased inbreeding in fragmented systems with small population sizes has been associated with selection against homozygotes (Raijmann et al. 1994, Kang et al. 2005, Honnay and Jacquemyn 2007). If the population is isolated and self-pollinating as reproductive assurance the effects of genetic drift would result in the erosion of alleles over time. Selection against homozygotes can ensure that diversity is retained by possessing heterozygous genotypes balancing selection and reducing diversity lost through drift. In this study *P. australis* was observed to have a greater proportion of fruit set in populations with greater heterozygosity compared to those populations with greater homozygosity.

Self-fertilisation may replace outcrossing in a population when the fitness of a self-fertilising morph exceeds that of an outcrossing morph (Asmussen et al. 1998). From an evolutionary angle, autogamy can occur for reasons including: providing reproductive assurance when mates and pollinators are scarce, when spontaneously self-pollinating genotypes produce more offspring than those which do not (Darwin 1876, Baker 1955), as well as a gene-level advantage termed the 'automatic selection' hypothesis (Busch and Delph 2012). The automatic selection hypothesis is the advantage that a self-fertilising plant has for passing on two sets of genes to offspring, whereas only one set of genes are transferred in outcrossing (Fisher 1941, Schoen et al. 1996, Busch and Delph 2012). This strong selection leads to fixation of genotypes that are completely autogamous, unless there is a counteracting selective force such as inbreeding depression (Fisher 1941). The transmission advantage hypothesis is a special case of reproductive assurance, and hence it is applicable less often in natural populations (Busch and Delph 2012). There are very few, if any (Mazer et al. 2004) empirical studies supporting this hypothesis. Stone et al. (2014) found that the automatic transmission advantage strongly favours self-compatible plants of *Witheringia solanacea* compared to self-incompatible plants, even in the face of high inbreeding depression, because an increase in self-fertilisation does

not decrease siring offspring. Further investigation into the breeding system of *P. australis* may reveal evolutionary intricacies of the species.

## 4.5 Conclusions

Despite the occurrence of small and isolated populations of *P. australis*, the species is not inbred; however there is strong evidence of clonal populations. This study has shown that *P. australis* possesses a mixed mating system; self-pollination contributes to the clonality of populations. Individuals with high heterozygosity may clone more than more homozygous plants within the populations which may counteract the low diversity and a small array of genotypes as a result of founding events (Chapter 3). Despite the potential for fewer seed to be produced in the smaller fruit of the spontaneous self-pollinated plants, the offspring of heterozygous adults spread the existing genotypes within populations. If 'outbred' populations and individuals (those heterozygous individuals) produce greater proportions of fruit set there is potential to further perpetuate a heterozygous genotype and vigour. Conservation of small and large populations in the wild is therefore ideal in order to preserve the ability of the species to continue to naturally evolve. For restoration purposes, avoiding sourcing propagation material and in particular seed from one plant or population alone will decrease the chances that clonal plants are sourced, thereby increasing the chances that genetic diversity will be captured in ex situ populations.

# Chapter 5: Population dynamics and potential climate change impacts on the survival of the endangered orchid *Phaius australis*

## 5.1 Introduction

Precipitation patterns have changed, average global temperature has risen by 0.74°C, and global sea level has risen by 0.19 m over the last 100 years (IPCC 2013b). Future climate change is increasingly recognised as one of the greatest global threats to biodiversity because species distribution on medium spatial scales is primarily controlled by climate (Hilbert et al. 2007, Kingsford et al. 2009, Garcia et al. 2014). In Australia, average temperatures are projected to increase by 1-5°C by 2100 with a higher frequency and longer duration of heat waves (IPCC 2013b, CSIRO and BOM 2014, Reisinger et al. 2014). Rainfall is predicted to decrease by 30% in parts of eastern Australian but increase by 20%in northern Australia with droughts expected to become more frequent (IPCC 2013a, CSIRO and BOM 2014). Sea level around the Australian coastline is predicted to rise 0.28-0.98m by 2100 (IPCC 2013a, CSIRO and BOM 2014). To persist in a changed climate, species may have to adapt genetically or respond plastically to new climatic conditions in situ, or migrate to track an optimal 'climatic niche' through dispersal, or any combination of all three (Bradshaw and McNeilly 1991, Thomas et al. 2004, Parmesan 2006, Gienapp et al. 2008, Bellard et al. 2012).

While high levels of genetic diversity or a distribution of genetic diversity across a species climatic range, combined with strong directional selection pressures may allow selection and adaptation to take place, climate change is expected to be variable in the short-term (Bradshaw and McNeilly 1991, Jump et al. 2009). Thus adaptive mechanisms may take generations to take place, far too slow for long-lived, sessile organisms to keep pace with changes (Bradshaw and McNeilly 1991, Jump et al. 2009, Benito Garzón et al. 2011, Hoffmann and Sgró 2011).

Range shifts of species to more suitable climate or habitat in different latitudes, altitudes or habitat in response to recent climate change have been well documented globally (Walther et al. 2005, Kelly and Goulden 2008, Catling and Oldham 2011, Saintilan et al. 2014, Lenoir and Svenning 2015). Despite some documented shifts, the velocity of movement required by species to track a climatic niche may be much faster than previously thought, exceeding the generation time for long lived stationary species, such as perennial flora (Corlett and Westcott 2013, Vanderwal et al. 2013). An increasing volume of literature suggests that range shifts may be less characterised by a leading-edge expansion and a trailing-edge retraction on a wide

scale and instead be more obvious on a local scale through differing performance of a species in local habitats (Sletvold et al. 2013, Lenoir and Svenning 2015).

If flora begin to migrate to track a preferred climate, immediate observable responses include shifts in phenology (e.g. timing of flowering and fruiting), such as the earlier season of flowering and spring phenology events of trees reported as occurring in some parts of the world (Menzel et al. 2006, Root and Schneider 2006). Plant reproduction systems may be likewise strongly influenced by rising temperatures, or drought conditions as the climate changes, resulting in lower fecundity (Jump and Peñuelas 2005, Hedhly et al. 2009). A greater risk of extinction may face species if successful seed set is reduced due to interrupted plant-pollinator interactions because of phenological shifts in flowering plants (Cayan et al. 2001, Jump and Peñuelas 2005, Parmesan 2006, Bellard et al. 2012).

Species occurring over large geographical areas may have populations with uneven ecological advantages due to the difference in abiotic conditions (*e.g.* light, moisture and temperature) across a wide geographical range. This can result in an abundance at the centre of the distribution or where conditions are most optimal, and abundance declining towards the edges (Hengeveld and Haeck 1982, Brown et al. 1996, Sagarin and Gaines 2002, Jump and Woodward 2003). For example, Hidas et al. (2013) found a reduced number of *Tesseropora rosea* recruits at the southern (poleward, cooler) edge of the distribution in Australia, limiting colonisation further south. Orchids may exhibit uneven populations within the landscape due to ecological specialisation to abiotic conditions or symbiosis with fungal mycorrhizae essential to parts of a species' lifecycle (Arditti 1992, Otero and Flanagan 2006, Swarts et al. 2010). Hence, because temperature and moisture availability are critical in seed germination and seedling survival, plant recruitment dynamics may also be impacted by climate change (Woodward and Williams 1987, Arditti 1992, Winkler et al. 2009, Walck et al. 2011). When conditions change and become less than optimal in parts of a current distribution, a species may undergo a reduction in population size resulting in eventual local extinction (Lenoir et al. 2008, Thomson and Parker 2008, IPCC 2013b). This may be intermediately observed as alterations in recruitment and reproduction patterns, changes in spatial structure, and a decrease in population growth, regeneration or density in areas where climate becomes less suitable for survival (Barry et al. 1995, Jiguet et al. 2010, Dolanc et al. 2013). Conversely, increased density or floral outputs may be observed in parts of a species range where climate becomes more climatically suited for survival and reproduction of a species (Jiguet et al. 2010, Dolanc et al. 2013). Plant species that have supressed reproductive or growth activity where higher latitudes and altitudes limit

their distribution compared to their counterparts at lower latitude or altitude, may benefit from increasing temperatures if they have the ability to expand their numbers (Pearson and Dawson 2003, Jump and Peñuelas 2005, Murphy et al. 2010). Understanding the fundamental biological traits of threatened species such as population structure, survivorship and fecundity is important for assessing how well it is currently surviving and regenerating in a particular habitat (IUCN 1998, Keith 2000, Vallee et al. 2004). This becomes particularly important when population size is small and extinction risk is increased through susceptibility to intrinsic and extrinsic genetic, demographic and environmental stochastic factors (Lande 1993, Young and Clarke 2000, Oostermeijer et al. 2003, Lienert 2004, Broadhurst and Young 2007, Newman et al. 2013). Minimum viable population size (MVP) is an estimated abundance threshold below which a population will have an unacceptable risk of extirpation (Shaffer 1981, Soulé 1987, Traill et al. 2007). MVP estimates range from 50 to thousands of individuals depending on the species and are commonly used for conservation assessments at a global or national level (Reed and Frankham 2003, Traill et al. 2007, Traill et al. 2010, IUCN 2011).

Stage or age-structured population matrices and population viability analysis (PVA) are systematic approaches used in population ecology to identify the life-history processes of populations that may increase the risk of extinction (Caswell 2001, Akçakaya 2004, Keith et al. 2008). PVA models that utilise species-specific biological and ecological data can be highly effective at showing declines in populations that occur in different locations providing a rapid assessment of priority areas requiring management action or enhancement for survival (Menges 2000, Crone et al. 2011, Knight 2012). Beyond assessing vulnerability to extinction, PVA can be used to identify MVP as well as crucial viability points within a plant's lifecycle at which it has highest vulnerability (Burgman and Lamont 1992, Tremblay et al. 2009, Raghu et al. 2013, Renton et al. 2013). PVA is often recommended in recovery plans for endangered species but it is a method that is still underutilised or not implemented for all species (Lindenmayer et al. 2003, Auld et al. 2007, Driscoll et al. 2010, Crone et al. 2011).

PVA has also been successful at identifying populations along a species distribution that are exhibiting current growth or decline in the context of climate change (Maschinski et al. 2006, Knight 2012, Watts et al. 2013). Simmons et al. (2012) investigated populations of an endangered palm for changes in growth rates at different elevation gradients in response to different climatic conditions. In this way PVA can be used to identify habitats of optimal climatic conditions for growth and prioritise locations for reintroductions or augmentations in the future (Thomas 2011, Knight 2012, Maschinski et al. 2012a).

Matrix modelling has also been used to quantify how critical the environmental drivers of vital rates are to population growth and then identify the critical point(s) within the lifecycle that have the highest vulnerability to climate (Davison et al. 2010, Toräng et al. 2010, Knight 2012, Renton et al. 2013). The most effective use of PVA is for evaluating or ranking the responses, extinction risks and decline, due to different scenarios (Fieberg and Ellner 2001, McCarthy and Cary 2002, Akçakaya 2004, Keith 2004). Thus, with knowledge of how current climate affects vital rates, predictions can be made on how future changes may affect a species compared to current viability (Andrello et al. 2012, Molano-Flores and Bell 2012, Swab et al. 2015). Detailed evaluations such as these are required in order to understand if species are pre-adapted to climatic changes, to gauge if a response to climate change is already occurring and accordingly, inform conservation strategies (Williams et al. 2008, Lindenmayer et al. 2010).

In Australia, human population expansion in northern New South Wales and south-east Queensland has resulted in high biodiversity value heaths, forested wetlands, swamps and littoral rainforests being reduced to fragments of their pre-European settlement size (Groves 1994, Shoo et al. 2014, DE 2015a). Coastal littoral rainforests and wetlands are among the most vulnerable plant communities in Australia to climate change (DECCW 2010, Laurance et al. 2011b, Traill et al. 2011). This is partially due to the stresses that fragmentation places on these communities and the vulnerability of the specific hydrological regimes that maintain these ecosystems, sea level rise, and the increases in extreme weather events that may impact such low lying coastal areas (Hilbert et al. 2007, DECCW 2010).

The swamp orchid (Orchidaceae) *Phaius australis* F.Muell. has a disjunct distribution across a climatic (latitude) gradient of 2 000 km along the east coast of Australia, from north coast New South Wales to north Queensland (Benwell 1994, Jones 2006; Chapter 2). While the majority of populations occur in coastal wetlands, there are some populations confined to inland springs or soaks fed by groundwater. *Phaius australis* occurs is listed as 'Endangered' under the federal *Environment Protection and Biodiversity Conservation Act 1999* due to illegal collection, habitat loss, invasive species, and geographic distribution in fragmented locations (Chapter 2; Jones 2006, DE 2015a). Small population sizes in isolated fragmented remnants are documented for the species which is thought to have lost 95% of its populations since European settlement (Benwell 1994, DE 2015a; Chapter 2). Orchids can be acutely susceptible to changes in ecosystems affecting the ability of seedlings to germinate and survive to adulthood (Dixon et al. 2003, Swarts and Dixon 2009). Hence, populations of *P. australis* are

vulnerable to the changes in climate that may affect the soil water moisture or the salinity in low lying wetland and spring habitats (Dixon et al. 2003, Swarts and Dixon 2009).

*Phaius australis* has a sympodial growth pattern whereby seeds germinate into small seedlings that grow into larger plants with an active growing shoot that forms a pseudobulb over time (Jones 2006, Freeman et al. 2011). Upon flowering the active shoot with inflorescence dies, reduced to an inactive pseudobulb, with a new shoot. Inactive pseudobulbs remain attached to the plant with growth continuing on new shoots that eventually form pseudobulbs and flower. Once a plant has the energy storage of several inactive pseudobulbs, more than one active pseudobulb can form with multiple growing directions with increased inflorescence production. Plants can occasionally produce more than one inflorescence per active pseudobulb. Inflorescence stalks can be over two metres tall; four to twenty flowers are produced in August-October (Jones 2006, Freeman et al. 2011). Flowers are 7-10 cm across but can be as large as 17 cm, opening one or two at a time (Dockrill 1992, Jones 2006).

Unlike many orchids which are triggered by minimum temperature cues, flower initiation for *Phaius australis* occurs with decreased day length (Arditti 1992) in winter (June, July, August) and thus flower initiation and phenology is unlikely to be affected as seasonal temperatures becomes more variable. The flowering season is from late August in North Queensland (North), early to mid-September in South East Queensland (Mid) to early October in inland Queensland, and mid to late October in New South Wales (Benwell 1994, Jones 2006; pers. observations). Fleshy seed capsules of *P. australis* develop during late spring and summer (October, November, December, January) and are fully ripe after 120-150 days, splitting to release seed in late December, January and February depending on location (pers. observation; Chapter 4). While abundantly produced, orchid seeds are minute (<1mm) and lack a protective endosperm reducing the longevity of viable seed. Therefore, the timeframe for *P. australis* seed germination is a relatively short 3 month timeframe (January, February, March), with seed germination rates for the closely related *P. tankervilleae* found to drop to less than 5% after a year of storage at 25°C (Hirano et al. 2009). This suggests that the precipitation and warm temperatures at the end of the Australian summer (late January, February and March) are important for seed germination and seedling development as well as the vegetative growth phase of adult *P. australis* plants.

Theory predicts that species are adapted physically to local climatic conditions and orchids often have a high specificity to their habitat due to mycorrhiza fungi required for plant growth thought to contribute to the persistence in small, hyper-dispersed populations (Otero and

Flanagan 2006, Phillips et al. 2011). Genetic analysis of *P. australis* undertaken as part of this study showed no genetic structuring in different parts of the species range and therefore there may be minimal genetic selection for specific climates (Chapter 3). However, some morphological variation in *P. australis* has been informally documented across the range, which could be attributed to habitat specificity (Benwell 1994). Future climate change may be of benefit for creating new climatically ideal habitat for *P. australis*, given the genetic evidence of historic colonisation of the species poleward from the tropics (Chapter 3; Jump and Peñuelas 2005).

This study asks if there are differences in the reproductive abundance, reproductive success and population structure across the climatic range of *P. australis*. Specifically, it will examine differences in population growth rate and extinction risk across 1) the climatic range of the species from north Queensland to New South Wales and 2) between populations of small, medium and large sizes. The study also questions if *P. australis* tracking a southwards climatic niche. Is there evidence of a range retraction at the northern edge of the species range or evidence of expansion occurring at the southern edge of the species range? Biologically relevant climate variables will be identified, then incorporated into the PVA models to test the effect climate change may have on the population growth and extinction risk of *P. australis* across a climatic range and between populations of different sizes. The results will be interpreted in terms of conservation management implications and identifying key locations for population enhancements.

## 5.1 Methods

*Field survey methods*

Thirty-three *P. australis* populations were surveyed for demographic and population structure from across the species distribution on the east coast of Australia range from Rossville, North Queensland to South West Rocks in New South Wales (1800km; Figure 5.1). Sites were selected in a stratified design to achieve replication of populations across four biogeographic regions size and replication within regions of small (less than 50 individuals) or large (greater than or equal to 50 individuals) categories. Sampling was thus undertaken at five sites in the North Queensland (North), twelve sites in the middle of the range (Mid), nine sites between

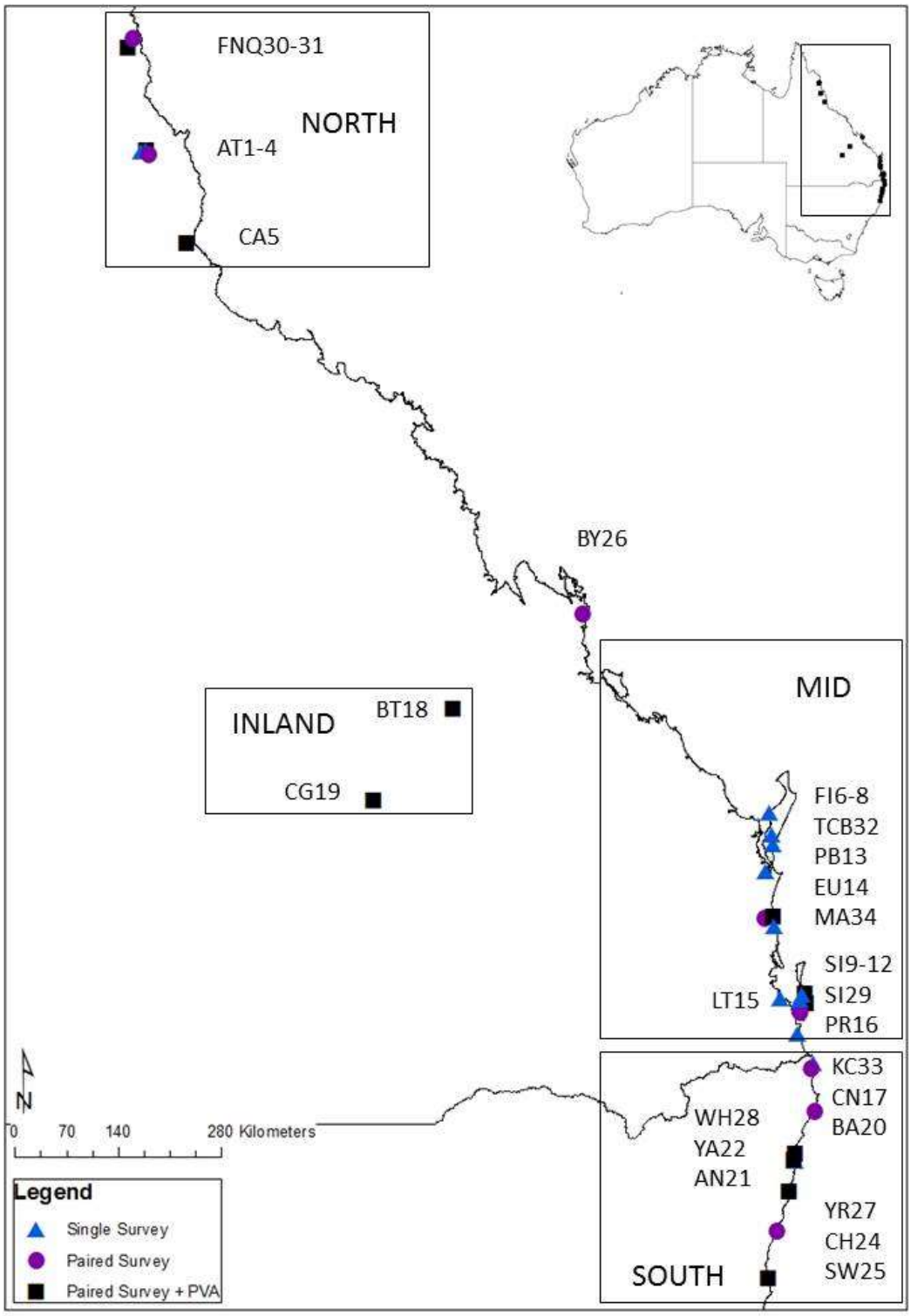


Figure 5.1. Map overview of the distribution of *P. australis* on the east coast of Australia, showing the locations of single survey, multiple population demographic surveys and populations used in PVA across four regions (North, Mid, South and Inland).

the Tweed and Hastings Rivers in New South Wales and at two inland sites (Figure 5.1). Replication was reduced for the inland region as only two populations are known.

Populations were sampled on foot during the flowering and fruiting season between August 2012 and January 2013 to aid identification of plants. Populations were located in the field utilizing a combination of GPS coordinates from historic records, maps, verbal descriptions or local guides. Searches were undertaken by systematically surveying the entire area of potential habitat in a series of continuous parallel belt transects 10 m wide (Cropper 1993). All *Phaius* plants were documented across the geographic range of a site with geographic extent of populations recorded with a GPS. At all locations, the starting location and elevation (metres above sea level, m asl) of each population/site was recorded using the GPS. The relative location of each individual plant within the site was mapped using compass direction (degrees) and distance (metres) between clumps and plants measured with a LaserDisto measure (Leica geosystems). These were later converted using Cosine and Sine trigonometry to XY coordinates in metres based on the start point for later spatial distribution analyses.

All *Phaius* plants were assessed across the geographic range of a site. In sparse, smaller populations, the location of each individual was marked with a GPS. For more extensive populations, each individual was recorded through the best representation of the population up to 90 individuals. Beyond 90 individuals, the number of plants, average height and number of inflorescences were recorded for the clump of plants.

The complex sympodial growth pattern of *P. australis* was simplified to two juvenile size classes and eight adult size classes and each individual was recorded according to: J1, no pseudobulb formed <0.1 m; J2, no pseudobulb formed >0.1 m; A1, one active pseudobulb, no sign of previous or current flowering; A2, one active pseudobulb, previous or current flowering; A3, one inactive pseudobulb with one active pseudobulb with no sign of previous or current flowering; A4, one inactive pseudobulb with one active pseudobulb, previous or current flowering; A5, two inactive pseudobulb with one active pseudobulb with no sign of previous or current flowering; A6, two inactive pseudobulbs with one active pseudobulb, previous or current flowering; A7, three inactive pseudobulbs with one or more active pseudobulb with no sign of previous or current flowering; A8, three inactive pseudobulbs with one or more active pseudobulb with previous or current flowering.

Each individual plant was classified as either too small for reproduction (size classes J1, J2, A1) or of reproductive age size classes (A2-A8). Active reproduction was recorded as the number of

inflorescences on each plant and floral output as the number of flowers per inflorescence. Populations were revisited three months after initial surveys and fruit set was recorded for each inflorescence within the population. The number of flower scars and the number of fruiting bodies (capsules) were recorded for each inflorescence, graded as healthy, unhealthy, split or dead. Healthy capsules were swollen, green and full; unhealthy capsules were wrinkled, shrivelled, not full, showing signs of insect attack, light green to yellow; and dead capsules were shrivelled brown/black capsules not mature size.

A subset of the large and small populations within regions were selected under a stratified sampling design and surveyed one year later (August to October 2013; Figure 5.1) utilizing the same methods to provide paired baseline stage transition and fecundity data for the population viability analysis data. Fruit set was not recorded in the second year due to time limitations and was therefore assumed to be equivalent to the previous year. Paired sites comprised three large and two small populations in the North, two large and two small populations in the Mid, two large and five small populations in the South and one large and one small population in the Inland regions (Table 5.1; Figure 5.1). Recent disturbances such as fire, animal herbivory and human impacts were recorded.

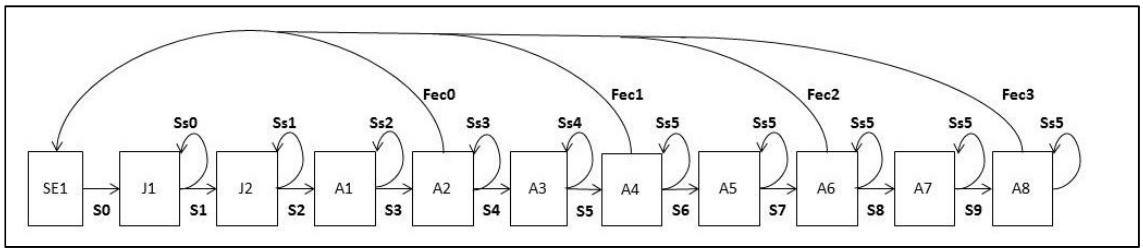


Figure 5.2. Life-cycle diagram for *P. australis*, where, $Fec_i$, fecundity rates; $Ss_i$, stage-specific survivorship and $S_i$, stage transition survival rates. J1, no pseudobulb formed <0.1 m; J2, no pseudobulb formed >0.1 m; A1, one active pseudobulb, no sign of previous or current flowering; A2, one active pseudobulb, previous or current flowering; A3, one inactive pseudobulb with one active pseudobulb with no sign of previous or current flowering; A4, one inactive pseudobulb with one active pseudobulb, previous or current flowering; A5, two inactive pseudobulb with one active pseudobulb with no sign of previous or current flowering; A6, two inactive pseudobulbs with one active pseudobulb, previous or current flowering; A7, three inactive pseudobulbs with one or more active pseudobulb with no sign of previous or current flowering; A8, three inactive pseudobulbs with one or more active pseudobulb with previous or current flowering.

*Population demographic analysis*

Population GPS points were mapped on ArcGIS (ESRI, version 10.2), with latitude and longitude in UTM extracted for later analyses. The area of occurrence (*Ar*) in $m^2$ was derived utilising boundary GPS coordinates for each population and the number of individuals in each population (*N*) recorded. Potential patch size ($P_P$) was calculated from field data, field observations and landscape level dynamics estimated using Google Earth converted from $m^2$ to hectares (ha). Population distance-to-nearest neighbour (*pNN*) in kilometres (km), population isolation ranking (*IR*) and population density measurements (*D*) per hectare (Ha) were extracted from GPS coordinates of populations following the methods detailed in Chapter 3. Carrying capacity (*K*) for each population later used in PVA was calculated by multiplying maximum observed density by $P_P$.

Size-class data were plotted as histograms to visualise a snapshot of information on population structure. Plots were examined following the methods of Oostermeijer et al. (1994) and Kéry and Gregg (2003) where populations were classified into three different population types: (a) 'dynamic' populations, characterized by higher densities of seedlings and juveniles relative to the adult ages states; (b) 'stable' populations with adult age stages prevailing, but with low densities of seedling and juveniles; and (c) 'senile' populations, consisting of only adult flowering and adult vegetative individuals.

For each population, the proportion of plants of reproductive age (*%R*), the proportion of the reproductive aged plants that were flowering (*%F*), the number of inflorescences per plant and the number of flowers per inflorescence were calculated for each year and averaged across both years. Flower scars and fruiting bodies (pods) were converted to the proportion of fruit set per inflorescence and the average proportion of fruit set per inflorescence *(%S*) per population was calculated. Population averages were calculated for the number of inflorescences per plant (*Inf*) and the number of flowers per inflorescence (*F/Inf*) over both years.

*Bioclimatic variables*

The major determinants of plant population growth include the reproductive processes of flower initiation, seed development, seed germination, seedling development and seedling survival, all of which are expected to be affected by climate (Arditti 1992, Jump and Peñuelas 2005, Menzel et al. 2006). *Phaius australis* and closely related tropical congener *P.*

*tankervilleae* are known to favour wet environments with higher temperature and are not cold resistant (Su 2000, Jones 2006) thus soil moisture levels and water availability through precipitation and groundwater is presumed to be a strong determinant for seedling survival of *P. australis* (Benwell 1994, Jones 2006). Therefore baseline bioclimatic layers affecting soil moisture including temperature and precipitation at time of seed germination and seedling and adult growth were investigated in this study.

Baseline bioclimatic layers for the study area (Queensland and New South Wales) were generated using SimClim 2013 (CLIMsystems 2013). SimCLIM is a computer-based, fine-resolution climate database and modelling system that can be used to examine the effects of climate variability and change over time and space (Warrick 2009; CLIMsystems 2013). In the programme, observed monthly temperature and precipitation that is sourced from the Australian Bureau of Meteorology (BoM) is averaged over a 30 year period to 2010 and interpolated to 0.25 lat/long grid for baseline surface predictions (CLIMsystems 2013). The baseline dataset was downscaled using the standard statistical downscaling methods supplied in SimClim 2013 (CLIMsystems 2013). Additionally, twelve temperature i-Buttons (Thermochron) were deployed at a sub-sample of sites across the species distribution to gather data about site conditions over a 12 month period in order to compare accuracy with the data gathered from the BoM.

Three sets of climatic variables most crucial for reproduction (seed capsule survival, establishment and growth) and therefore population growth were selected for *P. australis*: (1) average maximum temperature (°C) of three hottest months (December, January, February; $T_{MAX}$), (2) average precipitation (mm) of the wet months (January, February, March; $Pr_{JFM}$) both of which coincide with the three months of seed release across the species range; and (3) average precipitation of the driest quarter (June, July, August at all sites; $Pr_{JJA}$), which coincides with flower initiation. A baseline ASCII image was imported into ArcGIS (ESRI, version 10.2) and climatic data for each variable was extracted for each of the paired populations and tabled.

Spearman's rank correlation tests were undertaken to test the strength of the relationship between the climatic variables $T_{MAX}$, $Pr_{JJA}$, $Pr_{JFM}$ at each population and the *P. australis* fecundity values (the number of inflorescences per plant (*Inf*), the number of flowers per inflorescence (*F/Inf*), percentage fruit set (*%S*) for each population) and the proportion of recruits per population (J1, J2). Where correlations were found to be significant, linear regression was performed to describe the relationship. Regressions were later used in calculations of transition values in the climate change PVAs.

*Climate surface analysis to model future climate change*

Major climatic statistics based on the emission scenarios from the Intergovernmental Panel on Climate Change (IPCC) Fifth Assessment Report (AR5; IPCC 2013a) were utilised to predict the effect of climate change on the population dynamics of *P. australis*. Future climate change projections were developed for 2010, 2030, 2050 and 2070 with elections of global circulation models (GCM) and AR5 IPCC emission scenarios (IPCC 2013a) were undertaken for the study area using SimClim 2013 (CLIMsystems 2013). A linked-model approach was used for developing climate change scenarios in SimClim and projections were downscaled using the standard statistical method (CLIMsystems 2013).

An ensemble of four GCM models was used to develop future climate surfaces. These included MIROC 5.0 (Center for Climate System Research, Japan), CSIRO mk3.6 (Commonwealth Scientific and Industrial Research Organisation, Mark 3.6 Climate Model, Australia), GFDL CM3 (Geophysical Fluid Dynamics Laboratory, USA) and MIUB ECHO-G (Meteorological Institute of the University of Bonn, Germany. These models have been considered most suitable for assessing temperature and precipitation climate change in the Australian region (Perkins et al. 2007, Smith and Chandler 2010, Perkins et al. 2014). An ensemble of GCM models that are selected for the region and purpose reduces uncertainties and clarifies the nature and scale of the climate change projections (Beaumont et al. 2008, Beaumont et al. 2009, Perkins and Pitman 2009). Downscaling of these models for regional simulations such as those used in this study has been found as at least as good or better as GCMs utilised on the continental scale (Perkins et al. 2014).

Two emission scenarios (Representative Concentration Pathways, RCP's) from the IPCC AR5 (IPCC 2013a) were evaluated using the GCM ensemble: RCP 2.6 and RCP 8.5. The RCP2.6 represents a greenhouse gas concentration trajectory that peaks in 2010-2020, with emissions declining substantially thereafter; while in RCP 8.5 greenhouse gas emissions continue to rise throughout the 21$^{st}$ century (IPCC 2013a). Low climate sensitivity was utilised in the RCP 2.6 projections and high climate sensitivity was utilised in the RCP 8.5 projections (CLIMsystems 2013). To explore the range of uncertainty in projections of future climate surfaces, sensitivity analyses were conducted for each climate variable by generating predictions for the 5$^{th}$ percentile, median and 95$^{th}$ percentile climate values so that the convergence of future climate simulation by different GCM models for a given emissions scenario could be assessed.

Climate surface analysis was undertaken in SimClim 2013 (CLIMsystems 2013) for each simulation scenario (RCP 2.6, RCP 8.5) for each climatic variables (1) average maximum

temperature (°C) of three hottest months (December, January, February; $T_{MAX}$), (2) average precipitation (mm) of the wet months (January, February, March; $Pr_{JFM}$); and (3) average precipitation of the driest quarter (June, July, August at all sites; $Pr_{JJA}$) at 2010 (closest current) and for each 10 year time step to 2070 and saved as an ASCII file in SimClim 2013 (CLIMsystems 2013).

Each simulation scenario (RCP 2.6, RCP 8.5), and climatic variable output were imported into ArcGIS (ESRI, version 10.2) as a raster file, clipped to define the same spatial extent of the study area, and presented as individual maps to assess changes in these variables over the projected time period under the different climate change scenarios in a spatial context. The 5$^{th}$, median and 90$^{th}$ percentiles of each climatic variable were also presented to evaluate GCM model sensitivity. The predicted climatic variables at each time step under each scenario were then extracted for each currently known *P. australis* site using the species population occurrence points and plotted separately to show potential trends and fluctuations of these variables under different climate change scenarios.

Tabled values for each climatic variable derived from SimClim 2013 (CLIMsystems 2013) for the year 2010 were compared for accuracy against the current climatic averages derived from the closest Australian Bureau of Meteorology (BOM) station. The difference in maximum temperature (°C) for climatic variable 1 and the differences in precipitation (mm) for climatic variables 2 and 3 were calculated for each time step compared to current (2010) were calculated and tabled for each time step for each climate change scenario.

*Modelling and population viability analysis*

Based on field observations, data obtained from this study, previously published data and data obtained from orchid growers, a life-history model was developed for *P. australis* (Figure 5.2). *P. australis* produces sexually by seeds and asexually by continued growth of pseudobulbs; hence the ramet is utilised as the demographic unit in this study and is used interchangeably with 'plant'. A total of 11 stages were integrated into the model, including the two juvenile and eight adult stages utilised in population structure investigations and a seed stage in order to take into account the effects that future climate change may have on seedling germination. The numerous juvenile (non-pseudobulb, non-reproductive, J1, J2), sub-adult (pseudobulb, non-reproductive, A1) and adult stages (pseudobulbs, reproductive, A2-A8), were necessary to facilitate sufficient transitions between life history stages in the matrix and differing fecundity

values in the model structure (Figure 5.2). The life-history of the species as parameterised assumes the following factors: (1) any mycorrhizal fungi species required for germination, growth and reproduction occur in empty available habitat and the presence of such fungi will not be affected by future climate change; (2) a plant does not make backwards transitions and (3) there was no dormancy.

A baseline stage-matrix was developed for use in a RAMAS GIS v5.0 (Applied Biomathematics 2005) simulation model, with the matrix transition based on the life-history model (Figure 5.2). A summary of the model parameters and their source and justification are presented in Appendix 3. The relevant fecundity transition ($Fec_i$), stage-specific survivorship ($Ss_i$) and stage transition survival rates ($S_i$) were calculated as follows (Figure 5.2):

Fecundity transitions ($Fec_i$) for reproductive adult stages represent the net, annual, viable contribution of each adult towards seed to be germinated in the seed stage (SD1; Figure 5.2). To overcome the assumption of similar reproduction across all years, the average fecundity values across 3 years of data collection were utilised where possible, with two years as a minimum. Estimates for $Fec_i$ were modified from Keith (2004) and Simmons (2012) using the formula $Fec_i$ = *RO-Inf* * *RO-F/Inf* * *%S* **nS*; where *RRO-Inf* is the relative output of inflorescences of each reproductive adult stage; *RRO-F/Inf* is the relative output of number of flowers per inflorescence of each reproductive adult stage, *%S* is proportion of fruit set per inflorescence and *nS* is the estimated number of seed per fruit. Relative output of inflorescences and flowers per inflorescence were calculated for each reproductive adult stage compared to the final adult stage (A8). This was because there were significantly greater number of inflorescences per plant (ANOVA, $f$ = $p$<0.05) and number of flowers per inflorescences (ANOVA, $f$ = p<0.05) for the final Adult stage (A8) compared to other reproductive stages (A2-A7). The estimated number of seed per fruit was calculated as the 90% of the average seed volume per fruit from Chapter 4 divided by calculated seed volume from Arditti (1992), which is approximately 212 509 seed per fruit. Fecundity transitions ($Fec_i$) were calculated for each individual population matrix (*%R*, *%F*, *Inf*, *F/Inf*, *%S*) to reflect the potential climatic and ecological effects of each location on fecundity (Chapter 4).

Calculation of the $Ss_i$ (stage specific survivorship) and $S_i$ (stage-transition survivorship) parameters was based on Caswell (2001) and Akçakaya (1999) using a Lefkovitch matrix constructed via a survivorship frequency table utilising the paired data available from all paired populations other than WH28. Paired data was excluded from large population (WH28) due to the 'harvesting' effect of feral animal activity reducing the accuracy of paired data. The

formula $S_i = n_{(t+1)} / n_{(t)}$ was used, where $n_t$ is the abundance of a given stage class at time *t*, and $n_{(t+1)}$ equals the abundance in a given stage class at time *t+1*. The only exception was the input value for $Ss_1$, and $S_1$ which is the probability of seeds in the seed bank stage surviving to stay seeds in the proceeding time-step ($Ss_1$) and the probability of seeds to germinate and transition into seedlings ($S_1$). The value for *Ss1* was estimated as $Ss_1$ = Seed bank x *v* x *g* where *v*, is the seed viability rate estimated for congener *P. tankervilleae* and *g*, is germination rates after storage at 25°C for 1 year according to Hirano (2009). The value for $S_1$ was estimated as $S_1$= (mean J1) / (Seed bank * *v*), where mean J1 was the average number of individuals in life-stage J1 over the study duration. Estimated values for $Ss_1$, and $S_1$ were adjusted following the outcomes of the sensitivity analysis and model validation.

Calculated values of the stage-matrix elements ($Fec_i$, $Ss_i$, $S_i$) were input into RAMAS GIS v5.0 (Applied Biomathematics 2005) to form the structure for a population viability analysis (PVA) model to investigate the impacts of future climate change on *P. australis* populations of three different starting population sizes, approximately 10 individuals, approximately 50 individuals and over 90 individuals, replicated across the four climatic regions of the species. A total of 12 populations were utilised in the PVA models; one population of each starting population size in the north (three total); one population of approximately 10 individuals and two populations with over 90 individuals in the mid region (three total); one population with over 90 individuals, one population of approximately 50 individuals and two populations of approximately 10 individuals in the South and one population each of approximately 50 individuals and over 90 individuals in the inland region (Table 5.1).

Additional paired populations were excluded for several reasons, small populations at Barney Springs and Rossville (AT4, FNQ30) in north Queensland were excluded as they had less than 5 individuals of which none were reproductive in both years surveyed. Four additional small populations were excluded in PVA's as during the timeframe of the study, due to reduced population size from human or environmental impacts (EU14, BA20, CH24) or uncertainty in bioclimatic region (CNR17).

The baseline stage structure-matrix was run with sex structure set to 'all individuals' (mixed) and the matrix standard deviation was set to ±10% to allow for uncertainties arising from measurement errors (Akçakaya and Atwood 1997, Akçakaya 2005). Constraints were activated in the model structure with all relevant survivorships and fecundity values indicated in Figure 5.2 set to 1 or 0 respectively to ensure the realism of the simulated survival rates (Akçakaya 2005). The demographic stochasticity function was enabled, which means that the survivors

were sampled from a Poisson distribution (Akçakaya 2005). The environmental stochasticity distribution was set to lognormal with a perfect within-population correlation upon fecundities, carrying capacities and survivorships. All time steps were included in the stochasticity risk calculations.

Initial abundances of juvenile and adult stages were input according to the field-based demographic data from 2012. Initial abundance for the seed bank stage was calculated based upon 2012 population size, fecundity across all reproductive stages. The seed bank stage was omitted from the final population sizes with all other stages included. Carrying capacities were set at very high values to mitigate the influence of inaccurate estimates on the outcome of the PVA as per Regan and Auld (2004). This was a reasonable assumption as field observations and data from this research suggest it is unlikely that *P. australis* would approach carrying capacity type abundance at any of the research populations (Regan and Auld 2004). Relative survival and fecundity were both set to a neutral rate of 1 for all populations. All stages except seed bank were included as contributors to population totals for the final model output. The seed bank stage was configured to have no basis for density dependence, with ceiling type density dependence selected to affect survival rates. Ceiling-type density dependence assumes that the vital rates (the stage matrix) are not affected by the effects of density until the population reaches the Ceiling level (*K*).

*Model validation and sensitivity analysis*

Sensitivity analysis was conducted on all parameters that were not calculated from the field data, including SS1, the initial seed bank abundance (Appendix 3). The relevant parameters were input into the baseline matrix at rates 10% above or below their original input values. The models were then run for a 100-year simulation duration with 1000 replicates at each time-step. For each input parameter tested, the percentage change in final population abundance that resulted from the 10% increase or decrease in input parameter magnitude was calculated (Appendix 4).

The model was validated using all the baseline settings and the input parameter with paired baseline data available from paired populations. The outcomes from these trials were compared to the paired baseline data to facilitate adjustment of the stage-matrix where necessary, particularly for parameters not calculated from field data. Validation of the model was necessary to ensure that the outputs from the model have real-world applicability and

reflected the historical outcomes from the baseline data. The outcomes of the sensitivity analysis were utilised during the validation of the model. The final stage matrix for current climate values, are presented in Appendix 2, with a summary of their sources presented in Appendix 3.

*Model simulation values*

The PVA model was used to examine the effects of climate change on the population dynamics and population viability of *P. australis*. The *P. australis* PVA models assumes that climate change does not affect the mycorrhizal fungus that is required for seed germination and growth or any other symbionts of other parts of the plants' life cycle (Clements 1988, Otero and Flanagan 2006, Rasmussen and Rasmussen 2009). While there is evidence for historical gene flow between *P. australis* populations reported in Chapter 2 and knowledge of rare, long distance dispersal of orchid seed from literature (Arditti and Ghani 2000, Molnár et al. 2011), the *P. australis* PVA models assume no dispersal between *P. australis* populations. This is in order to reduce the complexity of the scenario (Fieberg and Ellner 2001, Lindenmayer et al. 2003, Driscoll et al. 2010, Crone et al. 2011).

Model time-steps of an initial seven years (2013-2020), then six 10-year time-steps (2021-2080) were utilised with the climate change surface analyses for the 2013 to 2080 timeframe generated in SlimCLIM 2013 (CLIMsystems 2013). A final 33-year time-step was utilised to bring model duration to 100 years. The PVA model was run for the set time-step and the final stage abundances at the end of the model duration extracted. The stage matrix vital rates were recalculated to represent the effect of projected climate change on the vital rates of *P. australis*. PVA models were run for the duration of the next time-step based on the recalculated vital rates and using the final stage abundance values extracted from the previous time step. The PVA model was run with 1000 replicates at each time-step. Calculations of stage matrix vital rates to represent how projected climate change may affect population growth of *P. australis* were calculated as follows:

(1) Average maximum temperature (°C) of three hottest months (December, January, February; $T_{MAX}$) occurs at the same time as seed dispersal, therefore increased maximum temperature at this phenological time is predicted to reduce seed germination, growth and survival. The current warmest $T_{MAX}$ February is 33°C recorded at Carnarvon Gorge, which has a high seedling proportion. Therefore, an increased mortality of 10% was incorporated into the

calculations for transition values *S0*, *S1*, *S2* and stage specific survival values *Ss0* and *Ss1* for every 1°C rise in temperature over 33°C since the 2010 baseline climate. A decrease of mortality 10% was calculated for every 1°C rise in temperature since 2010 until predicted temperatures reached 33°C. This is because it is assumed in this model that the ideal growing conditions for *P. australis* are the warmer and humid conditions in the northern and oldest part of the species range population compared to the south (Chapter 2, 3).

(2) Average precipitation (mm) of the wet months (January, February, March; $Pr_{JFM}$): reduced precipitation at this phenological time is predicted to reduce the ability for seed to germinate, growth and survival. Orchid seeds require moisture to germinate; therefore an increased mortality of 5% was incorporated into the calculations for transition values *S1*, *S2* and stage specific survival values *Ss0* and *Ss1* for every 100 mm reduction of precipitation in the wet months ($Pr_{JFM}$) since 2010. For each time step, the change in the number of flowers per inflorescence (*F/Inf*) was recalculated based on a regression equation and the new value was input into the fecundity calculation $Fec_i$ = *RO-Inf* * *RO-F/Inf* * *%S* **nS* for the PVA model.

(3) Average precipitation of the driest quarter (June, July, August at all sites; $Pr_{JJA}$): For each time step, the change in the number of inflorescences per plant (*Inf*) was recalculated based on a regression equation and the new value was input into the fecundity calculation $Fec_i$ = *RO-Inf* * *RO-F/Inf* * *%S* **nS* for the PVA model.

*Data analysis of the PVA model outputs*

Quasi-extinction probability and population trajectory values were exported from RAMAS GIS v5.0 (Applied Biomathematics 2005) for each of the paired populations under each climate emissions simulation scenario and plotted to visualise outcomes over the 100-year model duration. The threshold for quasi-extinction was treated as zero because of the species' ability to self-pollinate and hence maintain a population with minimal numbers (Chapter 4). Cumulative quasi-extinction probability at the end of the model duration ($P_{QE}$) was recorded. Population sizes at 2012, number of adults (*ANi*), final population size in 2112 ($N_F$) and final number of adults ($N_Fa$) in 2012 were calculated for each population under each simulation scenario in order to examine PVA outcomes. A proportional population size change with respect to initial population size value (*ΔNpi*) was calculated for each population under each simulation scenario with the formula $\Delta Np = (N_n - N_i)/N_i$, where $N_i$ is the initial population size. The proportional change in adults in respect to initial population size value (*ΔNpa*) was

calculated for each population under each simulation scenario with the formula $\Delta Npa = (N_F a – aN_i)/aN_i$, where $aN_i$ is the initial number of adults.

As with the demographic data analysis, a Kruskal-Wallis test was used to examine any differences in proportional population size change (*ΔNpi*) and proportional change in adults (*ΔNpia*) between the climatic regions and between the initial population size grouping (small, medium, large), with ANOVA rank comparison used to identify differences between groups. Differences in *ΔNpi* and *ΔNpia* were tested between the climate scenarios (no change, RCP 2.6, RCP 8.5) with a Kruskal-Wallis test and ANOVA rank comparison. A Kruskal-Wallis test and ANOVA rank comparison were also used to test for differences in the resultant *ΔNpi* and *ΔNpia* of each climate scenarios (no change, RCP 2.6, RCP 8.5) due to initial population size (small, medium, large).

## 5.3 Results

*Demographic analysis*

Population sizes (*N*) ranged from two individuals at Moon Point (FI7) through to 696 at Flinders Beach (SI11) with plant density ranging from 1 to 5500 plants per hectare (Byfield BY26 and Kingscliff KC33 respectively; Table 5.1).

All three major patterns of size class distributions were identified in the population snapshots (Figure 5.3a-b). Five populations across the species range were classified as 'dynamic': the Walkamin population, Tin Can Bay, Lota, Yuraygir and Carnarvon Gorge (AT1, TCB32, LT15, YR27, CG19; Figure 5.3a-b). All dynamic populations were large in size, with at least 69 individuals (Table 5.1). The second inland population at Blackdown Tableland (BT18) was border line 'dynamic' (BT18; Figure 5.3b). Nine small populations in the north, mid and south regions were classified as 'senile', including Rossville (FNQ30, not graphed), Cardwell (CA5), Barney Springs (AT4), Byfield (BY27), two Fraser Island populations (FI7, FI8) and Ballina (BA20; Figure 5.3a-b). All remaining populations were 'stable' with a relatively even distribution of juvenile and adult plants, including Shipton's Flat (FNQ31), Marcoola (MA34), three Stradbroke Island populations (SI9, SI10, SI29), Cudgen (CN17) Woody Heads (WH28), Angourie (AN21), Coffs Harbour (CH24) and South West Rocks (SW25; Figure 5.3a-b). Populations in this study described as 'stable' and 'senile' have previously been found to have large heterozygote excess

Table 5.1. Summary of location, population demographic parameters for 33 *P. australis* populations; *Ar*, population area of occupancy (ha); *N*, population size in 2012, *D*, density of plants per hectare (ha); *pNN*, population first nearest neighbour distance (km); *PP*, potential population size in hectares; *K*, carrying capacity. Overall average given; Bold = repeat surveys, + PVA models.

| Region | Pop'n | Location | N | *Ar* (ha) | *D* (plants/ha) | *pNN* (km) | *PP (ha)* | *K* |
|---|---|---|---|---|---|---|---|---|
| North | **+ FNQ31** | Shipton's Flat | 59 | 0.015 | 3919.48 | 12.94 | 0.29 | 1137 |
| | FNQ30 | Rossville | 1 | 0.0001 | 1 | 12.94 | | |
| | **+ AT1** | Walkamin, Atherton Tableland | 252 | 0.432 | 583.41 | 0.3 | 1.13 | 659 |
| | AT2 | Walkamin, Atherton Tableland | 338 | 0.735 | 459.42 | 0.3 | | |
| | AT3 | Walkamin, Atherton Tableland | 591 | 1.081 | 547.31 | 6.49 | | |
| | **AT4** | Tolga, Atherton Tableland | 8 | 0.009 | 8.423 | 8.26 | 0.02 | 0 |
| | **+ CA5** | Cardwell | 4 | 0.154 | 25.97 | 128.92 | 7.10 | 184 |
| North mean | | | 209 | 0.40 | 924 | 26.2 | 2.14 | 495.20 |
| Mid | **BY26** | Byfield National Park | 3 | 0.0001 | 1 | 209.18 | | |
| | FI6 | Pile Valley, Fraser Island | 96 | 4.442 | 21.61 | 29.5 | | |
| | FI7 | Moon Point, Fraser Island | 2 | 0.787 | 2.54 | 13.65 | | |
| | **FI8** | Dilli Village, Fraser Island | 8 | 0.003 | 8 | 13.65 | | |
| | TCB32 | Tin Can Bay | 151 | 0.546 | 275.63 | 36.56 | | |
| | **+ PB13** | Peregian Beach | 5 | 0.046 | 109.58 | 11.08 | 0.39 | 43 |
| | **EU14** | Eumundi | 17 | 0.001 | 5 | 11.08 | 0.31 | 2 |
| | MA34 | Marcoola | 12 | 0.014 | 870.22 | 13.33 | | |
| | LT15 | Lota | 224 | 0.653 | 343.52 | 23.82 | | |
| | **+ SI9** | 18 Mile Swamp, Stradbroke Is | 330 | 1.549 | 213.03 | 2.68 | 2.18 | 464 |
| | **+ SI10** | Flinders Swamp, Stradbroke Is | 286 | 1.899 | 150.64 | 2.68 | 3.80 | 572 |
| | SI11 | Amity Swamp, Stradbroke Is | 696 | 3.743 | 185.81 | 7.44 | | |
| | SI12 | Myora, Stradbroke Is | 113 | 2.197 | 51.34 | 9.33 | | |
| | **SI29** | Canaipa Passage, Stradbroke Is | 110 | 0.146 | 752.04 | 15.65 | 0.32 | 241 |
| | PR16 | Pine Ridge | 136 | 0.473 | 287.34 | 28.18 | | |
| Mid mean | | | 146 | 1.10 | 218 | 28.52 | 1.40 | 264.30 |
| South | KC33 | Kingscliff | 22 | 0.004 | 5500 | 7.45 | | 0 |
| | **CNR17** | Cudgen | 17 | 0.123 | 137.71 | 7.45 | 0.25 | 34 |
| | **BA20** | Ballina | 6 | 0.008 | 717.31 | 57.61 | 2.32 | 1661 |
| | **+ WH28** | Woody Heads | 64 | 6.118 | 10.46 | 7.98 | 6.51 | 68 |
| | AN21 | Angourie | 170 | 2.007 | 84.71 | 1.68 | | 0 |
| | **+ YA22** | Yamba | 6 | 0.003 | 2400 | 1.68 | 0.09 | 216 |
| | **+ YR27** | Yuraygir | 369 | 0.369 | 997.6 | 42.38 | 0.71 | 708 |
| | **CH24** | Coffs Harbour | 12 | 0.01 | 1200 | 87.78 | | 0 |
| | **+ SW25** | South West Rocks | 21 | 0.02 | 1033.97 | 87.78 | 0.53 | 548 |
| South mean | | | 76 | 0.96 | 1342 | 33.53 | 1.73 | 539.26 |
| Inland | **BT18** | Blackdown Tableland | 219 | 0.116 | 1879.81 | 159.09 | 0.26 | 881 |
| | **CG19** | Carnarvon Gorge | 69 | 0.178 | 387.49 | 159.09 | 0.32 | 124 |
| Inland mean | | | 144 | 0.15 | 1133 | 159.09 | 0.29 | 302.61 |
| **Overall mean** | | | 138 | 0.87 | 724.1 | 13.76 | 1.6 | 420.2 |

greater than -0.5 (FNQ31, CA5, PB13, YA22, SW25; Figure 5.3a-b, Table 5.1; Chapter 4). There were no significant differences ($p$>0.05) in the proportion of individuals in any life stages (including juvenile) between the regions. Similarly, there was no significant correlations ($p$>0.05) between any life stages and the three climatic variable ($T_{MAX}$, $Pr_{JFM}$, $Pr_{JJA}$) or latitude.

Across all populations, 73% of all plants (1226 individuals) were of reproductive age (*%R*). The average proportion of reproductive aged individuals in populations (*%R*) was 65% but this was variable among populations ranging from 56.1% at Woody Heads (WH28) to 100% at several populations (AT1, BY26, FI7, FI8, KC33; Table 5.2). The species was reproductively active throughout the range with an overall average of 40.6% of reproductive aged plants flowering (*%F*). This ranged from no flowering individuals in any year at Barney Springs on the Atherton Tableland (AT4) to 100% at small populations in Eumundi and Kingscliff (EU14, KC33; Table 5.2). Across all populations, plants that were flowering produced an average of 1.3 inflorescences (*Inf*), with an average of 10.6 flowers on each inflorescence (*F/Inf*; Table 5.2). Some populations had flowering plants with an average of more than 2 inflorescences per plant (CG19, YR27, PB13, AT1, AT2) and two populations had inflorescence with an average of more than 20 flowers on them (*Fl/Inf*; Table 5.2). Average fruit set (*%S*) was 49.2% but highly variable and ranged from 0% at Coffs Harbour and South West Rocks to 92% at Yamba (YA22; Table 5.2).

There was a weak but significant negative correlation between decreasing dry season precipitation ($Pr_{JJA}$) in the baseline (2010) climate and the average number of flowers per inflorescence (*F/Inf*, *rho*=-0.077, $p$<0.05). However the regression equation was not significant ($t$=-0.397, $p$>0.05) so this attribute was not worked into PVA parameter adjustments. Increased precipitation in the wet season ($Pr_{JFM}$) of the 2010 climate was weak but significantly correlated with increased average number of flowers per inflorescence (*F/Inf*, *rho*=0.052, $p$<0.05); an indication that sites with greater wet season precipitation are more fecund in the output of flowers. The regression equation *F/Inf*=10.702 + (0.003 * $Pr_{JFM}$) was significant ($t$=3.290, $p$<0.01)

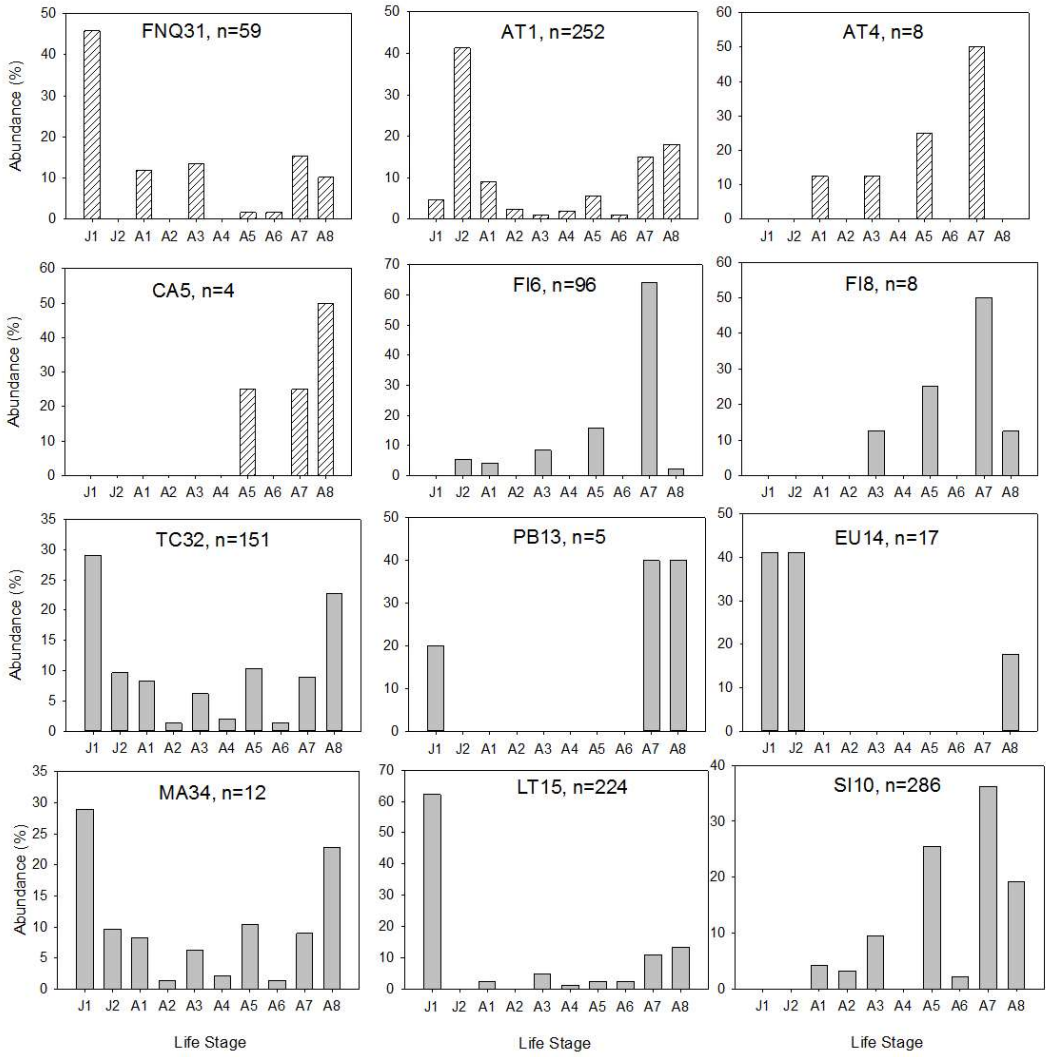


Figure 5.3.a) Stage-class distributions of *P. australis* populations that were surveyed over multiple years; population size in 2013 is given; populations FNQ30, BY26 and FI7 excluded due to very low population size (n). Bar fill indicates region: diagonal lines, north; light grey, mid.

In contrast to expectations, there was weak but significant correlation between decreasing dry season precipitation ($Pr_{JJA}$) at baseline (2010) climate with an increase in the number of inflorescences per plant (*Inf*; Spearman's *rho* = -0.251, $p<0.01$). This could be an indication that reproductive output may be triggered by drier winter conditions. The regression equation $Inf=1.953 + (-0.002 * Pr_{JJA})$ was significant ($t=-6.050$, $p<0.01$, r=0.194). For each time step, the change in the number of inflorescences per plant (*Inf*) was recalculated based on the climatic regression equation and the new value was input into the fecundity calculation $Fec_i$ = *RO-Inf* * *RO-F/Inf* * *%S* *nS* for the PVA model.

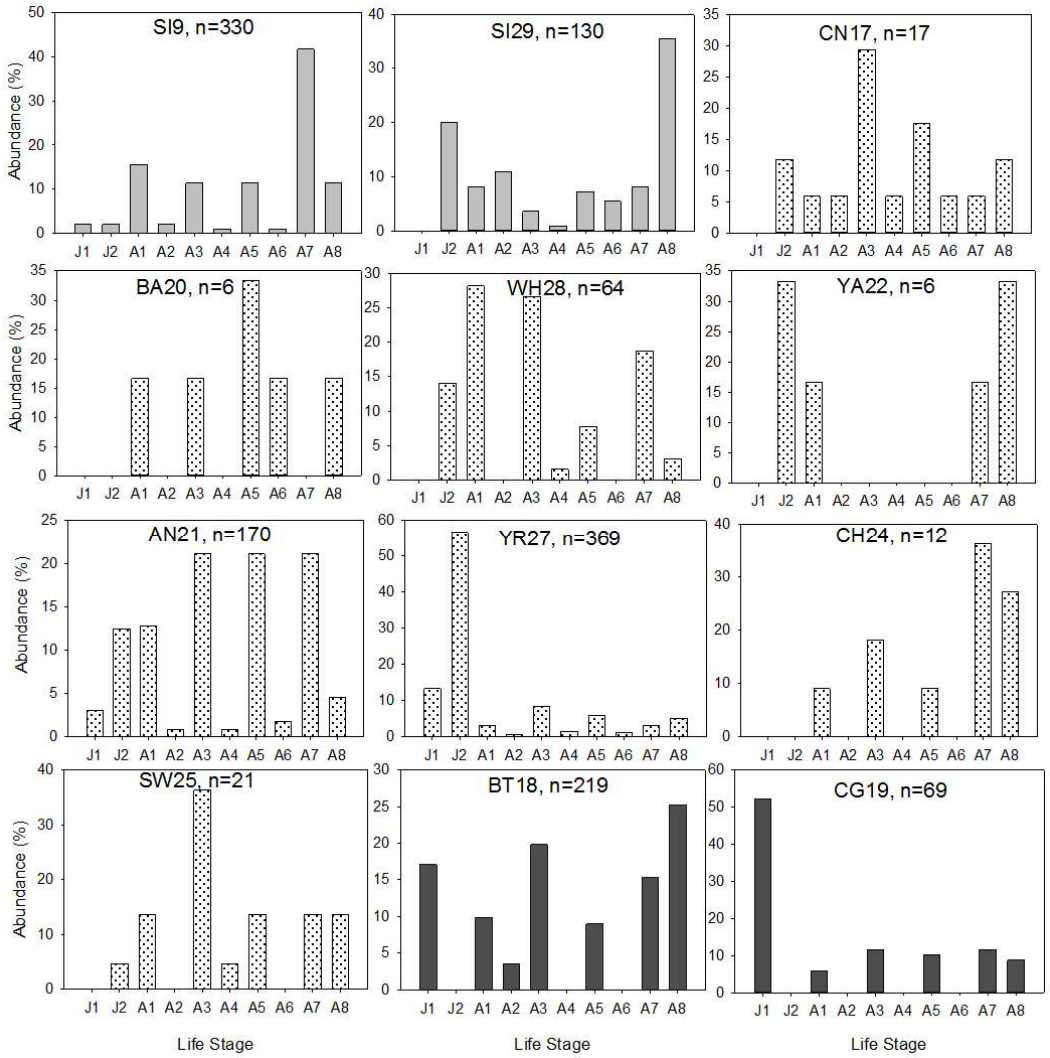


Figure 5.3.b) Stage-class distributions of *P. australis* populations that were surveyed over multiple years; population structure and size is given for 2012; populations FNQ30, BY26 and FI7 excluded due to very low population size (n). Bar fill indicates region: light grey, mid; dotted fill, south; dark grey, Inland.

Table 5.2. Population fecundity parameters for 33 *P. australis* populations across the species range, overall average given with standard deviation in parentheses; *%R*, proportion of plants of reproductive age; *%F*, the proportion of the reproductive aged plants that were flowering; *Inf*; average number of inflorescences per plant; *F/Inf*, number of flowers per inflorescence and *%S*, proportion of fruit set per inflorescence. Averages given, standard deviation in parentheses.

| Region | Population | N | *%R* | *%F* | *Inf* | *F/Inf* | *%S* |
|---|---|---|---|---|---|---|---|
| North | FNQ31 | 59 | 59.5 | 40.9 | 0.8 | 17.6 | 53.0 |
| | AT1 | 252 | 100.0 | 78.6 | 2.1 | 14.5 | 24.0 |
| | AT2 | 338 | 57.9 | - | 2.2 | 13.9 | 10.0 |
| | AT3 | 591 | - | - | 0.7 | 8.9 | 11.3 |
| | AT4 | 8 | 62.5 | 0 | 0.0 | - | - |
| | CA5 | 4 | 75.0 | 66.7 | 0.8 | 3.5 | - |
| **North mean** | | 209 (232) | 59.2 (33.0) | 51.5 (9.16) | 1.4 (1.1) | 9.7 (2.8) | 24.6 (20.0) |
| Mid | BY26 | 3 | 100.0 | 100.00 | 1.0 | 12.0 | |
| | FI6 | 96 | 82.8 | 5.2 | 1.3 | 10.3 | 24.0 |
| | FI7 | 2 | 100.0 | 100.0 | 1.0 | 7.5 | 44.0 |
| | FI8 | 8 | 100.0 | 12.5 | 1.0 | 3.5 | 59.0 |
| | TCB32 | 151 | 72.3 | 64.4 | 1.3 | 22.9 | 67.0 |
| | PB13 | 5 | 80.0 | 75.0 | 2.0 | 16.8 | 53.0 |
| | EU14 | 17 | 60.0 | 100.0 | 1.0 | 12.5 | .0 |
| | MA34 | 12 | 90.9 | 10.0 | 1.6 | 11.25 | 63.0 |
| | LT15 | 224 | 69.2 | 74.1 | 1.3 | 11.3 | 67.0 |
| | SI9 | 330 | 80.9 | 3.9 | 0.9 | 13.6 | 22.0 |
| | SI10 | 286 | 94.7 | 26.9 | 0.9 | 11.3 | 11.0 |
| | SI11 | 696 | - | - | 0.4 | 15.4 | - |
| | SI12 | 113 | - | - | 0.9 | 4.2 | - |
| | SI29 | 110 | 84.9 | 83.5 | 1.5 | 13.1 | 69.0 |
| | PR16 | 136 | - | - | 0.2 | 11.0 | - |
| **Mid mean** | | 146 (186) | 67.7 (36.9) | 52.84 (36.47) | 1.3 (0.4) | 9.4 (3.6) | 30.4 (36.9) |
| South | KC33 | 22 | 100.0 | 100 | 1.1 | 13.5 | 47.0 |
| | CNR17 | 17 | 82.4 | 85.7 | 1.9 | 14.1 | 62.0 |
| | BA20 | 6 | 83.3 | 30.0 | 1.2 | 4.5 | - |
| | WH28 | 64 | 56.1 | 15.6 | 1.0 | 6.5 | - |
| | AN21 | 170 | 71.6 | 19.2 | 1.2 | 7.2 | - |
| | YA22 | 6 | 60.0 | 66.7 | 1.2 | 11.2 | 92.0 |
| | YR27 | 369 | 64.6 | 67.9 | 1.7 | 10.9 | 12.0 |
| | CH24 | 12 | 81.8 | 55.6 | 1.0 | 10.9 | 0 |
| | SW25 | 21 | 63.6 | 42.9 | 1.2 | 9.0 | 0.0 |
| **South mean** | | 76 (122) | 62.6 (25.6) | 50.4 (33.7) | 1.3 (0.4) | 9.4 (3.6) | 33.5 (28.5) |
| Inland | BT18 | 219 | 76.8 | 60.3 | 1.4 | 13.8 | - |
| | CG19 | 69 | 68.4 | 42.3 | 1.7 | 10.8 | 14 |
| **Inland mean** | | 144 (106) | 72.6 (5.9) | 51.3 (12.7) | 1.9 (0.7) | 13.9 (1.5) | 14.0 |
| **Overall mean** | | 138 (31) | 65.0 (31.2) | 40.6 (36.0) | 1.2 (0.8) | 10.9 (0.8) | 33.5 (28.5) |

Table 5.3. Climate parameters for 33 *P. australis* populations, current average maximum temperature (°C) of three hottest months (December, January, February; $T_{MAX}$); average precipitation (mm) of the wet months (January, February, March; $Pr_{JFM}$); average precipitation of the driest quarter (June, July, August; $Pr_{JJA}$). Correlations with fecundity measures given and significance levels indicated (*$p$<0.05, **$p$<0.01). Bold type indicates significant correlation after Bonferroni correction for multiple tests (α<0.0125).

| Region | Pop'n | N | $T_{MAX}$ (°C) | $Pr_{JFM}$ (mm) | $Pr_{JJA}$ (mm) |
|---|---|---|---|---|---|
| North | FNQ31 | 59 | 30.8 | 1591.0 | 360 |
| | AT1 | 252 | 31.61 | 549.6 | 40 |
| | AT2 | 338 | 31.61 | 549.6 | 40 |
| | AT3 | 591 | 31.61 | 549.6 | 60 |
| | AT4 | 8 | 28.9 | 670.3 | 75 |
| | CA5 | 4 | 31.5 | 1119.0 | 95 |
| Mid | BY26 | 3 | 29.4 | 704 | 186 |
| | FI6 | 96 | 29.3 | 588 | 249 |
| | FI7 | 2 | 30.1 | 411 | 228 |
| | FI8 | 8 | 29.5 | 579 | 256 |
| | TCB32 | 151 | 29.6 | 558 | 254 |
| | PB13 | 5 | 29.1 | 212.4 | 289 |
| | EU14 | 17 | 29.1 | 238.0 | 268 |
| | MA34 | 12 | 29.4 | 490 | 281 |
| | LT15 | 224 | 29.7 | 485 | 212 |
| | SI9 | 330 | 29.9 | 274.6 | 299 |
| | SI10 | 286 | 31.5 | 274.6 | 314 |
| | SI11 | 696 | 28.7 | 274.6 | 299 |
| | SI12 | 113 | 28.9 | 274.6 | 282 |
| | SI29 | 110 | 29.1 | 274.6 | 262 |
| | PR16 | 136 | 29.3 | 581.0 | 236 |
| South | KC33 | 22 | 28.8 | 527.0 | 202 |
| | CNR17 | 17 | 28.2 | 675.0 | 245 |
| | BA20 | 6 | 28.2 | 214.3 | 285 |
| | WH28 | 64 | 26.7 | 183.9 | 232 |
| | AN21 | 170 | 26.7 | 183.9 | 232 |
| | YA22 | 6 | 26.7 | 183.9 | 232 |
| | YR27 | 369 | 28.7 | 135.7 | 228 |
| | CH24 | 12 | 27 | 233.8 | 263 |
| | SW25 | 21 | 26.9 | 183.0 | 252 |
| Inland | BT18 | 219 | 31.6 | 103.4 | 86 |
| | CG19 | 69 | 32.2 | 94 | 87 |
| | *%R* | - | -0.125 | **-0.368*** | 0.016 |
| | *%F* | - | 0.075 | -0.012 | 0.003 |
| | *Inf* | - | 0.065 | -0.078 | **-0.251 **** |
| | *F/Inf* | - | 0.168** | 0.052 | **0.077**** |
| | *%S* | - | **-252*** | **-0.259*** | **0.293*** |

*Current climate*

The average maximum temperature (°C) of the three hottest months (December, January, February; $T_{MAX}$) was coolest in the south of the species range 26.7°C at Yamba, Angourie and Woody Heads (WH28, YA22, AN21), while the warmest temperatures of 32.2°C occurred at the inland Carnarvon Gorge population (CG19; Table 5.3). As a result, decreasing latitude (UTM) was significantly correlated with decreasing $T_{MAX}$ (*rho*=0.836, $p$<0.05). Average precipitation (mm) of the wet months (January, February, March; $Pr_{JFM}$) was greatest in north Queensland at Shipton's Flat (FNQ31, 1591 mm) and Cardwell (CA5; 1119 mm; Table 5.3). Precipitation at the Atherton Tableland populations (AT1-4) is less than other North region populations (FNQ31, CA5) making it more comparable to those in the mid species range (Mid region; BY26, FI6-8, TCB32, MA34, PB13, EU14, LT15; Table 5.3). The lowest wet season precipitation occurs in the inland areas of Blackdown Tableland (BT18) and Carnarvon Gorge (CG19), making these populations the warmest with lowest precipitation (Table 5.3).

Thus, increased wet season precipitation ($Pr_{JFM}$) was weak but significantly correlated (*rho*=0.447, $p$<0.05) with increasing latitude (UTM) north. The average driest quarter precipitation (June, July, August at all sites; $Pr_{JJA}$) was greatest in the middle and south of the species range (*e.g.* SI10 314 mm, PB13 289 mm, BA20 285, CH24 263 mm; Table 5.3) and lowest (less than 100 mm) at several northern sites (AT1-4, CA5). Decreases in $Pr_{JJA}$ was also weakly but significantly correlated (*rho*=-0.405, $p$<0.05) with distance south.

There were some weak but significant correlations between reproduction and climate. The number of flowers per inflorescences weakly but significantly declined from north to south (*Inf*; *rho*= -0.208, $p$<0.01; Table 5.3). There were no other significant correlations with range southwards (*Lat*). There was a significant difference (*H*=13.09, $p$<0.05) in the average number of flowers per inflorescence between regions with mid having greater than the north and south regions (Table 5.2). There were no other significant differences in reproductive measures between regions. The number of inflorescences per plant was weakly negatively correlated with the average precipitation in the driest quarter (*F/Inf*, $Pr_{JJA}$; *rho*= -0.251, $p$<0.01; Table 5.3). The average proportion fruit set per plant (*%S*) was significantly negatively correlated with average maximum temperature of the hottest months ($T_{MAX}$, *rho* =-0.252, $p$<0.05), and the average precipitation of the wettest quarter ($Pr_{JFM}$, *rho*=-0.259, $p$<0.05) but significantly positively correlated with the average precipitation in the driest quarter ($Pr_{JJA}$; *rho*=0.293, $p$<0.05; Table 5.3). There were no other significant correlations between the three climatic variables ($T_{MAX}$, $Pr_{JFM}$, $Pr_{JJA}$) and fecundity measures (*%R*, *%F*, *Inf*, *F/Inf*; Table 5.3).

*Future climate predictions*

Both models predict increases in the projected future average maximum temperatures of the hottest months ($T_{MAX}$) however temperature increases are greater under the RCP 8.5 model (Figure 5.4a,b). $T_{MAX}$ is predicted to increase at the *P. australis* populations by 0.17-0.24°C 2040 under the RCP 2.6 emissions scenario in 2040 (Figure 5.4a). By comparison, $T_{MAX}$ is predicted to increase 1.3-1.8°C at the *P. australis* populations by 2040 in the RCP 8.5 emissions scenario (Figure 5.4b). Projected maximum temperatures do not exceed 33°C at any site under the RCP 2.6 model and returned to close to present by 2070; therefore PVA matrices under this scenario had increased survivability of the seedbank germination and juvenile stages in the first 30 years (Figure 5.4a; Appendix 5).

The situation is different under the RCP 8.5 model with $T_{MAX}$ projected to increase between 2.9-4.1°C by 2070 (Figure 5.4b). The greatest increases in $T_{MAX}$ are projected for populations in the inland region (CG19, 4.0°C; BT 18, 4.1 °C) and all populations in the north region (FNQ31, AT3, CA5, 3.3°C; Figure 5.4b). Under the RCP 8.5 model, $T_{MAX}$ is projected to exceed 33°C in 2020 at CG19, in 2030 at CA5 and BT18, and in 2060 at AT3; and continue to increase in further decades (Figure 5.4b). Therefore populations CG19, CA5 and BT18 had increased mortality in the seedbank germination and juvenile stages in the PVA matrices (Appendix 6). Increases in $T_{MAX}$ at all remaining populations were not predicted to exceed 33°C (Figure 5.4b). The PVA matrices for all other populations without predictions of $T_{MAX}$ exceeding 33°C had increased survivability in the seedbank germination and juvenile stages (Appendix 6).

Both emissions models predict increases in average precipitation of the wettest quarter ($Pr_{JFM}$) at all populations in the South and Inland regions but decreases at all populations the north and mid region (Figure 5.5a, b). Increases in $Pr_{JFM}$ are predicted to be very small (0.6-3.3 mm) for the south and inland populations, with very small decreases (0.3-3.3 mm) at all north and mid populations by 2040 in the RCP 2.6 emissions model, returning to close to current by 2070 (Figure 5.5a). As a result of small changes in $Pr_{JFM}$ there are very small increases in the number of inflorescences produced per plant at the South and Inland populations and very small decreases in the number of inflorescences produced per plant incorporated into the RCP 2.6 PVA calculations for the north and mid populations (Figure 5.5a; Appendix 5).

Under the RCP 8.5 emissions model, there are greater increases predicted in $Pr_{JFM}$ at the South and Inland populations (0.6-29.2 mm by 2040; 1.2-59.8 mm by 2070) compared to the RCP 2.6 emissions model (Figure 5.5a, b). The RCP 8.5 model also predicts greater decreases in $Pr_{JFM}$ compared with the RCP 2.6 model at the north and mid populations (3-124.8 mm by 2040;

48.5-255.9 mm by 2070; Figure 5.5b). The greatest decreases in $Pr_{JFM}$ by 2070 are predicted to occur at FNQ31, CA5 and AT3 (-255.9 mm, -219.3 mm -107 mm respectively; Figure 5.5b). The predicted changes in $Pr_{JFM}$ in the RCP 8.5 emissions model result in increased number of inflorescences (*Inf*) produced per plant for the south and inland populations and decreases *Inf* produced per plant for the north and mid populations incorporated into the RCP 8.5 PVA calculations, that are more pronounced than the RCP 2.6 emissions model (Figure 5.5b; Appendix 6).

The RCP 2.6 emissions model predicted very small decreases in the average precipitation in the driest quarter at all populations ($Pr_{JJA}$; 0.06-2.57 mm) by 2040 returning to current at 2070, while the RCP 8.5 model predicts small decreases continuing through to 2070 (2.5-42.9 mm) in $Pr_{JJA}$ (Figure 5.6a, b). The exception is Cardwell in the north region, where small increases in $Pr_{JJA}$ are predicted at the peak of the emissions scenario models (CA5; 0.82 mm RCP 2.6, 13.8 mm RCP 8.5; Figure 5.6a, b). Both models predict the greatest decreases in $Pr_{JJA}$ at the southernmost population (SW25; Figure 5.6a, b). Under both emissions scenarios, the decreases in $Pr_{JJA}$ result in increased number of flowers produced per inflorescences (*Fl/Inf*) worked into the PVA matrix model calculations at all populations other than CA5, where increases in precipitation decrease the *Fl/Inf* in the PVA model calculations (Appendix 5, 6).

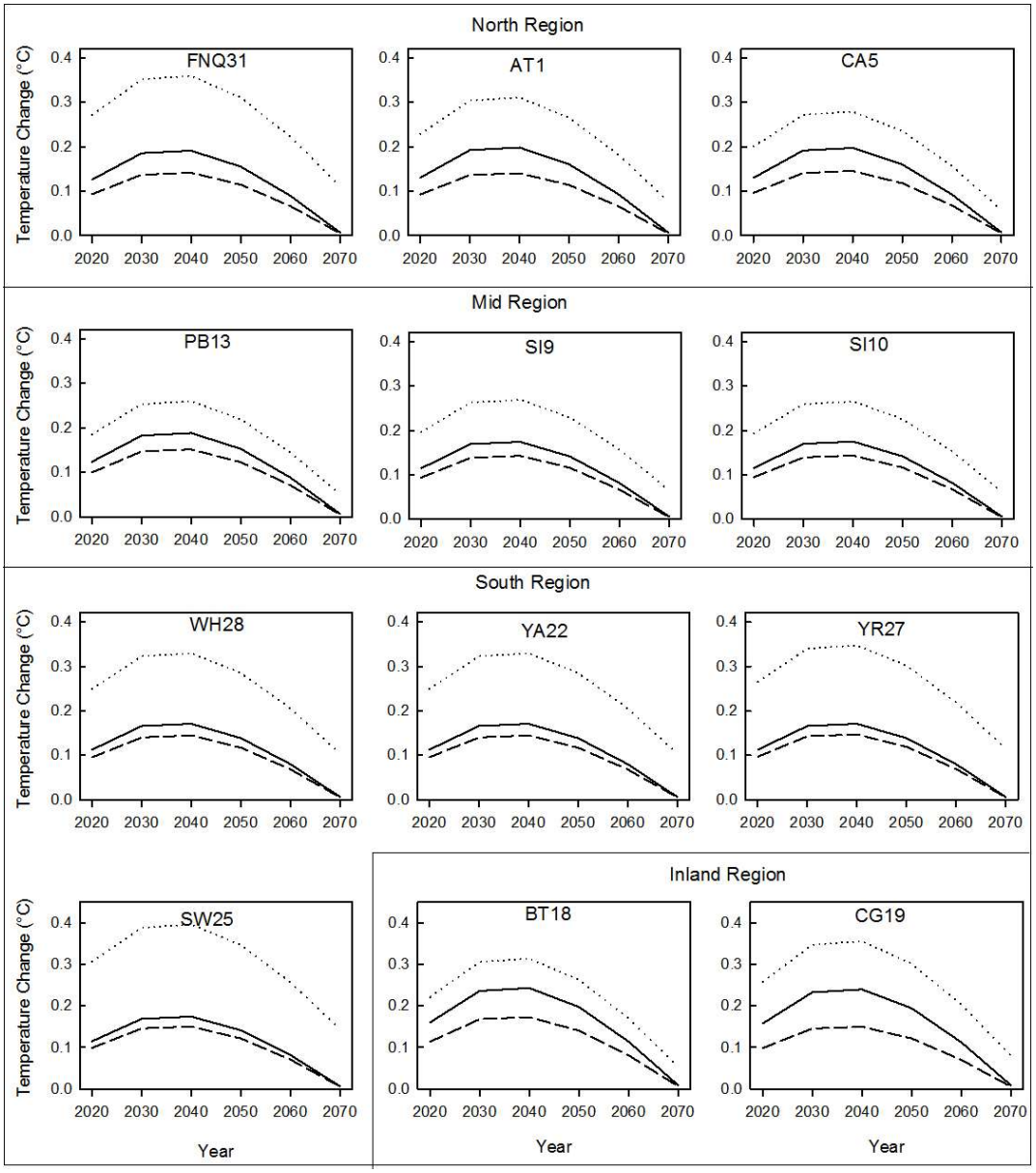


Figure 5.4. Predicted changes in average maximum temperature (°C) of the hottest months (December, January, February) at 12 *P. australis* sites from 2020 to 2070 for emission scenario RCP 2.6; 5th and 95th percentiles of climate ensemble models given. For all, dashed lines indicate 5th percentile, solid lines indicate 50th percentile, dotted lines indicate 95 percentile.

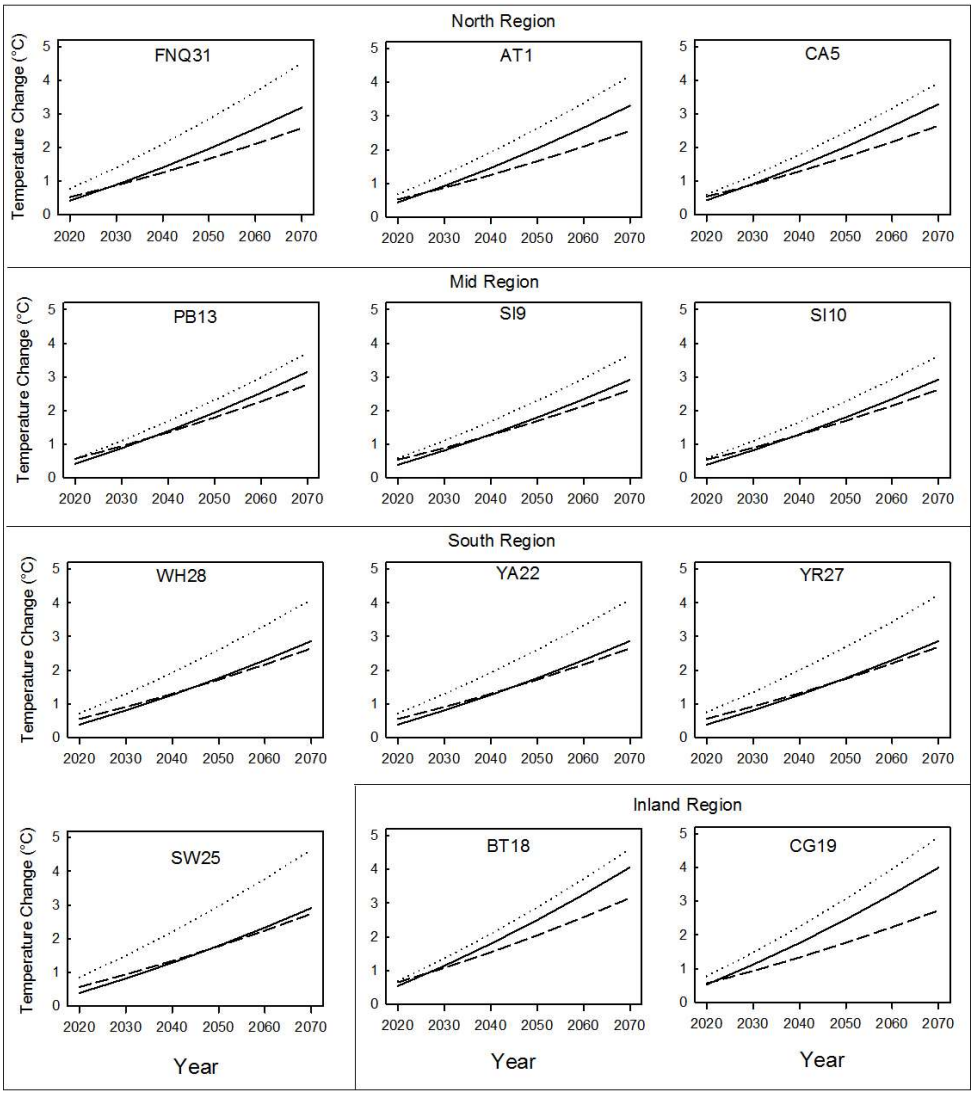


Figure 5.5 Predicted changes in average maximum temperature (°C) of the hottest months (December, January, February) at 12 *P. australis* sites from 2020 to 2070 for emission scenario RCP 8.5; 5th and 95th percentiles of climate ensemble models given. For all, dashed lines indicate 5th percentile, solid lines indicate 50th percentile, dotted lines indicate 95 percentile.

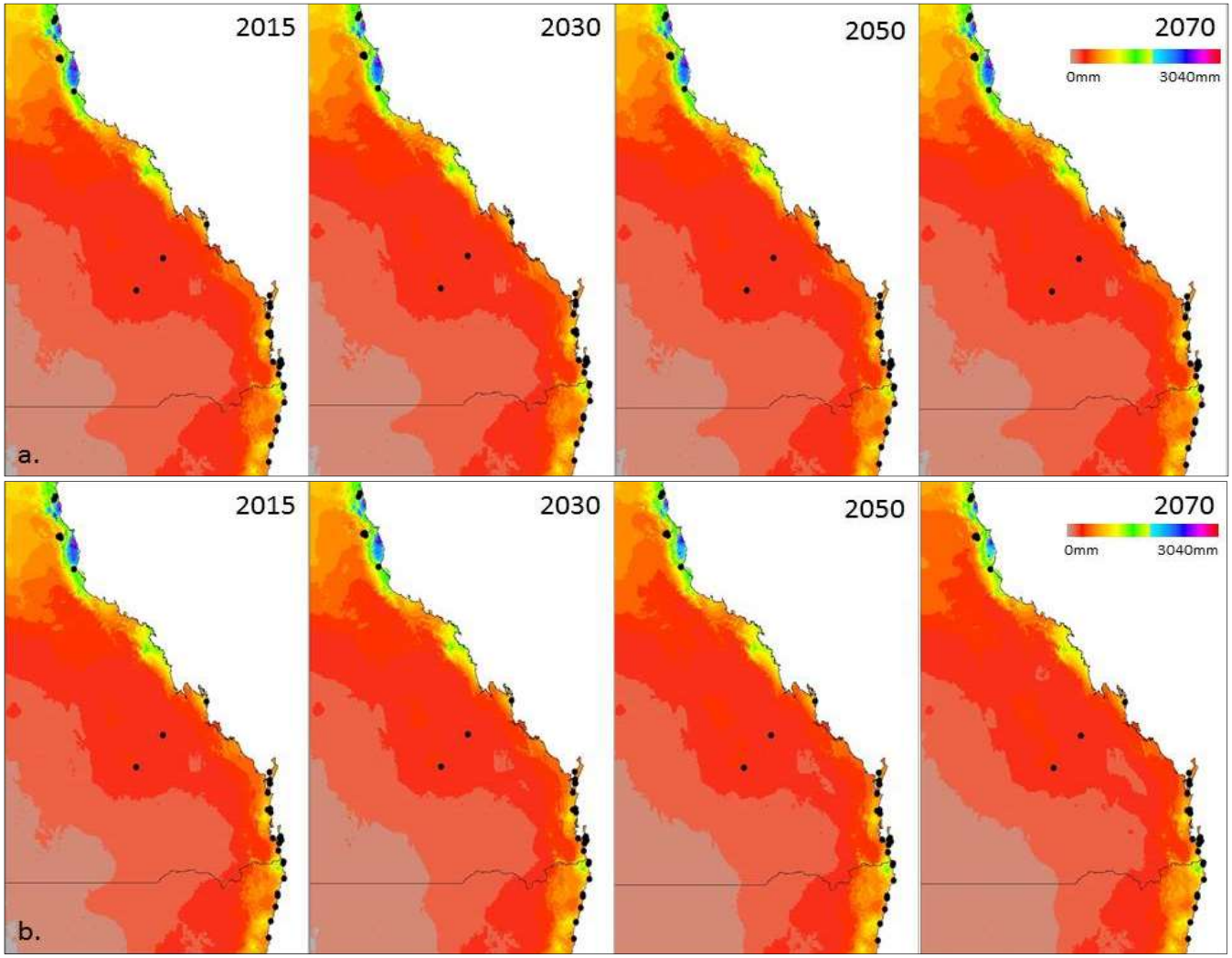


Figure 5.6. The predicted change in average precipitation (mm) of the wettest months (January, February, March) from 2020 to 2070 for the median prediction of climate ensemble models across the east coast of Australia under emission scenario a) RCP 2.6; and b) RCP 8.5. Locations of *P. australis* sites are indicated.

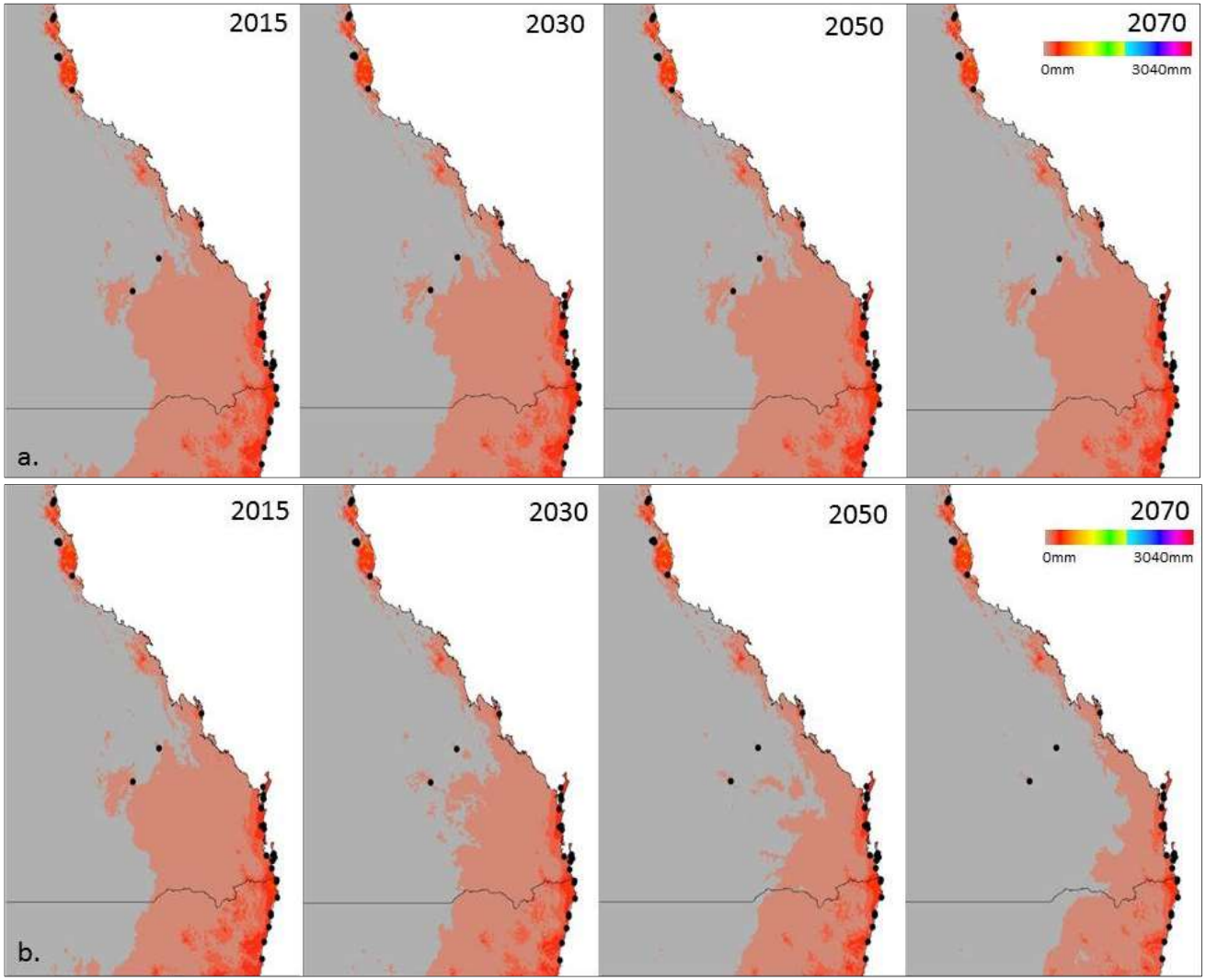


Figure 5.7. The predicted change in average precipitation (mm) of the driest months (June, July, August) from 2020 to 2070 for the median prediction of climate ensemble models across the east coast of Australia under emission scenario a) RCP 2.6; and b) RCP 8.5. Locations of *P. australis* sites are indicated.

Table 5.4. Summary of initial population size (overall $N_i$ and adult $N_ia$), and final population size at the end of the PVA model duration (overall $N_F$, and adult $N_Fa$) and the proportional change in population size (overall $\Delta N_p$, and adult $\Delta N_pa$) for 12 *P. australis* populations. Overall means and means for the regions are given; standard deviations in parenthesis. Significant differences for proportional change between initial population sizes groupings are given (Kruskall Waliis H values, *=$p$<0.05); superscripts indicate initial population size groups that were significantly different (10 = < 10 initial adults, 50 = 30-60 initial adults, 100= 90+ initial adults). No change, is no change in emissions model; RCP 2.6, RCP 2.6 emissions scenario model; and RCP 8.5, RCP8.5 emissions scenario model.

| | | Overall | | | | | | | Adult | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | No change | | RCP 2.6 | | RCP8.5 | | | No change | | RCP 2.6 | | RCP8.5 | |
| Region | Population | $N_i$ | $N_F$ | $\Delta N_p$ | $N_F$ | $\Delta N_p$ | $N_F$ | $\Delta N_p$ | $N_ia$ | $N_Fa$ | $\Delta N_pa$ | $N_Fa$ | $\Delta N_pa$ | $N_Fa$ | $\Delta N_pa$ |
| North | FNQ31 | 59 | 65 | 0.10 | 62 | 0.14 | 53 | -0.24 | 32 | 26 | -0.18 | 25 | -0.22 | 23 | -0.30 |
| | AT1 | 200 | 2137 | 9.68 | 2280 | 10.40 | 2198 | 8.08 | 108 | 676 | 5.23 | 723 | 5.69 | 701 | 5.49 |
| | CA5 | 4 | 4 | -0.29 | 4 | -0.28 | 2 | -0.40 | 4 | 3 | -0.23 | 3 | -0.30 | 2 | -0.49 |
| | **mean** | | | 3.17 (5.64) | | 12.41 (21.85) | | 9.71 (17.39) | | | 1.40 (3.35) | | 1.87 (3.32) | | 1.57 (3.39) |
| Mid | PB13 | 5 | 3 | -0.32 | 5 | 0.00 | 5 | 0.08 | 4 | 4 | -0.09 | 4 | -0.03 | 4 | -0.01 |
| | SI9 | 96 | 549 | 3.21 | 577 | 5.02 | 3461 | 35.05 | 92 | 209 | 1.27 | 218 | 1.37 | 1111 | 11.07 |
| | SI10 | 94 | 543 | 5.71 | 654 | 5.96 | 3214 | 8.71 | 94 | 245 | 1.61 | 253 | 1.69 | 1009 | 3.35 |
| | **mean** | | | 2.89 (3.06) | | 3.66 (3.21) | | 23.11 (19.95) | | | 0.93 (0.90) | | 1.01 (0.91) | | 7.27 (6.31) |
| South | WH28 | 64 | 44 | -0.44 | 45 | -0.30 | 71 | 0.11 | 55 | 29 | -0.47 | 30 | -0.46 | 39 | -0.29 |
| | YA22 | 6 | 6 | -0.37 | 9 | 0.45 | 9 | 0.43 | 4 | 3 | -0.26 | 4 | 0.04 | 4 | 0.00 |
| | YR27 | 363 | 1257 | 2.46 | 1153 | 2.18 | 3842 | 9.59 | 109 | 442 | 3.05 | 398 | 2.65 | 1352 | 11.41 |
| | SW25 | 22 | 16 | -0.44 | 17 | -0.25 | 19 | -0.12 | 21 | 12 | -0.44 | 12 | -0.43 | 13 | -0.40 |
| | **mean** | | | 0.30 (1.44) | | 0.52 (1.16) | | 2.50 (4.73) | | | 0.47 (1.72) | | 0.45 (1.49) | | 2.65 (5.84) |
| Inland | BT18 | 111 | 4838 | 35.76 | 4843 | 42.63 | 2404 | 20.82 | 92 | 925 | 9.06 | 919 | 8.99 | 379 | 4.12 |
| | CG19 | 69 | 36 | -0.68 | 33 | -0.52 | 17 | -0.76 | 33 | 14 | -0.59 | 12 | -0.63 | 9 | -0.71 |
| | **mean** | | | 17.54 (25.77) | | 21.05 (30.51) | | 10.03 (15.25) | | | 4.23 (6.82) | | 4.18 (6.80) | | 1.71 (3.42) |
| **Overall mean** | | | | 4.54 $^{100}$ (10.34) | | 7.70 $^{100}$ (15.34) | | 10.71 $^{100}$ (14.88) | | | 1.45 $^{100}$ (3.01) | | 1.57 $^{100}$ (2.96) | | 3.38 $^{100}$ (5.04) |
| **H (Ni pop sizes)** | | | | 8.48* | | 8.79* | | 8.26* | | | 9.69** | | 8.48* | | 8.48* |

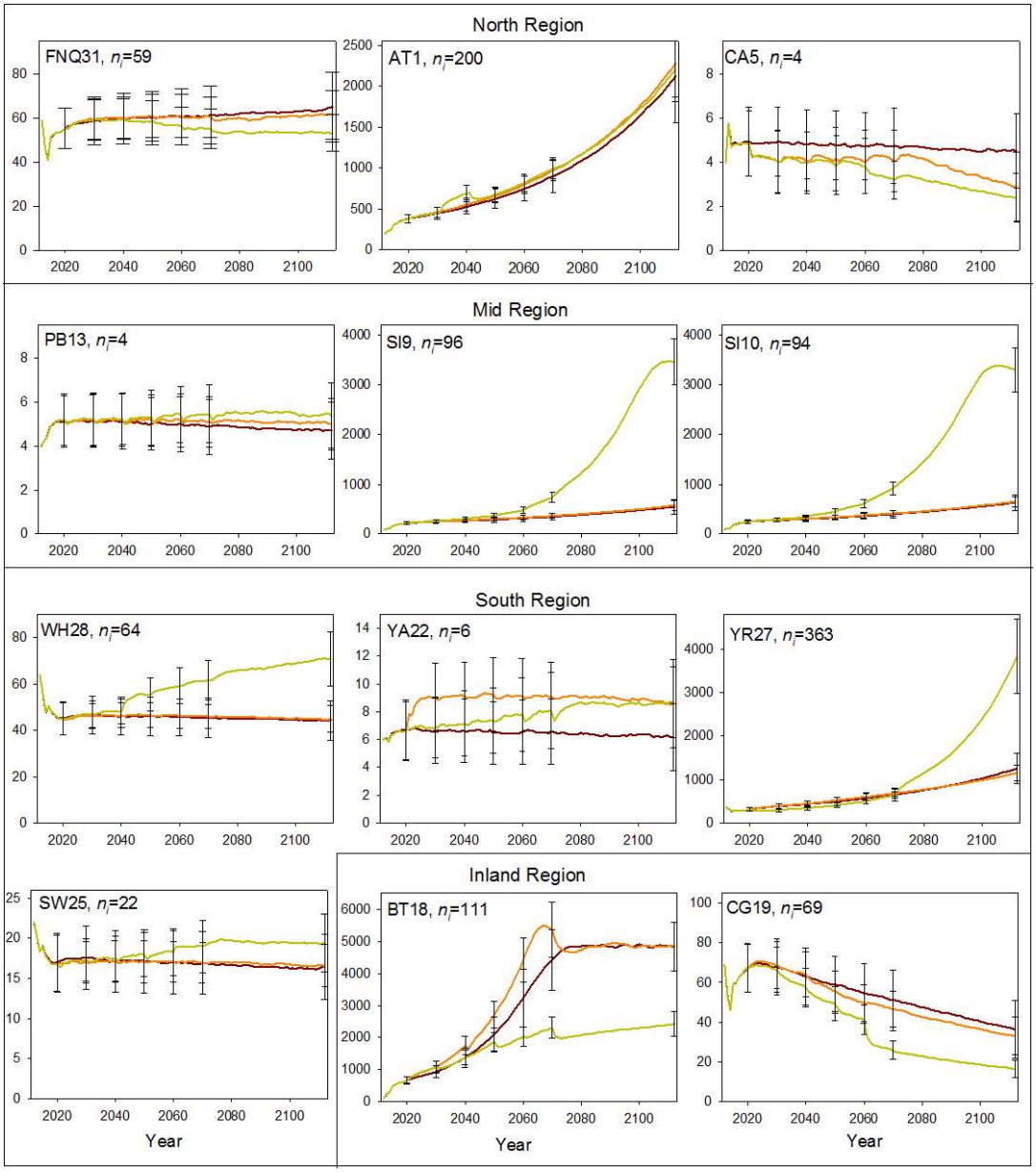


Figure 5.8. Trajectory summaries for 12 *P. australis* populations from 2012 to 2113. For all, 'no change' model in red, RCP 2.6 emissions scenario model in orange, and RCP 8.5 emissions scenario model in yellow; x axis, indicates year; y axis, population abundance; $n_i$, initial population size in 2012.

*PVA models under current climate*

There was no difference in the threshold size for reproduction and no difference in the growth rate of individual plants across the range of *P. australis*. The PVA sensitivity analysis indicated highest model vital rate sensitivity in the seedling recruitment and juvenile transition stages (*S0, S1, S2*; Appendix 4). Very few current populations exhibited an increase in population size over the model duration under current climate conditions (Table 5.4; Figure 5.7).

Table 5.5. Estimated population growth rate (lambda *λ*) for 12 *P. australis* sites currently and under the no change model, then from 2020 to 2070 for the PVA matrices calculated from the biological effects of climate change predictions from emission scenarios RCP 2.6 and RCP 8.5.

| Region | Population | Ni | Current / No Change | 2020 | 2030 | 2040 | 2050 | 2060 | 2070 |
|---|---|---|---|---|---|---|---|---|---|
| | **RCP 2.6** | | | | | | | | |
| North | FNQ31 | 59 | 1.0188 | 1.0196 | 1.0196 | 1.0200 | 1.0197 | 1.0199 | 1.0188 |
| | AT1 | 200 | 1.0312 | 1.0324 | 1.0329 | 1.0329 | 1.0326 | 1.0321 | 1.0312 |
| | CA5 | 4 | 0.9981 | 0.9980 | 0.9982 | 0.9982 | 0.9981 | 0.0998 | 0.9977 |
| Mid | PB13 | 5 | 1.0034 | 1.0036 | 1.0037 | 1.0037 | 1.0036 | 1.0036 | 1.0035 |
| | SI9 | 96 | 1.0227 | 1.0235 | 1.0239 | 1.0239 | 1.0237 | 1.0233 | 1.0227 |
| | SI10 | 94 | 1.0221 | 1.0230 | 1.0233 | 1.0233 | 1.0231 | 1.0227 | 1.0221 |
| South | WH28 | 64 | 1.0062 | 1.0065 | 1.0067 | 1.0067 | 1.0066 | 1.0064 | 1.0062 |
| | YA22 | 6 | 1.0139 | 1.0144 | 1.0147 | 1.0147 | 1.0146 | 1.0143 | 1.0139 |
| | YR27 | 363 | 1.0212 | 1.0270 | 1.0275 | 1.0275 | 1.0273 | 1.0268 | 1.0224 |
| | SW25 | 22 | 1.0048 | 1.0049 | 1.0049 | 1.0049 | 1.0049 | 1.0049 | 1.0046 |
| Inland | BT18 | 111 | 1.0300 | 1.0317 | 1.0326 | 1.0326 | 1.0319 | 1.0312 | 1.0301 |
| | CG19 | 69 | 1.0147 | 1.0157 | 1.0164 | 1.0147 | 1.0157 | 1.0155 | 1.0147 |
| | **RCP 8.5** | | | | | | | | |
| North | FNQ31 | 59 | 1.0188 | 1.0190 | 1.0183 | 1.0174 | 1.0161 | 1.0146 | 1.0131 |
| | AT1 | 200 | 1.0312 | 1.0323 | 1.0404 | 1.0321 | 1.0321 | 1.0320 | 1.0296 |
| | CA5 | 4 | 0.9981 | 0.9987 | 0.9965 | 0.9951 | 0.9938 | 0.9927 | 0.9915 |
| Mid | PB13 | 5 | 1.0034 | 1.0040 | 1.0050 | 1.0062 | 1.0076 | 1.0095 | 1.0117 |
| | SI9 | 96 | 1.0227 | 1.0255 | 1.0288 | 1.0329 | 1.0409 | 1.0479 | 1.0594 |
| | SI10 | 94 | 1.0221 | 1.0249 | 1.0282 | 1.0343 | 1.0417 | 1.0502 | 1.0574 |
| South | WH28 | 64 | 1.0062 | 1.0073 | 1.0065 | 1.0078 | 1.0151 | 1.0181 | 1.0212 |
| | YA22 | 6 | 1.0139 | 1.0159 | 1.0183 | 1.0212 | 1.0246 | 1.0288 | 1.0212 |
| | YR27 | 363 | 1.0212 | 1.0198 | 1.0240 | 1.0275 | 1.0315 | 1.0364 | 1.0509 |
| | SW25 | 22 | 1.0048 | 1.0054 | 1.0065 | 1.0078 | 1.0095 | 1.0116 | 1.0143 |
| Inland | BT18 | 111 | 1.0300 | 1.0667 | 1.0529 | 1.0446 | 1.0314 | 1.0239 | 1.0130 |
| | CG19 | 69 | 1.0147 | 1.0129 | 1.0095 | 1.0060 | 1.0027 | 0.9968 | 0.9938 |

Large populations with over 90 initial individuals in the north (AT1) and inland regions (BT18) exhibited the greatest proportional change in final population size relative to initial population size (*ΔNp*; Table 5.4). These populations had the largest estimated population growth rate (lambda *λ*) and the greatest proportional change in final adult population size relative to initial number of adults (*ΔNpa*; Table 5.4). Other populations in the north and inland regions however, experienced decreases in population size and in the number of adults over the model duration (CA5, CG19; Figure 5.7, Table 5.4-5). High lambda values (*λ*) and resulting *ΔNp* and *ΔNpa* values were not just confined to the lower latitudes. Three large populations across the remaining part of the species range,

Stradbroke Island populations SI9 and SI10 as well as south population YR27 large and positive *ΔNp* and *ΔNpa* (Table 5.4) reflecting high lambda values (*λ*; SI9, 1.0222; SI10, 1.0130; YR27, 1.0212; Table 5.5). As a result under the 'no change' model scenario, the *ΔNp*, *ΔNpa* was not significantly different between the regions ($p$>0.05). Indicating that the difference in climate ($T_{MAX}$, $Pr_{JFM}$, $Pr_{JJA}$) across the species range is not affecting the growth or decline of the species. There is no evidence of range retraction at the north edge of range or expansion at the south edge of the species range.

The Shipton's Flat population (FNQ31) in the north region had relatively high lambda value (*λ*=1.0188) and a small but positive *ΔNp* values; however there was a negative change in *ΔNpa* (Table 5.4-5; Figure 5.7). This indicates that population growth is predominately due to the addition of young recruits (J1 and J2) that do not survive to the older life stages (Table 5.5). This (FNQ31) and all other populations of small and medium initial population sizes, experienced decreases to some degree in population size over the model duration under current climate conditions (Table 5.4; Figure 5.7). As a result, there was significantly greater loss of individuals and adults in populations with an initial population size under 10 individuals compared to populations with an initial population size greater than 60 (*ΔNp*, $H$=8.51, $p$<0.05; *ΔNpa*, $H$=8.795, $p$<0.01; Table 5.4).

The greatest loss of population size was at the inland isolated Carnarvon Gorge population (CG19), which is predicted to lose 68% of the overall population and 59% in adults over the model duration (Table 5.4; Figure 5.7). The lambda value for this population is 1.0147, which despite being greater than some populations, resulted in greater proportional loss of individuals (Table 5.5). Smaller *Phaius* populations at the south part of the species range exhibited a 44% decline in population size and a loss of 44% of adults in South West Rocks (SW25) and 47% loss of adults in Woody Heads (WH28; *ΔNp*, *ΔNpa*; Table 5.5). Low seedbank transition value (0.00004) combined with low inflorescence and flower production contributes to the declining population at WH28 (*Inf*, *Fl/Inf*, $S_0$; Table 5.2, Appendix 2). Similarly, an extremely low seedbank transition value ($S_0$=0.00001) coupled with moderate *Inf*, *F/Inf* production affects declining population size at SW25 Table 5.2, Appendix 2). The extremely small population at Cardwell WH28, *λ*=0.9981) had the lowest lambda value across all populations with a loss of one of the four adult individuals over the model duration, indicating that recruitment levels and survival rates are not great enough to enable the population to grow.

Despite there being several populations of extremely small size (less than seven individuals; CA5, PB13, YA22), no populations went extinct over the model duration of 100 years (Figure 5.7). Only YA22 had a cumulative extinction risk probability ($P_{QE}$) greater than 0, though at 0.002, the actual risk of extinction is still very low (Appendix 7). This is a reflection of the low mortality rates that were observed in repeated surveys over several years. Across all sites, there was no recorded mortality of

adult individuals in the A2-A8 stage classes in any year except due to fire at several sites. Populations with the lowest change in population size and growth rates (FNQ31, CA5, PB13, YA22, SW25) have previously been found to have large heterozygote excess greater than -0.5 (Figure 5.7, Table 5.4-5; Chapter 4).

*PVA under RCP 2.5 and RCP 8.5 emissions scenarios*

Both climate models predicted increases in the average maximum temperatures of the hottest months ($T_{MAX}$), increases in precipitation of the wettest quarter ($Pr_{JFM}$) at south and inland populations, but decreases in precipitation of the wettest quarter ($Pr_{JFM}$) at north and mid region and decreases in average precipitation of the driest quarter ($Pr_{JJA}$; Figure 5.4-6a, b). Changes were greater under the RCP 8.5 scenario which continued to change to 2070, compared to the RCP 2.6, with temperatures and precipitation returning to current by the 2070 (Figure 5.4-6a, b). These climate factors affected the survivability of the seedbank germination ($S_0$), juvenile stages ($Ss_0$, $S_1$, $Ss_1$, $S_2$), the production of inflorescences (*Inf*) and the number of flower per inflorescence (*F/Inf*), thus affecting fecundity values ($Fec_0$, $Fec_1$, $Fec_2$, $Fec_3$) in the PVA model matrices (Appendix 5-6).

Population viability analysis for the RCP 2.6 scenario resulted in small increases in population growth (lambda $\lambda$) and positive proportional change in final population size ($\Delta Np$) and proportional change in final adult population size ($\Delta Npa$) that was equal to or greater than the 'no change' PVA results at most populations (Figure 5.7, Table 5.4-5). For example, lambda values for AT1 at the northern part of the species range increased and peaked at 1.0329 in 2040, resulting in a 4% greater increase in overall population size and adults at the end of the model duration compared to 'no change' ($\Delta Np$= 10.40, $\Delta Npa$=5.69; Table 5.4-5). A similar pattern was observed for SI9 and SI10 in the mid region and BT18 in the Inland region with marginal increases in $\lambda$, $\Delta Np$, $\Delta Npa$ compared to the 'no change' model (Table 5.4-5). In contrast to expectations, $\Delta Np$ increased by 43% with adults increasing by 4% in an extremely small population YA22 at the end of the model duration, a more positive outcome than the model of the current climate (Table 5.4-5).

There were some populations however where the RCP 2.6 PVA model outcomes were not an improvement on the 'no change' models. These results were inconsistent across the species range, with no significant difference ($p$>0.05) in the change in final population size and number of adults ($\Delta Np$ and $\Delta Npa$) between regions. What appears to be of greater importance again is the initial population size, with significant greater (positive) changes in population size ($\Delta Np$ and $\Delta Npa$) at large populations (e.g. AT1, SI9, SI10) compared to smaller populations ($\Delta Np$, $H$=8.481, $p$<0.05; $\Delta Npa$,

*H*=8.481, *p*<0.05). For example, at the most northern population FNQ31, $\lambda$ increased to 1.0200, then dropped resulting in a marginally greater increase in *ΔNp* compared to 'no change', but a greater decrease in *ΔNpa* at the end of the model duration compared to the 'no change' PVA (FNQ31; Table 5.4). Similarly marginal increases in $\lambda$ at south populations WH28 and SW25 still resulted in a loss of overall individuals (*ΔNp*) and adults (*ΔNpa*) though less than that of the 'no change' model (Table 5.4). The RCP 2.6 model resulted in reduced loss of overall individuals (ΔNp) but a greater loss of adults (*ΔNpa*) at inland CG19 (Table 5.4-5) The population that was affected the most by the RCP 2.6 emissions PVA was CA5 in the north region resulting in decreases in population size less than that of the current climate model (*ΔNp*= -0.28) but a loss of one of 4 adults at the end of the model duration (*ΔNpa*=-0.30; Table 5.4).

There was a greater effect on *P. australis* PVA's from the RCP 8.5 emissions scenarios across the species range, because the climatic changes ($T_{MAX}$, $Pr_{JFM}$, $Pr_{JJA}$) are expected to be greater and longer lasting than that of the RCP 2.6 emissions scenario. Climate is predicted to change in favour of *P. australis* at some populations. Mid region populations SI9 and SI10 there are predicted to be minimal decreases in $Pr_{JFM}$ and $Pr_{JJA}$ and while $T_{MAX}$ increases, it is not predicted to exceed 33°C (Figures 5.4-6a, b). As a result, lambda values increase throughout the model duration resulting in a much greater increase in population size in comparison to the 'no change' model as well as over 300% increase in adults (*ΔNp* = 35.05, *ΔNpa* =3.35; Table 5.4-5). Extremely small population in the mid region PB13 is predicted to have an 8% increase in *ΔNp* due to climate change and while *ΔNpa* is still a negative, the effect is not as great as under other models (Table 5.4). Precipitation ($Pr_{JFM}$) is predicted to increase by greater than 50 mm combined with decreases in $Pr_{JJA}$ precipitation results in increased fecundity values at YR27 (Appendix 6). The addition of predicted increases in $T_{MAX}$ also increases the 'hot house' survival potential of the J1 and J2 stage classes (Appendix 6). As a result, the RCP 8.5 emissions model results in a 43% growth of the overall population through recruitment of juveniles, but an even retaining of adults (*ΔNpa* =0; Table 5.4). Similar climate predictions for the remaining south populations result in growth 43% increase in *ΔNpa* through the recruitment of more juveniles, but an even retention of adults (*ΔNpa*) at YA22 compared to that of the 'no change' model (Table 5.4). At WH28 the RCP 8.5 emissions PVA predict an 11% growth of the overall population through the recruitment of juveniles but still predicts a loss of 29% of adults (Table 5.4).

While no populations went extinct over the model duration, not all populations benefited from the changes in climate projected under the RCP 8.5 emissions scenario (Figure 5.7). Both populations in the Inland region are predicted to be negatively affected by the RCP 8.5 emissions scenario. Over the model timeframe the population at remote Carnarvon Gorge (CG19) is predicted to decline by over

75% (*ΔNp*) and lose over 70% of adults (*ΔNpa*; Figure 5.7, Table 5.5-6). While still having a positive population change in overall population and numbers of adults, the larger population BT18 was also predicted to have a lower *ΔNp* and *ΔNpa* under the RCP 8.5 scenario PVA compared to the 'no change' and RCP 2.6 models (Figure 5.7, Table 5.4). Despite these results in the Inland region under the RCP 8.5 PVA, again there were no significant ($p$>0.05) differences in *ΔNp* and *ΔNpa* between the regions ($p$>0.05; Table 5.4).

As with the 'no change' and the RCP 2.6 emissions PVA, there was a significantly greater positive change in *ΔNp* ($H$=8.256, $p$<0.05) and *ΔNpa* ($H$=8.481, $p$<0.05) in populations with initial starting size over 90, compared to that of the smaller size groupings (Table 5.4). For example, the northern most population FNQ31 is predicted to have 24%loss in overall population size and 30% loss of individuals under the RCP 8.5 scenario PVA, a severe change compared to other models (*ΔNp*, *ΔNpa* Figure 5.7, Table 5.4). Likewise, the climate predictions for extremely small population CA5 in the north region is for increased temperatures exceeding 33°C and a 200 mm change in rainfall (Figures 4-5a, b). This reduces fecundity ($Fec_{0\text{-}3}$) and increases mortality in the seedbank and juvenile stages compared to the 'no change' model, resulting in a 40% decrease in population size and 49%decrease in adult plants at the end of the model duration (Table 5.4). At the southernmost population SW25, the increased lambda under the RCP 8.5 PVA is not enough to counteract low recruitment rates and the small starting population size (Appendix 2).This results in *ΔNp* and *ΔNpa* decreasing at the end of the model duration at SW25, though less than that of the current climate and RCP 2.6 model *ΔNp*=-0.12, *ΔNpa*=-0.40; Table 5.4).

When all population sizes, regions and climate scenario PVA models are considered, there was no significant difference in the change in overall population size (*ΔNp*) and the number of adults (*ΔNpa*) due to climate scenario ($p$>0.05). When split by population size, there was again no significant difference in the change in overall population size (*ΔNp*) and the number of adults (*ΔNpa*) due to climate scenario in any population size grouping ($p$>0.05). When climate scenario was disregarded, there was a significant difference in the change in overall population sizes (*ΔNp*; $H$=25.938, $p$<0.01) and in the proportional change in the number of adults (*ΔNpa*; $H$=27.354, $p$<0.01) between population sizes that were large, compared to small and moderate sizes.

## 5.4 Discussion

*Does climate or population size affect the reproductive characteristics, population structure or population growth of Phaius australis?*

Species distribution on medium spatial scales is primarily controlled by climate because temperature and moisture play a critical role in seed germination and seedling survival, plant vigour as well as fecundity (Woodward and Williams 1987, Lawton 1993). Reduced fecundity and / or recruitment have been observed for some species at the climatic edges of their range (Jump and Woodward 2003, Vaupel and Matthies 2012, Hidas et al. 2013). The decrease in demographic and fecundity parameters in turn affect population dynamics, population growth rates and as a result, species may be less abundant or have declines in demography or population gradually towards the climatic range margins (Sagarin and Gaines 2002). This study identified that there are considerably lower maximum temperatures in the warmest months ($T_{MAX}$) and lower precipitation in the wet season ($Pr_{JFM}$) in the southern part of the species range compared to the north in the season that is vital for seed dispersal, germination and seedling growth. Combined with the genetic evidence of post-colonisation dispersal southwards (Chapter 3), it was predicted that recruitment, fecundity and population growth may be more favourable at the wetter, warmer 'hot house' in the north of the species range. For example, Hidas et al. (2013) found less recruitment at the southern (poleward) edge of a species distribution in Australia, limiting colonisation of the species further south. In contrast, while north sites did have a greater output of flowers, there was very minimal, weak evidence of declines in fecundity (*Fl/Inf*; Table 5.2) along the distribution of *P. australis* from north to south.

Again, while there were differences in population structure, density, fecundity, reproduction, population growth rate and the change population size between individual populations across the range of *P. australis*, these were not attributable to the climate gradient. For example, one of the furthest south *P. australis* populations (Yuraygir, YR27) was among the largest and most dense populations (Table 5.1). Similarly, there were no significant differences in the population growth rate, the proportional change in adult population size or the overall population size across the model duration. This is similar to the findings of Aikens and Roach (2014) who found that abundance and vital-rate contributions did not decline consistently towards the edge of the species range. Genetic structure across a species climatic distribution can result in individuals being adapted to the climatic conditions at that point of the species range (Byrne et al. 2013, Saeki et al. 2014). The lack of demographic, fecundity and population growth differences between the climatic regions complements the earlier findings of minimal genetic structure for *P. australis* according to climate

range (Chapter 3). These results also indicate that the lower genetic diversity identified in the south region is not negatively affecting population growth (Chapter 3).

While the physiological responses of plants to climate are likely to be very important drivers of population-abundance and persistence, there may be other factors that also influence survival and performance of populations. For example, the threshold size for flower initiation in *Orchis purpurea* is almost three times greater in shaded environments compared to light environments making the difference between a stable and growing population (Jacquemyn et al. 2010). The successful seed germination and establishment of new orchid plants is generally reported to be low, due to relationships with fungi including often specific mycorrhizal fungi required for seed growth and germination (Clements 1988, Rasmussen and Rasmussen 2009, Swarts et al. 2010). The specificity of mycorrhizal fungi is thought to contribute to the small, hyper-dispersed populations common to orchid species, and their rarity and ecological specialisation within the landscape (Otero and Flanagan 2006, Swarts and Dixon 2009, Swarts et al. 2010). There is great habitat variation between many of the locations of *P. australis* populations and even in the micro-habitat matrix within populations (Chapter 2). Several populations occur on low sand plains in coastal swamps (SI9-12, 29; TCB32, PB13, AN21, YA22, WH28), known to have acidic water (Laycock 1975), while populations on the Atherton Tableland in the north region occur on alkaline soils (Malcolm et al. 1999). Several populations occur on creek lines (FNQ31; CG19; AT4), while others have a water source that is aquifer fed (SI9-12, 29; Sparshott and Bostock 1993). Similarly, *P. australis* populations range from occurring in full sun (AT1-3) to almost complete shade (AT4, FI6, WH28). Thus factors other than climate that may influence the abundance, diversity and composition of both *P. australis* and any mycorrhizal symbionts warrant further investigation.

*Is there evidence of a leading-edge expansion and a trailing-edge retraction?*

Temperatures across Australia have warmed by 0.9 °C and patterns of precipitation have changed since 1910 (CSIRO and BOM 2014). Plant species at higher latitudes may benefit from increasing temperatures at the limits of their range if they have the ability to expand (Jump and Peñuelas 2005, Pearson and Dawson 2005, Murphy et al. 2010). Some range-expansions and retractions have been observed around the world (Catling and Oldham 2011, Lenoir and Svenning 2015). Altered recruitment and reproduction patterns, changes in spatial structure, declines in population growth, regeneration and abundance in areas of less suitable climate and increases in areas that are now more climatically suited may indicate intermediate range shifts (Jiguet et al. 2010, Dolanc et al. 2013). For example, Sletvold and Agren (2015) estimated that longer, warmer summers in the future

may increase the survival and fecundity of two orchids *Dactylorhiza lapponica* and *Gymnadenia conopseae*. Thus, it was also postulated that *P. australis* may show characteristics of southern edge expansion as a positive response to 21st century climate change (Maggini et al. 2011). This study found no evidence of the intermediate responses of climate range shift occurring for *P. australis* at the north or south ends of the species range. There were neither significant decreases in population structure, density, fecundity, reproduction or population growth rate at the northern edge compared to the south.

The possession of genetic diversity and phenotypic plasticity spread across a species range may buffer individuals against climatic fluctuations in the short term, eventually leading to diversification and divergence of individuals within populations, adjusted to altered climatic conditions in the long term (Pfennig et al. 2010, Benito Garzón et al. 2011, Richter et al. 2012). A plastic response to rainfall was observed in the leaf thickness and size for *Eucalyptus tricarpa* and water use efficiency was found to be highly plastic in *E. salubris* across both species' wide climatic range, potentially increasing the fitness of this species by extending the species range of responses to environmental conditions (Byrne et al. 2013, McLean 2014). There were no differences in fecundity, demographic structure or growth for *P. australis* that were strongly correlated with climatic conditions ($Pr_{JFM}$,$Pr_{JJA}$, $T_{MAX}$). However there was variable demography and fecundity across all populations and additional anecdotal evidence of leaf plasticity in different conditions (Benwell 1994) indicate the potential ability of *P. australis* to respond to different conditions.

It is possible that the magnitude of 21st century climate change experienced in recent times has not provided enough selection pressure to result in trailing-edge decreases or leading-edge increases for *P. australis*, particularly given the lack of adaptation for specific climatic conditions observed for the species and the broad climate range. The variation in temperature (5°C) between the north and the south edges of the distribution of *P. australis* is far greater than the 0.9 °C increase observed in the last 115 years (CSIRO and BOM 2014). All *P. australis* populations occur in on groundwater dependant ecosystems and such ecosystems may buffer populations from the effect that increased frequency and severity of drought may have on soil moisture (Chapter 2; Benwell 1994, Freeman et al. 2011; IPCC 2013b, CSIRO and BOM 2014). These are complex hydrological systems that may have the ability to buffer flora against small changes in precipitation, including annual seasonal fluctuations (Freeman et al. 2011). Congener *Phaius tancarvilleae* also has an extremely wide distribution across a broad climatic range (Nepal to the South Pacific Islands; Dockrill 1992), resulting in the potential for a wide thermal tolerance. Greater changes in phenology, fecundity, recruitment and population growth at the leading and trailing-edges are expected for narrow range endemics

adapted to local conditions in response to contemporary climate change, compared to species with a broader range and climatic tolerance (Woodward and Williams 1987, Lawton 1993, Maschinski et al. 2006, Chitale et al. 2014, McCallum et al. 2014, Lenoir and Svenning 2015).

*How will future climate change affect population growth and extinction risk of Phaius australis?*

The choice of climate model and emissions scenarios affects the projected changes to climate in the future, reflecting both the nature of uncertainties in global circulation models as well as future emissions trajectories (Perkins and Pitman 2009, IPCC 2013a). Equally, the climate models utilized for the species range in this study predicted great differences in climate predictions: the increase in the average maximum temperature of the three hottest months ($T_{MAX}$; 'best case' emissions peak 0.17-0.24°C, 'worst case' emissions peak 2.8-4.6°C), changes to average precipitation in the wet season ($Pr_{JFM}$; 'best case' emissions peak +2.5mm to -12mm; 'worst case' emissions peak +59mm south to -256mm north) and dry-season precipitation shift ($Pr_{JJA}$; 'best case' peak +0.82 to -2.57mm; 'worst case' peak +13.8 mm to -57 mm). As a result, the selection of climatic model and emissions scenario affects the results of any further biological modelling undertaken based on climate model predictions (Beaumont and Hughes 2002, Beaumont et al. 2008). Consistent with this and logical given the large differences between the IPCC emissions scenarios, the PVA results for *P. australis* differed between the two climate emissions scenarios and the no change scenario (IPCC 2013b). Current global emissions are tracking on par or above the RCP 8.5 worst case scenario; hence discussion will focus towards the results of the worst case scenario PVA (IPCC 2013a).

Contemporary climate change has already had some effect on reproduction and vital rates of population growth of other species (Lenoir et al. 2008, Chambers et al. 2013, Lenoir and Svenning 2015). Thus, it was expected that direct changes to vital rates based on climate change predictions would have a considerable impact on the predicted survival of *P. australis*. All populations beside AT1 and PB13 had a greater response, both positive and negative, to the 'worst case' scenario compared to the 'no change' and 'best case' scenarios when climate change predictions were added to the PVA. However in contrast to expectations, the changes were not consistent across the species range. There was no significant difference in outcomes between the current climate and the two climate change scenario PVA. Maschinski et al. (2006) used PVA to compare the population viability and extinction risk of the endangered Arizona cliffrose *Purshia subintegra* using past-100 year climate conditions and two climate change scenarios of increased aridity. Their study found that the current population is declining and will be under greater risk of extinction with the prolonged periods of drought predicted under future climate change (Maschinski et al. 2006). Similarly,

*Pediocactus bradyi* may not be resilient to a predicted hotter and drier climate in Southwestern United States (Shryock et al. 2014). Molano-Flores and Bell (2012) found increased population extinction through all PVA models across all future climate change emission scenarios utilised. Finally, increased population extinction risk for arid-zone tree species *Acacia peuce* due to environmental conditions expected under climate change (Raghu et al. 2013).

The greatest negative changes to population growth under the 'worst case' climate change scenario were at the inland Blackdown Tableland and Carnarvon Gorge populations. The predicted decline of *P. australis* at these locations is of particular concern given the individuals within both populations possess alleles that are restricted to the individual population (private alleles, Chapter 3). These populations are isolated from the abundance of other populations, occurring in unique microhabitats supported by spring fed groundwater supplies within the surrounding landscape. There are already other perceived threats to the continued existence of these populations, including feral animal impacts and fire. A population decline in the 'worst case' scenario PVA is also predicted for the isolated small north population in Cardwell (CA5) which occurs in a small remnant wetland fragment. For CA5 and CG19 in particular, predicted decreasing population size increases the risks of genetic erosion and extinction increase from other factors, and these risks become even higher when populations are fragmented (Young et al. 1996). Gene flow estimates are high for *P. australis* and primarily related to rare long distance seed dispersal at colonisation; however the chance of recolonization of these two populations by long distance dispersal from neighbouring populations would still be low (Arditti and Ghani 2000, Molnár et al. 2011). Similarly, *Callistemon teretifolius* in South Australia is expected to be adversely affected by climate change, due to population persistence in suitable habitat and limited gene flow between populations (McCallum et al. 2014).

The 'worst case' scenario PVA also predicted increases in population growth at three populations in the southern part of the species distribution (WH28; YR27; SW25; Figure 5.1; Figure 5.7). These increases in population growth rate resulting in positive changes to the trajectories (albeit small in magnitude at Southwest Rocks, SW25) demonstrate how vital rates due to climate change may be realised at different parts of the species range, particularly if range is wide. In addition, this highlights the potential benefit from increasing temperatures and changes in precipitation for species with supressed activity at the higher latitudes (Jump and Peñuelas 2005, Pearson and Dawson 2005, Murphy et al. 2010).

Do these mixed results mean that *P. australis* is not at risk from climate change? In a similar approach, Salguero-Gómez (2012) found that the increasing precipitation in Utah and the decreasing precipitation in Israel lead to increased population growth two desert plants under projected future

climate conditions. Their study highlighted that long-term demographic data combined with climatic data can lead to robust predictions about species persistence in a future climate (Salguero-Gómez et al. 2012). Likewise, other authors have found that while some species population sizes decreased, others increased in population size, despite habitat suitability potentially decreasing under projections of future climate (Fordham et al. 2013). Similarly, by applying the direct effects of climate in models of populations of *Carlina vulgaris*, Swab et al. (2015) provided different insights into species' responses to climate change than species distribution models alone; demonstrating increases in population viability even in the face of decreasing habitat. The *P. australis* PVA results adds to the growing body of literature establishing that it is important to consider the underlying demographic, habit or life-history traits of each individual population of a species' when considering persistence under climate change (Salguero-Gómez et al. 2012, Fordham et al. 2013, Swab et al. 2015).

The limitation of these PVA models in the context of predicting vulnerability to climate change is that they do not account for the potential impact of sea level rise reducing the availability of habitat of low-lying *P. australis* populations. While the PVA models predict increases in SW25 and WH28, the physical ability of these populations to expand may be limited by suitable habitat. For example, the southern-most population SW25 currently occupied 1/26 of the linear potential patch along a creek bank. The population at Woody Heads (WH28) similarly occurs in a very subtle 2 m ecotone between a fresh water wetland and the saltwater high tide mark. Populations that are most vulnerable to sea level rise because they occur within metres of the high tide mark include populations FI7, TCB32, SI12, SI29 WH28 in the mid region, and WH28, AN21 and SW25 in the South Region. Additionally, populations CA5, FI8, PB13, MA34, SI9, SI10, SI11, KC33, BA20, YA22, YR27 and CH24 occur within 50 m of the coastline, making these populations vulnerable to sea water inundation in the storm surge events predicted to increase as the severity of extreme weather events increases along the east coast of Australia (IPCC. 2013b).

There are additional factors to consider regarding the persistence of south populations: genetic diversity is significantly lower compared to the north, the number of populations and the average population sizes are smaller in the south, particularly compared to the middle of the species range (Table 5.1; Chapter 2). Genetic diversity between individuals and within populations provides the basis for adaptation to changed environmental conditions as well as potential resilience to pests and diseases (Reed and Frankham 2003, Frankham 2005). Thus the reduced genetic diversity and the relatively low number of *P. australis* individuals in the south region make southern populations more vulnerable to extinction.

*Is population size (small, medium and large) a greater threat to persistence than climate change?*

Plant populations occurring in small isolated fragments are more vulnerable to changes in the local environment and microclimates especially in the case of orchids which often have very specific microhabitat preferences (Saunders et al. 1991, Harper et al. 2005, Swarts and Dixon 2009). The importance of population size to the persistence of *P. australis* is highlighted in the results of the comparative PVA models. In each of the PVA model scenarios ('no change', 'worst case' and 'best case') the initial population size controls the outcome for each population, with significant greater (positive) changes in population size and the number of adults (*ΔNp* and *ΔNpa*) at large populations (over 90 individuals) compared to smaller populations (less than 10). Reduced population size can lead to an increased extinction risk by increasing susceptibility to intrinsic and extrinsic demographic and stochastic factors (Lande 1993, Oostermeijer et al. 2003, Newman et al. 2013). Habitat fragmentation is likely to reduce population sizes and so in turn, reduce the population viability of those species that remain in the fragment (Agren 1996, Matthies et al. 2004). Small population size was significantly correlated with decreased capsule set in West Australian orchid *Pterostylis sanguinea* (Newman et al. 2013). The loss of recruits, followed by a failure of recruitment for fen orchid *Liparis loeselii* was thought to influence a crash in population size at a Sutten Fen, despite the rapid regeneration from seed known for this species (Wheeler et al. 1998). Many *P. australis* populations have been lost in the past 50 years, increasing the isolation between populations that already had a disjunct and fragmented distribution (Benwell 1994; DE 2015a; Chapter 2). The results of the PVA analysis for *P. australis* support theory and empirical studies of small populations having a greater extinction risk than large populations.

None of the PVA models predicted extinction for any *P. australis* populations in the 100 year model duration despite extremely small initial population sizes at several populations. This is due to several factors. First, no mortality of adult plants above 'A2' was observed in several years of population surveys, supported by observations of small numbers of adult plants persisting in the same location for several decades (pers. comm. M. Mathieson) resulting in minimal mortality rates incorporated into model matrices. There are also low values for the seedbank germination vital rate ($S_0$) in the PVA matrices, consistent with observations of low seedling establishment in orchid literature (Appendix 1; Figure 5.2; Rasmussen 1995, Arditti and Ghani 2000). The germination of seed into seedlings ($S_0$) is the most sensitive vital-rate for *P. australis* also identified in the sensitivity analysis (Appendix 3). The evidence in the *P. australis* PVA models suggest that a population size of greater than 90 individuals is enough to counteract the low $S_0$ transition values, exceeding an equilibrium state to result in a change in population size greater than 300% of original, including an input at least 100% more adults (Table 5.4). Finally, autogamy of *P. australis* in natural populations may assist in

overcoming pollinator limitations and provide the ability for a population to grow, albiet comprised of genetically identical individuals (Hobbs and Yates 2003, Broadhurst and Young 2006, Leimu et al. 2006).

Minimum viable population size (MVP) is an estimation of the abundance thresholds below which population have an unacceptable risk of extirpation (Shaffer 1981, Soulé 1987, Traill et al. 2007). Common ranges of MVP estimates suggest that thousands of individuals may be required for long-term population persistence of vertebrates (Reed and Frankham 2003, Traill et al. 2007, Traill et al. 2010) but may be much lower for monoecious plants, and lower for self-pollinating species if evolutionary issues are excluded. Demographic stochasticity is thought to increase vulnerability at abundance or effective population size less than 50 individuals (Lande 1993, Brook et al. 2011). Consistent with MVP estimates of 50, this study has indicated populations of *P. australis* that do not drop below a threshold of 90 individuals may persist into the future and potentially increase in size, highlighting the vulnerability of small populations of *P. australis*. The results of the climate change PVA in this study support a minimum viable population for *P. australis* as greater than 90 individuals. For example, the worst case climate change scenario induced small increases to the $S_0$ vital rates at WH28 and the $Fec_{0\text{-}3}$ vital rates at SW25 resulted in small increases in population growth and adults demonstrating that vital rates are limiting the growth of these populations (Appendix 6; Figure 5.7; Table 5.4-5). Similarly, the worst case climate change scenario induced a decrease in vital rates at small populations CG19 and CA5, resulting in a quick and change a negative population growth trajectory, while at large BT18, the same changes to vital rates results in the maintenance of a positive population trajectory (Appendix 6; Figure 5.7; Table 5.4-5). Hence, small populations such as the isolated Carnarvon Gorge (CG19) population and the northern Cardwell population (CA5) may be at greatest risk to future climatic changes.

*Limitations of the models and further directions*

As with any predictive modelling, there are limitations and uncertainties with various aspects of this study (Lindenmayer et al. 2003, Roubicek et al. 2010). Due to the limited timeframe of this study, the vital rates for the PVA matrix have been calculated from three years of data collection. A longer timeframe of collecting data for PVA will always be better in order to account for the environmental variation between years that may result in variation in recruitment and fecundity with minimum timeframes of 10 years suggested (Fieberg and Ellner 2000, Ellner et al. 2002, Lindenmayer et al. 2003, Pfeifer et al. 2006). In the model construction phase, stage residency times were compared to species estimates from orchid growers, observations in the field, and the population stage

abundances at one and two years compared to data collected in the field, with adjustments made to stage transitions and survivorships where necessary. The final matrix was validated against long term population data at a 30 year model duration resulting in high confidence in backbone matrix for the PVA.

Climate predictions have not been modelled beyond 2070, as the uncertainty in GCM becomes greater over time (IPCC 2013a, Perkins et al. 2014). The SimCLIM predictions for precipitation ($Pr_{JFM}$, $Pr_{JJA}$) resulted in some variation between the 5$^{th}$, 50$^{th}$, 95$^{th}$ percentiles (Appendix 8-11). This is primarily because the input GCM models have been selected for their accuracy at specific parts of the latitude gradient of *P. australis* (Perkins et al. 2014). The advantage of selecting GCM that are accurate at specific parts of the gradient is to have a model that gives higher accuracy at particular point locations across a wide range. However, it does result in large variation between models when they are more accurate for different parts of the species range and as such relies on the median value for a region and range.

Predicted future climatic changes expected to increase the frequency and intensity of extreme weather events such as cyclones and storms as well as drought (IPCC 2013a, CSIRO and BOM 2014). Anecdotal evidence has indicated that droughts experienced in South East Queensland in the 1990's and early 2000's may have affected the growth and survival of *P. australis* in some populations (pers. comm. P. Bostock, C. Kelly). The lack of quantifiable and long term for such *P. australis* populations resulting in this element not being incorporated into the PVA in this study; if such data was obtained at a later date, the effect on population growth and survival of *P. australis* could easily be incorporated utilising the 'catastrophe' function in RAMAS GIS v5.0 (Applied Biomathematics 2005) as utilised in PVA for fire management (Keith 2004, Regan and Auld 2004, Conroy 2012).

Incorporating sea level rise (SLR) predictions which range 0.28-0.98 m by 2100 is beyond the scope of these PVA models (IPCC 2013a, CSIRO and BOM 2014). However, it should be noted that sea level rise may have an effect on several low lying populations, in particular: FI7 (Fraser Island) and TCB32 (Tin Can Bay) which occur in land that lies below sea level; all populations on Stradbroke Island (SI9-12, SI29) which occur within 2-25m of the high tide mark; and WH28 (Woody Heads) and SW25 (South West Rocks) occurring within metres of the high tide mark. Future use of models that incorporate digital elevation modelling in the GIS analysis, such as MaxEnt (Maximum Entropy; Phillips et al. 2006) may be of assistance with the identification of habitats that will no longer be suitable for *P. australis* in the future. The impact of predicted SLR on the population persistence of a threatened mammal in south east Queensland was undertaken in this manner (Traill et al. 2011).

Population viability analysis (PVA) is an important tool for conservation that uses abstract models to represent complex lifecycle systems to predict how a species will behave in the future with/without different impacts (Akçakaya 2004). PVAs are considered to be accurate for scenarios involving single populations with simple population dynamics as have been utilised here (Lindenmayer et al. 2003, Driscoll et al. 2010, Crone et al. 2011). PVAs have been successful for comparing conservation management strategies for several orchid species (Kéry and Gregg 2004, Tremblay et al. 2006, Winkler et al. 2009). The literal interpretation of model predictions is a criticism in the use of some PVA, hence this study has preferably examined trends in population growth and the positive or negative change in population size, avoiding the use of finite population size predictions (Lindenmayer et al. 2003, Crone et al. 2011). As a result, the comparative demographic approach and models presented here provided strong evidence that population size is an important factor for persistence of *P. australis* and that some populations may be under increased threat from future climate change.

## 5.5 Conclusions

Several main findings emerge from the research. The climatic differences (temperature $T_{MAX}$ and precipitation $Pr_{JFM}$, $Pr_{JJA}$) across the species latitude distribution do not result in differences in the population dynamics across the species range. The range of *P. australis* does not appear to be retracting or expanding via changes in the population dynamics as a result of climatic changes in the past century, potentially due to the species broad climatic distribution and therefore potential climatic tolerance. The magnitude of temperature and precipitation changes in the future predicted under the best (RCP 2.5) and worst (RCP 8.5) case emissions scenarios is less than the current climatic differences among the species latitude distribution. Future climate change is predicted to increase the population size of *P. australis* in some locations, but decrease population size in other locations. Hence, given that there is also little evidence of genetic structuring between regions the species is likely to be robust enough to withstand predicted future climatic change.

Population size is of greater concern for the persistence of *P. australis* as populations larger than 90 individuals are predicted to have greater population growth compared to populations with less than 90 individuals. While populations of less than 90 individuals may not necessarily go extinct if left alone, none were predicted to increase in size over a 100-year model duration. Small *P. australis* populations also possess lower genetic diversity, decreasing the theoretical ability to cope with abiotic and biotic changes (Chapter 3; Ellstrand and Elam 1993, Frankham 2005, 2010b). Thus, populations with a size less than 90 individuals (FNQ31, AT4, CA5, BY26, FI7-8, CN17, BA20, YA22,

CH24, SW25, CG19) are most threatened, due to intrinsic factors as well as climate change. In particular, isolated Carnarvon Gorge (CG19) and the northern Cardwell (CA5) populations are of great concern.

Finally, this research has shown that anthropogenic influences that may reduce population size could be more important determinants of the future survival of *P. australis* than climate change. A listed threat to populations that was also documented during surveys at two locations was the illegal removal of flower stalks, reducing reproductive capacity of populations (Chapter2; DE 2015a). Sensitivity to changes in reproduction was identified in the PVA model analysis, highlighting the importance of these vital rates to population growth. Of greater concern is anthropogenic damage to reduce population size through land use changes and the removal of whole individuals or entire populations. Maintaining or increasing current population sizes will be important for conservation of the species into the future given the greater vulnerability of small populations.

# Chapter 6: General Discussion and Conclusions

This body of work represents a unique combination of genetics, population viability analysis and climate change science that will be relevant on both a local and global scale. Habitat fragmentation, illegal collection and climate change are conservation issues that threaten the survival of biodiversity and, in particular, threatened species globally. This study examined how fragmentation (measured by population size and isolation), climate and climate change affect the potential viability of swamp orchid *Phaius australis* in Australia. Extensive research has previously been undertaken regarding species response to fragmentation (Broadhurst and Young 2006, Klank et al. 2010, Lauterbach et al. 2012, Newman et al. 2013, Chung et al. 2014) and climate change (Root et al. 2003, Keith et al. 2008, Loarie et al. 2008, Laurance et al. 2011a, Guerin and Lowe 2012, Vanderwal et al. 2013, Shryock et al. 2014, Lenoir and Svenning 2015). While there has been some research on the effects of fragmentation on coastal plant species, there has been very little research on coastal wetland species in the context of climate change (Traill et al. 2011), with the majority of studies undertaken on Wet Tropics or Subtropical rainforest species in Australia (Kitching et al. 2010, Hughes 2011, Laurance et al. 2011a, Vanderwal et al. 2013).

This study has used *P. australis* as a case study of a plant threatened by a variety of impacts and faces the additional stress of future climate change, to identify the range of responses that an organism may have and consequent priorities for conservation to enable the survival of a species under new conditions. This study provides:

- The first geo-referenced comprehensive record of the distribution and population sizes of *P. australis* and *P. bernaysii* since 1994, with accompanied lodged herbarium and genetics specimens;
- A combination of morphological, genetic and reproductive assessments to clarify the relationship of *P. australis* and *P. bernaysii*;
- The first population genetics study of *P. australis* that will aid in the successful protection and conservation of individual populations and the species as well as any future translocations or population enhancements;
- A preliminary investigation into the reproductive biology of *P. australis* that may contribute heterozygote selection and excess in populations; and
- One of only a few models for combining population viability analysis (PVA) with climate change science to predict a species response to expected climatic change.

Thus, it has contributed significantly to conservation science because it has explored the gaps in knowledge relating to population genetics, fragmentation and climate change as well as applied new methods that can contribute to future conservation practice.

## 6.1 The effects of fragmentation on *P. australis*

This first aim of this study was to clarify the taxonomy in order to accurately examine and communicate the distribution, population size and the threats to *P. australis* in Australia. The distribution of *P. australis* was surveyed across a 2000 km latitude gradient across the east coast of Australia and examined the size, threats and morphology of the species. The lack of genetic and morphological evidence for *P. tancarvilleae* populations in Australia supports current taxonomy that all populations are *P. australis* except for one mixed population of purple-flowered *P. australis* and yellow-flowered *P. bernaysii* (Chapter 2; Clements and Jones 2008, CHAH 2010, DE 2015a).

The second and third aims were to examine how fragmentation affects the demographics, reproduction, population growth and population genetics of *P. australis* by comparing small and large populations and populations of varying degrees of isolation across the species range. Habitat fragmentation is a global reality and there has been a comprehensive body of research on the effects that habitat fragmentation can have on threatened species (Aguilar et al. 2006, Lindenmayer and Fischer 2006). In particular, habitat fragmentation has been shown to decrease the population size and density, negatively affect reproductive processes, recruitment and population growth as well as reduce genetic diversity, gene flow and increase inbreeding in plants (Ellstrand and Elam 1993, Young et al. 1996, Lienert 2004, Leimu et al. 2006, Honnay and Jacquemyn 2007, Aguilar et al. 2008, Vranckx et al. 2012). The assembled evidence indicates that the population genetic diversity of *P. australis* was significantly lower in smaller populations as well as small, isolated populations (Chapter 3). Results of this study support those found for several other species, both Australian and around the world (Shapcott et al. 2005, Brownlie et al. 2009, Swarts et al. 2009, Chung et al. 2014, Shapcott et al. 2015). There was also evidence of limits to recruitment, population growth rate observed and senile population structure, potentially amplifying the impacts of stochastic processes on populations with less than 90 individuals (Chapter 5).

However, in this study there was also some evidence contrary to theory. Small populations have been reported to exhibit higher levels of homozygosity due to a reduction in plant-pollinator interactions (Ellstrand and Elam 1993, Wallace 2002, Leimu et al. 2006). Small *P. australis* populations were not consistently found to be inbred, with a trend instead towards greater excess

heterozygosity (Chapter 4). Similar results have been found in other studies (Kang et al. 2005, Geiger et al. 2014). There were also no negative effects of population size and isolation on the reproductive fitness of *P. australis*, a result similar to that found for *Macadamia integrifolia* (Neal et al. 2010), and contrary to theory (Chapter 4; Aguilar et al. 2006, Broadhurst and Young 2006). Reproductive biology studies have indicated that an autonomous self-pollinating system may be compensating for pollinator limitations in wild populations, as also found for the orchid *Neotinea maculata* (Duffy et al. 2009). Thus, the self-pollinating system may be reducing the effects of inbreeding, and there may be evidence of a heterozygote selection advantage for *P. australis* (number of reproductive individuals; Chapter 4; Kang et al. 2005).

This study identified high levels of genetic similarity between populations and little evidence of structuring based on population size or isolation despite the large distances between populations in the fragmented landscape. This is contrary to small population and isolation theory that predicts small populations and isolation between patches is likely to increase the effect of genetic drift resulting in population differentiation. For *P. australis* the observed genetic similarity is likely due to ancestral similarity with a wide distribution of common alleles and a clonal breeding system that may counter the effects of genetic divergence through mixed mating resulting in the maintenance of genetic similarities between *P. australis* populations.

## 6.2 The effect of climate and predicted climate change on *P. australis*

Climate change is recognised as one of the greatest global threats to biodiversity because species distribution, reproduction, seed germination, seedling and plant survival, and hence population dynamics, are primarily controlled by climate (Good 1931, Woodward and Williams 1987, Arditti 1992, Pearson and Dawson 2003, Hilbert et al. 2007, Kingsford et al. 2009, Winkler et al. 2009, Kingsford and Watson 2011, Walck et al. 2011, Bellard et al. 2012). Given the wide latitude distribution of *P. australis*, the second and fourth aims of this study were to examine how climate affects the population genetics, demographics, reproduction, population dynamics and population growth of *P. australis*. Theory predicts that a species will be more abundant, with denser populations and greater reproduction in the centre of a range and hence possess greater genetic diversity in the centre of a range, declining towards the range margins of suboptimal conditions, also called the 'abundant centre hypothesis' (Hengeveld and Haeck 1982, Brown et al. 1996, Sagarin and Gaines 2002, Jump and Woodward 2003, Duffy et al. 2009).

This study identified a gradual loss of diversity in *P. australis* along the poleward gradient; an evolutionary insight into the colonisation of the species from tropical north Queensland, southwards to what is possibly the edge of its bioclimatic envelope (Chapter 3). This is likely to have occurred as genetic diversity was lost through repeated founder effects as stepping stone colonisation events occurred across the landscape similar to that found in other species (Austerlitz et al. 1997, Bialozyt et al. 2006, Excoffier et al. 2009, Sandoval-Castro et al. 2014). Such a pattern of genetic diversity could be perpetuated with predicted climate change as additional poleward habitat becomes climatically suitable and provided that the species is physically able to migrate further south (Parmesan and Yohe 2003, Arenas et al. 2012, Bellard et al. 2012). In contrast to expectations, there was a lack of genetic differentiation between the north and the south edges of the distribution of *P. australis* (Chapter 3; Wallace and Case 2000, Lowe et al. 2004, Chung et al. 2005). This may be explained by long distance dispersal of self-pollinated seed, followed by exponential population growth in newly colonised sites (Hamrick and Godt 1996, Bialozyt et al. 2006, Eckert et al. 2008, Excoffier et al. 2009). While reproductive output was greater in *P. australis* populations at the centre of the species range (Chapter 4), there was minimal evidence of other demographic, population structure or population growth differences across the species range and this is indicative of a species with a wide thermal tolerance (Chapter 5). In most cases, population size was a more important factor in terms of genetic diversity, reproductive output, population growth and hence viability of populations than climate (Chapter 5). This suggests that future restrictions on population size related to development, fragmentation, predation and illegal collecting may be more significant for viability of the species than the direct impacts of climate change in the majority of locations.

The final aim of this study was to examine if recent climatic changes are having an impact on population dynamics and reproduction, and predict the viability of populations under future climatic conditions. Climate change is affecting species worldwide through shifts in phenology (Menzel et al. 2006, Root and Schneider 2006, Pearson et al. 2014, Beaumont et al. 2015), altered recruitment and reproduction patterns, changes in spatial structure, population growth, regeneration and abundance across the species geographic distribution in areas of less suitable climate (Barry et al. 1995, Dang et al. 2010, Jiguet et al. 2010, Murphy et al. 2010, Catling and Oldham 2011, Shimazaki et al. 2011, Dolanc et al. 2013).

In contrast to expectations, there was no evidence for any of these climate-change effects occurring for *P. australis*. This could be due to the wide thermal tolerance of the species and the comparable magnitude of recent temperature changes across the range, or due to low generation turnover

creating a lag between climate change and migration (Huntley 1991, Davis and Shaw 2001, Malcolm et al. 2002, Neilson et al. 2005).

The fragmentation of suitable habitat across the disjunct range of *P. australis* that may limit migration, and the lack of evidence of early migration, implies a greater importance for populations persisting *in situ*. The possession of a range of genotypes and moderate to high levels of genetic diversity occurring across different environmental conditions may assist survival by providing a species with a broader baseline for adaptation to new conditions (Bradshaw 1965, Bradshaw and McNeilly 1991, Davis and Shaw 2001, Gienapp et al. 2008). While genetic diversity is moderately low for this species it falls in the range of genetic diversity that has been identified for other terrestrial orchids (Swarts et al. 2009, Chung et al. 2013, Qian et al. 2013, Chung et al. 2014, Menz et al. 2015). The minimal evidence of genetic structuring between climatic regions may indicate that *P. australis* is likely to be genetically robust enough to withstand predicted future climatic changes.

Under predicted changes in climate, the PVA forecasts minimal changes for the majority of populations, with increases in population size at locations where climate may become more favourable (greater rainfall, warmer temperatures), but a small decrease in populations in less favourable locations over a model duration of 100 years (Chapter 5). Small populations with a population size less than 90 individuals may not necessarily go extinct over the 100 year projection if not otherwise disturbed in current climate and either of the future climate scenarios that were simulated (Chapter 5). However, none of the small populations (less than 10 individuals) were predicted to increase in size in a 100-year model duration. The small *P. australis* populations also possess lower genetic diversity, decreasing the genetic diversity on which selection can occur in order to cope with abiotic and biotic changes (Chapter 5; Ellstrand and Elam 1993, Frankham 2005, 2010b). Thus it is concluded that isolated small populations, and populations with less than 90 individuals, are at greatest risk in a future changed climate (Chapter 5).

## 6.3 Conservation management of *P. australis* in the context of a changing climate

Surveys over the duration of this study found some large (500 + individuals) and robust populations which have increased in size since original surveys from 1930-2008 (Chapter 2). However there are still many small populations, which are more vulnerable to extinction from environmental stochasticity than populations of greater than 90 individuals (Chapter 2). Most importantly, there was evidence of local extinctions, population sizes having declined in some locations and all populations face at least one threat or synchronous multiple threats. Threats include the illegal

collection of entire populations, as well as cutting and removal of inflorescence stalks which may have significant impacts on population dynamics, conservation of genetic diversity in wild populations, as well as directly causing local extinctions.

There is evidence to suggest that *P. bernaysii* is neither geographically or genetically distinct from *P. australis*, which may have potential taxonomic consequences. Regardless of taxonomy, from a conservation point of view, this significantly increases the conservation importance of the single known mixed *P. australis* / *P. bernaysii* population because this is the only population hosting and expressing the recessive genes of the *P. bernaysii* phenotype.

Arguably, the most significant finding of this study is that habitat fragmentation, particularly the reduction in population sizes has yielded negative effects on the persistence of *P. australis*. This highlights the importance of maintaining large populations across the species range, in order to maintain genetic diversity as well as to overcome the environmental and demographic stochasticity that may threaten small populations. Hence, mitigating further habitat fragmentation and minimising the loss or reduction of population size is imperative for conservation management. Climate change is predicted to have a negative effect on the survival of some populations. Reducing the potential threats that may decrease population size while protecting and maintaining population sizes is therefore of importance for the survival of the species. Regardless of climate change, maintaining the current levels of habitat connectivity between suitable occupied and unoccupied habitats is important for survival of *P. australis* across a disjunct landscape.

Several small, isolated and unique populations including Carnarvon Gorge (CG19), Blackdown Tableland (BT18), Shipton's Flat (FNQ31) and Cardwell (CA5) are therefore of great conservation value. The predictions of lower population growth in the future combined with the genetic distinctiveness of these populations, increases their conservation significance. Translocations and population enhancements are recognised as a last resort measure to conserve a species in the face of a changing future. It is worth noting that over the duration of this study, population enhancements and the establishment of new populations were undertaken by community groups at the southern edge of the species distribution. There is additional interest in the community to supplement small populations and to re-establish now-extinct populations. Given the threats to small populations, this may be an important aspect for the survival of the species. In all such cases, it is important to utilise the results of the genetics study to decide which populations should provide optimal sources for propagative material in order to enhance the viability, evolutionary trajectory and survival of the species in the long term. Ideally, material would be best sourced from a variety of

large populations within the same region in order to enhance genetic diversity, while maintaining any adaptation to local conditions.

### 6.4 Future research directions

The results of this study have highlighted several potential future research directions for the swamp orchid *Phaius australis* in Australia:

- Additional reproductive biology, pollinator observations and cross-pollination experiments could be undertaken in the mixed *P. australis* and *P. bernaysii* populations to provide a basis for understanding the evolutionary basis of the survival of the phenotypic form (*P. bernaysii*) in the wild.

- Population genetic surveys could be expanded to sample material from *P. tancarvilleae* populations in the region surrounding Australia including Pacific Islands, Papua New Guinea, Indonesia and South East Asia. Such studies would add a phylogenetic perspective to the relationship between *P. australis* and *P. tancarvilleae* and enable an evolutionary timeline to be established with important implications for the radiation of ancestral material over past period of climate change and environmental dislocations.

- Future use of species distribution modelling (SDM) such as MaxEnt (Maximum Entropy; Phillips et al. 2006) may be of assistance in clarifying the importance of current climate, abiotic and biotic factors to the species distribution. Such modelling would be particularly informative if future climatic and sea level rise predictions were coupled with digital elevation modelling such as LiDAR data and the population viability analysis in this study to identify the coastal habitats that will no longer be suitable for *P. australis* in the future (Midgley et al. 2006, Thuiller et al. 2008, Pacifici et al. 2015).

- *Phaius australis* occurs within and directly adjacent to fire prone habitat (Sparshott and Bostock 1993, Benwell 1994, QH 2014), however there is little understanding of the effect that fire and repeated fire regimes may have on the survival of the species. Timeframes ranging from five to six, eight to ten, and up to fourteen to twenty years are variously cited as most appropriate in terms of maintaining species richness in such habitats (Tran and Wild 2000, QH 2014). It would thus benefit management to research the effect that burning regimes aimed at maintaining biodiversity may have on the survival and persistence of *P. australis* populations as have been undertaken for other rare orchids and plant species (Regan and Auld 2004, Coates et al. 2006, Coates and Duncan 2009, Conroy 2012). As described in this study, the most effective use of PVA is for evaluating or ranking the response and extinction risks due to comparative activities,

impacts or different ecological management scenarios (Caswell 2001, Akçakaya 2004, Keith 2004, Regan and Auld 2004). Over the duration of this study, opportunistic surveys were undertaken pre- and post-fire in several populations affected by wildfires and this data has not been included in this body of work. Given the robust nature of the PVA presented in this study, opportunity exists to critically examine and to expand the survey data and thereby make a further contribution to the current body of knowledge regarding fire management for biodiversity and threatened species conservation (Driscoll et al. 2010).

## 6.5 Concluding remarks

This study represents a rare combination of population viability analysis and climate change science with genetics allowing the evaluation of likely species responses, predictions of species extinction risk, identification of priority conservation areas and development of management recommendations (Maschinski et al. 2006, Molano-Flores and Bell 2012, McCallum et al. 2014). Population surveys and PVA identified some large, robust populations with genetic variability which is a positive sign for the viability of populations and persistence of the species in the wild. While moderately low genetic variation was found for the species, the results are within the range that should be expected for orchids. The decline of diversity along the axis of colonisation, combined with a potential heterozygote selection advantage and self-pollination provides an evolutionary insight into the species which will be of assistance in planning conservation management plans for the species.

*Phaius australis* is potentially a species robust enough to tolerate predicted climatic changes across the majority of the range if not disturbed by threats that may impact population dynamics. Several populations have been identified that should be prioritised in climate change mitigation efforts. Additional studies incorporating sea level rise may identify other priority populations. The future predicted climate at other locations may be favourable for expansion of populations where the size is greater than approximately 90 individuals.

Finally and of utmost importance, the results of this study have highlighted that for *P. australis*, survival in the wild is about safety in numbers. Larger populations (more than 90 individuals) have greater genetic diversity and greater potential to expand than smaller populations. Conservation is best done *in situ* where conditions resemble those for which *P. australis* is adapted. In contrast conservation in private collections will inevitably contribute more harm than good for the survival of this species in the wild.

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## Appendices

Appendix 1: Allelic frequencies across 13 loci for33 *P. australis* populations (note 11 variable loci for this species). Private alleles indicated in bold.

| | ml-Pa03 | | | ml-Pa19 | | | ml-Pa31 | | | | ml-Pa44 | | | ml-Pa49 | | | ml-Pa59 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Allele** | **232** | **234** | **238** | **240** | **298** | **300** | **221** | **225** | **227** | **229** | **188** | **190** | **192** | **195** | **197** | **199** | **230** | **232** |
| **FNQ31** | 0.500 | 0.259 | 0.000 | 0.241 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 0.052 | 0.948 | 0.000 | 0.000 | 1.000 | 0.000 | 0.569 | 0.431 |
| **AT1** | 0.534 | 0.293 | 0.000 | 0.172 | 0.500 | 0.500 | 0.000 | 0.000 | 0.411 | 0.589 | 0.172 | 0.828 | 0.000 | 0.000 | 0.966 | ***0.034*** | 0.946 | 0.054 |
| **AT2** | 0.643 | 0.339 | 0.000 | 0.018 | 0.467 | 0.533 | 0.000 | 0.000 | 0.346 | 0.654 | 0.143 | 0.821 | 0.036 | 0.000 | 1.000 | 0.000 | 0.875 | 0.125 |
| **AT3** | 0.519 | 0.173 | 0.000 | 0.308 | 0.519 | 0.481 | ***0.019*** | 0.000 | 0.942 | 0.038 | 0.220 | 0.740 | 0.040 | 0.000 | 1.000 | 0.000 | 0.920 | 0.080 |
| **AT4** | 0.600 | 0.400 | 0.000 | 0.000 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 |
| **CA5** | 0.667 | 0.333 | 0.000 | 0.000 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 |
| **BY26** | 0.167 | 0.333 | 0.000 | 0.500 | 0.667 | 0.333 | 0.000 | 0.000 | 1.000 | 0.000 | 0.167 | 0.833 | 0.000 | *0.167* | 0.833 | 0.000 | 0.833 | 0.167 |
| **FI6** | 0.015 | 0.515 | 0.000 | 0.470 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 0.091 | 0.909 | 0.000 | 0.000 | 1.000 | 0.000 | 0.606 | 0.394 |
| **FI7** | 0.500 | 0.500 | 0.000 | 0.000 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 |
| **FI8** | 0.000 | 0.500 | 0.000 | 0.500 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 0.375 | 0.375 | 0.250 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 |
| **TCB32** | 0.000 | 0.518 | 0.000 | 0.482 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 0.054 | 0.911 | 0.036 | 0.000 | 1.000 | 0.000 | 0.893 | 0.107 |
| **PB13** | 0.000 | 0.500 | 0.000 | 0.500 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 0.375 | 0.625 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 |
| **EU14** | 0.000 | 0.500 | 0.000 | 0.500 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 0.250 | 0.500 | 0.250 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 |
| **MA34** | 0.000 | 0.500 | 0.000 | 0.500 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 0.222 | 0.778 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 |
| **LT15** | 0.400 | 0.200 | 0.000 | 0.400 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 0.081 | 0.919 | 0.000 | 0.000 | 1.000 | 0.000 | 0.581 | 0.419 |
| **SI9** | 0.000 | 0.500 | 0.000 | 0.500 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 0.097 | 0.903 | 0.000 | 0.000 | 1.000 | 0.000 | 0.984 | 0.016 |
| **SI10** | 0.000 | 0.500 | 0.000 | 0.500 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 0.100 | 0.900 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 |
| **SI11** | 0.000 | 0.517 | 0.000 | 0.483 | 0.466 | 0.534 | 0.000 | 0.000 | 1.000 | 0.000 | 0.100 | 0.883 | 0.017 | 0.000 | 1.000 | 0.000 | 0.600 | 0.400 |
| **SI12** | 0.000 | 0.500 | 0.000 | 0.500 | 0.517 | 0.483 | 0.000 | 0.000 | 1.000 | 0.000 | 0.161 | 0.839 | 0.000 | 0.000 | 1.000 | 0.000 | 0.774 | 0.226 |
| **SI29** | 0.350 | 0.200 | 0.000 | 0.450 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 0.047 | 0.953 | 0.000 | 0.000 | 1.000 | 0.000 | 0.703 | 0.297 |
| **PR16** | 0.000 | 0.516 | 0.000 | 0.484 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 0.125 | 0.875 | 0.000 | 0.000 | 1.000 | 0.000 | 0.625 | 0.375 |
| **KC33** | 0.000 | 0.500 | 0.000 | 0.500 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 |
| **CNR17** | 0.000 | 0.500 | 0.000 | 0.500 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 0.318 | 0.591 | 0.091 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 |
| **CNR17** | 0.000 | 0.500 | 0.000 | 0.500 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 0.318 | 0.591 | 0.091 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 |
| **BA20** | 0.000 | 0.500 | 0.000 | 0.500 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 0.417 | 0.583 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **AN21** | 0.000 | 0.486 | 0.000 | 0.514 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 0.111 | 0.889 | 0.000 | 0.000 | 1.000 | 0.000 | 0.597 | 0.403 |
| **YA22** | 0.000 | 0.500 | 0.000 | 0.500 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 |
| **CH24** | 0.000 | 0.500 | 0.000 | 0.500 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 |
| **SW25** | 0.500 | 0.444 | 0.000 | 0.056 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 0.222 | 0.778 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 |
| **YR27** | 0.515 | 0.091 | 0.000 | 0.394 | 0.500 | 0.500 | 0.000 | 0.000 | 1.000 | 0.000 | 0.045 | 0.955 | 0.000 | 0.000 | 1.000 | 0.000 | 0.636 | 0.364 |
| **WH28** | 0.050 | 0.467 | 0.000 | 0.483 | 0.520 | 0.480 | 0.000 | 0.000 | 1.000 | 0.000 | 0.150 | 0.850 | 0.000 | 0.000 | 1.000 | 0.000 | 0.967 | 0.033 |
| **BT18** | 0.000 | 0.481 | 0.000 | 0.519 | 0.528 | 0.472 | 0.000 | 0.000 | 1.000 | 0.000 | 0.192 | 0.769 | 0.038 | 0.000 | 1.000 | 0.000 | 0.923 | 0.077 |
| **CG19** | 0.000 | 0.426 | ***0.574*** | 0.000 | 0.500 | 0.500 | 0.000 | ***1.000*** | 0.000 | 0.000 | 0.167 | 0.833 | 0.000 | 0.000 | 1.000 | 0.000 | 0.944 | 0.056 |

| | ***ml-Pa12*** | ***ml-Pa02*** | | | | ***ml-Pa14*** | | | | ***ml-Pa24*** | | | ***ml-Pa27*** | | | ***ml-Pa57*** | | ***ml-Pa21*** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Allele** | **315** | **198** | **200** | **202** | **276** | **286** | **288** | **290** | **235** | **238** | **241** | **211** | **213** | **215** | **157** | **159** | **158** |
| **FNQ31** | 1.000 | 0.000 | 0.000 | ***1.000*** | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 |
| **AT1** | 1.000 | 0.648 | 0.352 | 0.000 | 0.000 | 0.460 | 0.540 | 0.000 | 0.000 | 0.385 | 0.615 | 0.250 | 0.750 | 0.000 | 1.000 | 0.000 | 1.000 |
| **AT2** | 1.000 | 0.327 | 0.673 | 0.000 | 0.000 | 0.192 | 0.808 | 0.000 | 0.000 | 0.607 | 0.393 | 0.222 | 0.778 | 0.000 | 1.000 | 0.000 | 1.000 |
| **AT3** | 1.000 | 0.980 | 0.020 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.043 | 0.022 | 0.935 | 0.019 | 0.981 | 0.000 | 1.000 | 0.000 | 1.000 |
| **AT4** | 1.000 | 1.000 | 0.000 | 0.000 | 0.000 | 0.875 | 0.125 | 0.000 | 0.000 | 0.000 | 1.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 |
| **CA5** | 1.000 | 0.333 | 0.667 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 |
| **BY26** | 1.000 | 0.000 | 1.000 | 0.000 | ***0.333*** | 0.000 | 0.667 | 0.000 | 0.000 | 0.333 | 0.667 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 |
| **FI6** | 1.000 | 0.059 | 0.941 | 0.000 | 0.000 | 0.891 | 0.109 | 0.000 | 0.000 | 0.000 | 1.000 | 0.912 | 0.088 | 0.000 | 1.000 | 0.000 | 1.000 |
| **FI7** | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 |
| **FI8** | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.250 | 0.000 | 0.750 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 |
| **TCB32** | 1.000 | 0.207 | 0.793 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.207 | 0.793 | 0.017 | 0.983 | 0.000 | 1.000 | 0.000 | 1.000 |
| **PB13** | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 |
| **EU14** | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 |
| **MA34** | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.333 | 0.667 | 0.000 | 1.000 | 0.000 | 1.000 |
| **LT15** | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.767 | 0.233 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 |
| **SI9** | 1.000 | 0.355 | 0.645 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.355 | 0.645 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 |
| **SI10** | 1.000 | 0.097 | 0.903 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.016 | 0.097 | 0.887 | 0.071 | 0.929 | 0.000 | 1.000 | 0.000 | 1.000 |
| **SI11** | 1.000 | 0.310 | 0.690 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SI12** | 1.000 | 0.032 | 0.968 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.083 | 0.917 | 0.000 | 1.000 | 0.000 | 1.000 |
| **SI29** | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 |
| **PR16** | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.970 | ***0.030*** | 1.000 |
| **KC33** | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | ***1.000*** | 1.000 | 0.000 | 1.000 |
| **CNR17** | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 |
| **CNR17** | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 |
| **BA20** | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 |
| **AN21** | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.028 | 0.972 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 |
| **YA22** | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 |
| **CH24** | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 |
| **SW25** | 1.000 | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 |
| **YR27** | 1.000 | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 |
| **WH28** | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.242 | 0.758 | 0.000 | 1.000 | 0.000 | 1.000 |
| **BT18** | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 0.982 | 0.018 | 0.000 | 0.000 | 1.000 | 0.054 | 0.946 | 0.000 | 1.000 | 0.000 | 1.000 |
| **CG19** | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 |

Appendix 2: Stage matrices used for the *Phaius australis* current climate PVA models.

| FNQ 31 | SE | J1 | J2 | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SE | | | | | 15997 | | 17798 | | 15580 | | 30745 |
| J1 | 0.00003 | 0.296 | | | | | | | | | |
| J2 | | 0.148 | 0.544 | | | | | | | | |
| A1 | | | 0.181 | 0.495 | | | | | | | |
| A2 | | | | 0.165 | 0.499 | | | | | | |
| A3 | | | | | 0.499 | | | | | | |
| A4 | | | | | | 0.998 | 0.499 | | | | |
| A5 | | | | | | | 0.499 | | | | |
| A6 | | | | | | | | 0.998 | 0.499 | | |
| A7 | | | | | | | | | 0.499 | | |
| A8 | | | | | | | | | | 0.998 | 0.990 |

| AT1 | SE | J1 | J2 | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SE | | | | | 14977 | | 16663 | | 14586 | | 28784 |
| J1 | 0.00006 | 0.296 | | | | | | | | | |
| J2 | | 0.148 | 0.544 | | | | | | | | |
| A1 | | | 0.181 | 0.495 | | | | | | | |
| A2 | | | | 0.165 | 0.499 | | | | | | |
| A3 | | | | | 0.499 | | | | | | |
| A4 | | | | | | 0.998 | 0.499 | | | | |
| A5 | | | | | | | 0.499 | | | | |
| A6 | | | | | | | | 0.998 | 0.499 | | |
| A7 | | | | | | | | | 0.499 | | |
| A8 | | | | | | | | | | 0.998 | 0.990 |

| CA5 | SE | J1 | J2 | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SE | | | | | 6569 | | 7308 | | 6397 | | 12624 |
| J1 | 0.00002 | 0.241 | | | | | | | | | |
| J2 | | 0.121 | 0.443 | | | | | | | | |
| A1 | | | 0.148 | 0.495 | | | | | | | |
| A2 | | | | 0.165 | 0.499 | | | | | | |
| A3 | | | | | 0.499 | | | | | | |
| A4 | | | | | | 0.998 | 0.499 | | | | |
| A5 | | | | | | | 0.499 | | | | |
| A6 | | | | | | | | 0.998 | 0.499 | | |
| A7 | | | | | | | | | 0.499 | | |
| A8 | | | | | | | | | | 0.998 | 0.990 |

| PB13 | SE | J1 | J2 | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SE | | | | | 89386 | | 99447 | | 87052 | | 171791 |
| J1 | 0.000001 | 0.296 | | | | | | | | | |
| J2 | | 0.148 | 0.544 | | | | | | | | |
| A1 | | | 0.181 | 0.495 | | | | | | | |
| A2 | | | | 0.165 | 0.499 | | | | | | |
| A3 | | | | | 0.499 | | | | | | |
| A4 | | | | | | 0.998 | 0.499 | | | | |
| A5 | | | | | | | 0.499 | | | | |
| A6 | | | | | | | | 0.998 | 0.499 | | |
| A7 | | | | | | | | | 0.499 | | |
| A8 | | | | | | | | | | 0.998 | 0.990 |

| SI10 | SE | J1 | J2 | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SE | | | | | 5384 | | 5990 | | 5243 | | 10347 |
| J1 | 0.00011 | 0.320 | | | | | | | | | |
| J2 | | 0.160 | 0.588 | | | | | | | | |
| A1 | | | 0.196 | 0.495 | | | | | | | |
| A2 | | | | 0.165 | 0.499 | | | | | | |
| A3 | | | | | 0.499 | | | | | | |
| A4 | | | | | | 0.998 | 0.499 | | | | |
| A5 | | | | | | | 0.499 | | | | |
| A6 | | | | | | | | 0.998 | 0.499 | | |
| A7 | | | | | | | | | 0.499 | | |
| A8 | | | | | | | | | | 0.998 | 0.990 |

| SI9 | SE | J1 | J2 | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SE | | | | | 9991 | | 11115 | | 9730 | | 19201 |
| J1 | 0.00011 | 0.300 | | | | | | | | | |
| J2 | | 0.150 | 0.55 | | | | | | | | |
| A1 | | | 0.183 | 0.495 | | | | | | | |
| A2 | | | | 0.165 | 0.499 | | | | | | |
| A3 | | | | | 0.499 | | | | | | |
| A4 | | | | | | 0.998 | 0.499 | | | | |
| A5 | | | | | | | 0.499 | | | | |
| A6 | | | | | | | | 0.998 | 0.499 | | |
| A7 | | | | | | | | | 0.499 | | |
| A8 | | | | | | | | | | 0.998 | 0.990 |

| YA22 | SE | J1 | J2 | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SE | | | | | 34032 | | 37862 | | 33143 | | 65406 |
| J1 | 0.00001 | 0.296 | | | | | | | | | |
| J2 | | 0.148 | 0.544 | | | | | | | | |
| A1 | | | 0.181 | 0.495 | | | | | | | |
| A2 | | | | 0.165 | 0.499 | | | | | | |
| A3 | | | | | 0.499 | | | | | | |
| A4 | | | | | | 0.998 | 0.499 | | | | |
| A5 | | | | | | | 0.499 | | | | |
| A6 | | | | | | | | 0.998 | 0.499 | | |
| A7 | | | | | | | | | 0.499 | | |
| A8 | | | | | | | | | | 0.998 | 0.990 |

| WH28 | SE | J1 | J2 | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SE | | | | | 3766 | | 4190 | | 3667 | | 7237 |
| J1 | 0.00004 | 0.296 | | | | | | | | | |
| J2 | | 0.148 | 0.544 | | | | | | | | |
| A1 | | | 0.181 | 0.495 | | | | | | | |
| A2 | | | | 0.165 | 0.499 | | | | | | |
| A3 | | | | | 0.499 | | | | | | |
| A4 | | | | | | 0.998 | 0.499 | | | | |
| A5 | | | | | | | 0.499 | | | | |
| A6 | | | | | | | | 0.998 | 0.499 | | |
| A7 | | | | | | | | | 0.499 | | |
| A8 | | | | | | | | | | 0.998 | 0.990 |

| YR27 | SE | J1 | J2 | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SE | | | | | 23865 | | 26551 | | 23242 | | 45866 |
| J1 | 0.00002 | 0.296 | | | | | | | | | |
| J2 | | 0.148 | 0.544 | | | | | | | | |
| A1 | | | 0.181 | 0.495 | | | | | | | |
| A2 | | | | 0.165 | 0.499 | | | | | | |
| A3 | | | | | 0.499 | | | | | | |
| A4 | | | | | | 0.998 | 0.499 | | | | |
| A5 | | | | | | | 0.499 | | | | |
| A6 | | | | | | | | 0.998 | 0.499 | | |
| A7 | | | | | | | | | 0.499 | | |
| A8 | | | | | | | | | | 0.998 | 0.990 |

| SW25 | SE | J1 | J2 | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SE | | | | | 11432 | | 12719 | | 11133 | | 21971 |
| J1 | 0.00001 | 0.296 | | | | | | | | | |
| J2 | | 0.148 | 0.544 | | | | | | | | |
| A1 | | | 0.181 | 0.495 | | | | | | | |
| A2 | | | | 0.165 | 0.499 | | | | | | |
| A3 | | | | | 0.499 | | | | | | |
| A4 | | | | | | 0.998 | 0.499 | | | | |
| A5 | | | | | | | 0.499 | | | | |
| A6 | | | | | | | | 0.998 | 0.499 | | |
| A7 | | | | | | | | | 0.499 | | |
| A8 | | | | | | | | | | 0.998 | 0.990 |

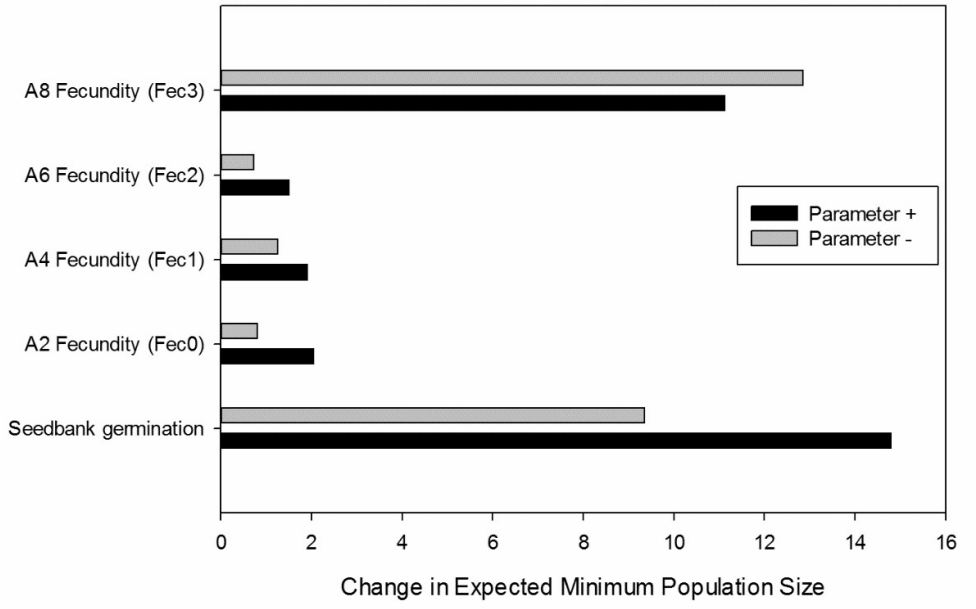
| BT18 | SE | J1 | J2 | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SE | | | | | 35689 | | 39706 | | 34757 | | 68590 |
| J1 | 0.00002 | 0.296 | | | | | | | | | |
| J2 | | 0.148 | 0.544 | | | | | | | | |
| A1 | | | 0.181 | 0.495 | | | | | | | |
| A2 | | | | 0.165 | 0.499 | | | | | | |
| A3 | | | | | 0.499 | | | | | | |
| A4 | | | | | | 0.998 | 0.499 | | | | |
| A5 | | | | | | | 0.499 | | | | |
| A6 | | | | | | | | 0.998 | 0.499 | | |
| A7 | | | | | | | | | 0.499 | | |
| A8 | | | | | | | | | | 0.998 | 0.990 |

| CG19 | SE | J1 | J2 | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SE | | | | | 27679 | | 30794 | | 26956 | | 53195 |
| J1 | 0.00002 | 0.296 | | | | | | | | | |
| J2 | | 0.148 | 0.544 | | | | | | | | |
| A1 | | | 0.181 | 0.495 | | | | | | | |
| A2 | | | | 0.165 | 0.499 | | | | | | |
| A3 | | | | | 0.499 | | | | | | |
| A4 | | | | | | 0.998 | 0.499 | | | | |
| A5 | | | | | | | 0.499 | | | | |
| A6 | | | | | | | | 0.998 | 0.499 | | |
| A7 | | | | | | | | | 0.499 | | |
| A8 | | | | | | | | | | 0.998 | 0.990 |

Appendix 3. Parameters used in the *P. australis* PVA models. Parameter code is given, the source of reference for the parameter and/or justification for their calculation is given.

| Parameter | Value | Source / Justification |
|---|---|---|
| S0, S1, S2, S3, S4, S5, S6, S7, S8, S9 | | Calculated using paired field data. Calculations were made using a Lefkovitch matrix, a survivor frequency table and the formula: $S_i = n_{(t+1)} / n_{(t)}$ was used, where $n_t$ is the abundance of a given stage class at time *t*, and $n_{(t+1)}$ equals the abundance in a given stage class at time *t+1* (Caswell 2000; Akcakaya et al. 1999) |
| Ss0 | | This rate set at extremely low value to reflect that 5% of viable seed survives 1 year |
| Ss1, Ss2, Ss3, Ss4, Ss5, Ss6, Ss7, Ss8, Ss9, Ss10 | | Calculated using paired field data. Calculations were made using a Lefkovitch matrix, a survivor frequency table and the formula: $S_i = n_{(t+1)} / n_{(t)}$ was used, where $n_t$ is the abundance of a given stage class at time *t*, and $n_{(t+1)}$ equals the abundance in a given stage class at time *t+1* (Caswell 2000; Akcakaya et al. 1999) |
| Fec0, Fec1, Fec2, Fec3 | | Estimated using the formula modified from Keith (2004) and Simmons (2012): $Fec_i$ = *RO-Inf* * *RO-F/Inf* * *%S* **nS* **v*; where *RRO-Inf* is the relative output of inflorescences of each reproductive adult stage; *RRO-F/Inf* is the relative output of number of flowers per inflorescence of each reproductive adult stage, *%S* is proportion of fruit set per inflorescence, *nS* is the estimated number of seed per fruit and *v*, is the seed viability rate estimated for congener *P. tankervilleae* according to Hirano (2009). This value is affected by the *.FCH file, which instigates a temporal trend in fecundity in the year following a fire. |

Appendix 4: Sensitivity analysis of *P. australis* PVA parameters that were not estimated from field data. The names of the parameter are given with +10% and -10% indicated. The resulting percentage change in final population abundance is given.



Appendix 5: Calculated stage matrix values for the 'best case scenario' *P. australis* PVA. Calculations derived from SimCLIM generated RCP 2.6 ($T_{MAX}$, $Pr_{JFM}$, $Pr_{JJA}$) outputs.

| | | S0 | Ss0 | S1 | Ss1 | S2 | Fec0 | Fec1 | Fec2 | Fec3 |
|---|---|---|---|---|---|---|---|---|---|---|
| FNQ31 | Current | 0.00003 | 0.148 | 0.296 | 0.181 | 0.544 | 15997 | 17798 | 15580 | 30745 |
| | 2020 | 0.00003 | 0.150 | 0.300 | 0.184 | 0.551 | 15957 | 17753 | 15540 | 30667 |
| | 2030 | 0.00003 | 0.148 | 0.302 | 0.185 | 0.554 | 15982 | 17781 | 15564 | 30715 |
| | 2040 | 0.00003 | 0.151 | 0.302 | 0.185 | 0.554 | 15981 | 17780 | 15564 | 30714 |
| | 2050 | 0.00003 | 0.150 | 0.301 | 0.184 | 0.552 | 15984 | 17783 | 15567 | 30720 |
| | 2060 | 0.00003 | 0.149 | 0.299 | 0.183 | 0.549 | 15990 | 17790 | 15572 | 30731 |
| | 2070 | 0.00003 | 0.148 | 0.296 | 0.181 | 0.544 | 15997 | 17797 | 15579 | 30744 |
| AT1 | Current | 0.00003 | 0.148 | 0.296 | 0.181 | 0.544 | 14977 | 16663 | 14586 | 28784 |
| | 2020 | 0.00003 | 0.150 | 0.300 | 0.184 | 0.551 | 14963 | 16647 | 14572 | 28757 |
| | 2030 | 0.00003 | 0.151 | 0.302 | 0.185 | 0.554 | 14960 | 16644 | 14569 | 28752 |
| | 2040 | 0.00003 | 0.151 | 0.302 | 0.185 | 0.554 | 14959 | 16643 | 14569 | 28751 |
| | 2050 | 0.00003 | 0.151 | 0.302 | 0.184 | 0.552 | 14963 | 16647 | 14572 | 28757 |
| | 2060 | 0.00003 | 0.150 | 0.299 | 0.183 | 0.549 | 14969 | 16654 | 14578 | 28769 |
| | 2070 | 0.00003 | 0.148 | 0.296 | 0.181 | 0.544 | 14976 | 16662 | 14585 | 28783 |
| CA5 | Current | 0.00002 | 0.148 | 0.296 | 0.181 | 0.544 | 7076 | 7873 | 6892 | 13600 |
| | 2020 | 0.00002 | 0.150 | 0.300 | 0.184 | 0.551 | 7071 | 7867 | 6887 | 13591 |
| | 2030 | 0.00002 | 0.151 | 0.302 | 0.185 | 0.554 | 7032 | 7823 | 6848 | 13514 |
| | 2040 | 0.00002 | 0.151 | 0.302 | 0.185 | 0.554 | 7057 | 7851 | 6873 | 13563 |
| | 2050 | 0.00002 | 0.151 | 0.301 | 0.184 | 0.552 | 7039 | 7831 | 6855 | 13528 |
| | 2060 | 0.00002 | 0.150 | 0.299 | 0.183 | 0.549 | 7055 | 7849 | 6870 | 13558 |
| | 2070 | 0.00002 | 0.148 | 0.296 | 0.181 | 0.544 | 7075 | 7871 | 6890 | 13597 |
| PB13 | Current | 0.000001 | 0.148 | 0.296 | 0.181 | 0.544 | 89386 | 99447 | 87052 | 171791 |
| | 2020 | 0.000001 | 0.150 | 0.300 | 0.184 | 0.551 | 89189 | 99229 | 86860 | 171413 |
| | 2030 | 0.000001 | 0.151 | 0.302 | 0.185 | 0.554 | 89579 | 99663 | 87240 | 172162 |
| | 2040 | 0.000001 | 0.151 | 0.302 | 0.185 | 0.554 | 89585 | 99669 | 87246 | 172173 |
| | 2050 | 0.000001 | 0.150 | 0.301 | 0.184 | 0.552 | 89548 | 99627 | 87209 | 172101 |
| | 2060 | 0.000001 | 0.149 | 0.299 | 0.183 | 0.549 | 89479 | 99551 | 87143 | 171970 |
| | 2070 | 0.000001 | 0.149 | 0.296 | 0.181 | 0.544 | 89393 | 99456 | 87059 | 171805 |
| SI9 | Current | 0.00011 | 0.148 | 0.296 | 0.181 | 0.544 | 10010 | 11137 | 9749 | 19238 |
| | 2020 | 0.00011 | 0.150 | 0.300 | 0.183 | 0.55 | 9991 | 11115 | 9730 | 19201 |
| | 2030 | 0.00011 | 0.151 | 0.301 | 0.184 | 0.553 | 10036 | 11166 | 9774 | 19288 |
| | 2040 | 0.00011 | 0.151 | 0.301 | 0.184 | 0.553 | 10037 | 11167 | 9775 | 19290 |
| | 2050 | 0.00011 | 0.150 | 0.300 | 0.184 | 0.551 | 10032 | 11161 | 9770 | 19280 |
| | 2060 | 0.00011 | 0.149 | 0.299 | 0.183 | 0.548 | 10023 | 11151 | 9761 | 19263 |
| | 2070 | 0.00011 | 0.148 | 0.296 | 0.181 | 0.544 | 10011 | 11138 | 9750 | 19240 |
| SI10 | Current | 0.00011 | 0.148 | 0.296 | 0.181 | 0.544 | 5290 | 5886 | 5152 | 10167 |
| | 2020 | 0.00011 | 0.150 | 0.300 | 0.183 | 0.55 | 5275 | 5869 | 5138 | 10139 |
| | 2030 | 0.00011 | 0.151 | 0.301 | 0.184 | 0.553 | 5310 | 5908 | 5171 | 10205 |
| | 2040 | 0.00011 | 0.151 | 0.301 | 0.184 | 0.553 | 5311 | 5908 | 5172 | 10206 |
| | 2050 | 0.00011 | 0.150 | 0.300 | 0.184 | 0.551 | 5307 | 5904 | 5168 | 10199 |
| | 2060 | 0.00011 | 0.149 | 0.299 | 0.183 | 0.548 | 5300 | 5896 | 5161 | 10185 |
| | 2070 | 0.00011 | 0.148 | 0.296 | 0.181 | 0.544 | 5291 | 5886 | 5153 | 10169 |

| | | S0 | Ss0 | S1 | Ss1 | S2 | Fec0 | Fec1 | Fec2 | Fec3 |
|---|---|---|---|---|---|---|---|---|---|---|
| WH28 | Current | 0.00004 | 0.148 | 0.296 | 0.181 | 0.544 | 3766 | 4190 | 3667 | 7237 |
| | 2020 | 0.00004 | 0.150 | 0.300 | 0.183 | 0.55 | 3756 | 4179 | 3658 | 7219 |
| | 2030 | 0.00004 | 0.151 | 0.301 | 0.184 | 0.553 | 3787 | 4213 | 3688 | 7278 |
| | 2040 | 0.00004 | 0.151 | 0.301 | 0.184 | 0.553 | 3787 | 4214 | 3688 | 7279 |
| | 2050 | 0.00004 | 0.150 | 0.300 | 0.184 | 0.551 | 3783 | 4209 | 3685 | 7271 |
| | 2060 | 0.00004 | 0.149 | 0.299 | 0.183 | 0.548 | 3776 | 4201 | 3677 | 7257 |
| | 2070 | 0.00004 | 0.148 | 0.296 | 0.181 | 0.544 | 3767 | 4190 | 3668 | 7239 |
| YA22 | Current | 0.00001 | 0.148 | 0.296 | 0.181 | 0.544 | 34032 | 37862 | 33143 | 65406 |
| | 2020 | 0.00001 | 0.150 | 0.300 | 0.183 | 0.554 | 33961 | 37784 | 33074 | 65270 |
| | 2030 | 0.00001 | 0.151 | 0.301 | 0.184 | 0.553 | 34190 | 38038 | 33297 | 65709 |
| | 2040 | 0.00001 | 0.151 | 0.301 | 0.184 | 0.553 | 34194 | 38043 | 33301 | 65717 |
| | 2050 | 0.00001 | 0.150 | 0.300 | 0.184 | 0.551 | 34164 | 38009 | 33272 | 65659 |
| | 2060 | 0.00001 | 0.149 | 0.299 | 0.183 | 0.548 | 34108 | 37947 | 33217 | 65552 |
| | 2070 | 0.00001 | 0.148 | 0.296 | 0.181 | 0.544 | 34038 | 37869 | 33149 | 65418 |
| YR27 | Current | 0.00003 | 0.148 | 0.296 | 0.181 | 0.544 | 23865 | 26551 | 23242 | 45866 |
| | 2020 | 0.00003 | 0.150 | 0.300 | 0.183 | 0.55 | 23837 | 26520 | 23215 | 45813 |
| | 2030 | 0.00003 | 0.151 | 0.301 | 0.184 | 0.553 | 23933 | 26627 | 23308 | 45997 |
| | 2040 | 0.00003 | 0.151 | 0.301 | 0.184 | 0.553 | 23935 | 26629 | 23310 | 46001 |
| | 2050 | 0.00003 | 0.150 | 0.300 | 0.184 | 0.551 | 23922 | 26615 | 23297 | 45975 |
| | 2060 | 0.00003 | 0.149 | 0.299 | 0.183 | 0.548 | 23898 | 26588 | 23274 | 45929 |
| | 2070 | 0.00003 | 0.148 | 0.296 | 0.181 | 0.544 | 23868 | 26555 | 23245 | 45872 |
| SW25 | Current | 0.00001 | 0.148 | 0.296 | 0.181 | 0.544 | 11432 | 12719 | 11133 | 21971 |
| | 2020 | 0.00001 | 0.150 | 0.300 | 0.183 | 0.55 | 11400 | 12684 | 11103 | 21910 |
| | 2030 | 0.00001 | 0.151 | 0.301 | 0.184 | 0.553 | 11483 | 12776 | 11183 | 22069 |
| | 2040 | 0.00001 | 0.151 | 0.301 | 0.184 | 0.553 | 11485 | 12777 | 11185 | 22072 |
| | 2050 | 0.00001 | 0.150 | 0.300 | 0.184 | 0.551 | 11475 | 12766 | 11175 | 22053 |
| | 2060 | 0.00001 | 0.149 | 0.299 | 0.183 | 0.548 | 11457 | 12746 | 11157 | 22019 |
| | 2070 | 0.00001 | 0.148 | 0.296 | 0.181 | 0.544 | 11434 | 12721 | 11135 | 21975 |
| BT18 | Current | 0.00007 | 0.296 | 0.148 | 0.544 | 0.181 | 35689 | 39706 | 34757 | 68590 |
| | 2020 | 0.00007 | 0.301 | 0.151 | 0.552 | 0.184 | 35666 | 39681 | 34735 | 68547 |
| | 2030 | 0.00007 | 0.303 | 0.152 | 0.557 | 0.186 | 35737 | 39760 | 34804 | 68683 |
| | 2040 | 0.00007 | 0.303 | 0.152 | 0.557 | 0.186 | 35738 | 39761 | 34805 | 68685 |
| | 2050 | 0.00007 | 0.302 | 0.151 | 0.554 | 0.185 | 35729 | 39751 | 34796 | 68668 |
| | 2060 | 0.00007 | 0.300 | 0.150 | 0.55 | 0.183 | 35712 | 39732 | 34780 | 68635 |
| | 2070 | 0.00007 | 0.297 | 0.148 | 0.544 | 0.181 | 35691 | 39708 | 34759 | 68594 |
| CG19 | Current | 0.00002 | 0.296 | 0.148 | 0.544 | 0.181 | 27679 | 30794 | 26956 | 53195 |
| | 2020 | 0.00002 | 0.301 | 0.150 | 0.552 | 0.184 | 27655 | 30768 | 26933 | 53150 |
| | 2030 | 0.00002 | 0.303 | 0.152 | 0.556 | 0.185 | 27719 | 30839 | 26995 | 53273 |
| | 2040 | 0.00002 | 0.296 | 0.148 | 0.544 | 0.181 | 27720 | 30841 | 26996 | 53276 |
| | 2050 | 0.00002 | 0.302 | 0.151 | 0.554 | 0.185 | 27712 | 30832 | 26989 | 53260 |
| | 2060 | 0.00002 | 0.300 | 0.150 | 0.55 | 0.183 | 27698 | 30816 | 26975 | 53233 |
| | 2070 | 0.00002 | 0.297 | 0.148 | 0.544 | 0.181 | 27680 | 30796 | 26957 | 53198 |

Appendix 6. Calculated stage matrix values for the 'worst case scenario' *P. australis* PVA. Calculations derived from SimCLIM generated RCP 8.5 ($T_{MAX}$, $Pr_{JFM}$, $Pr_{JJA}$) outputs.

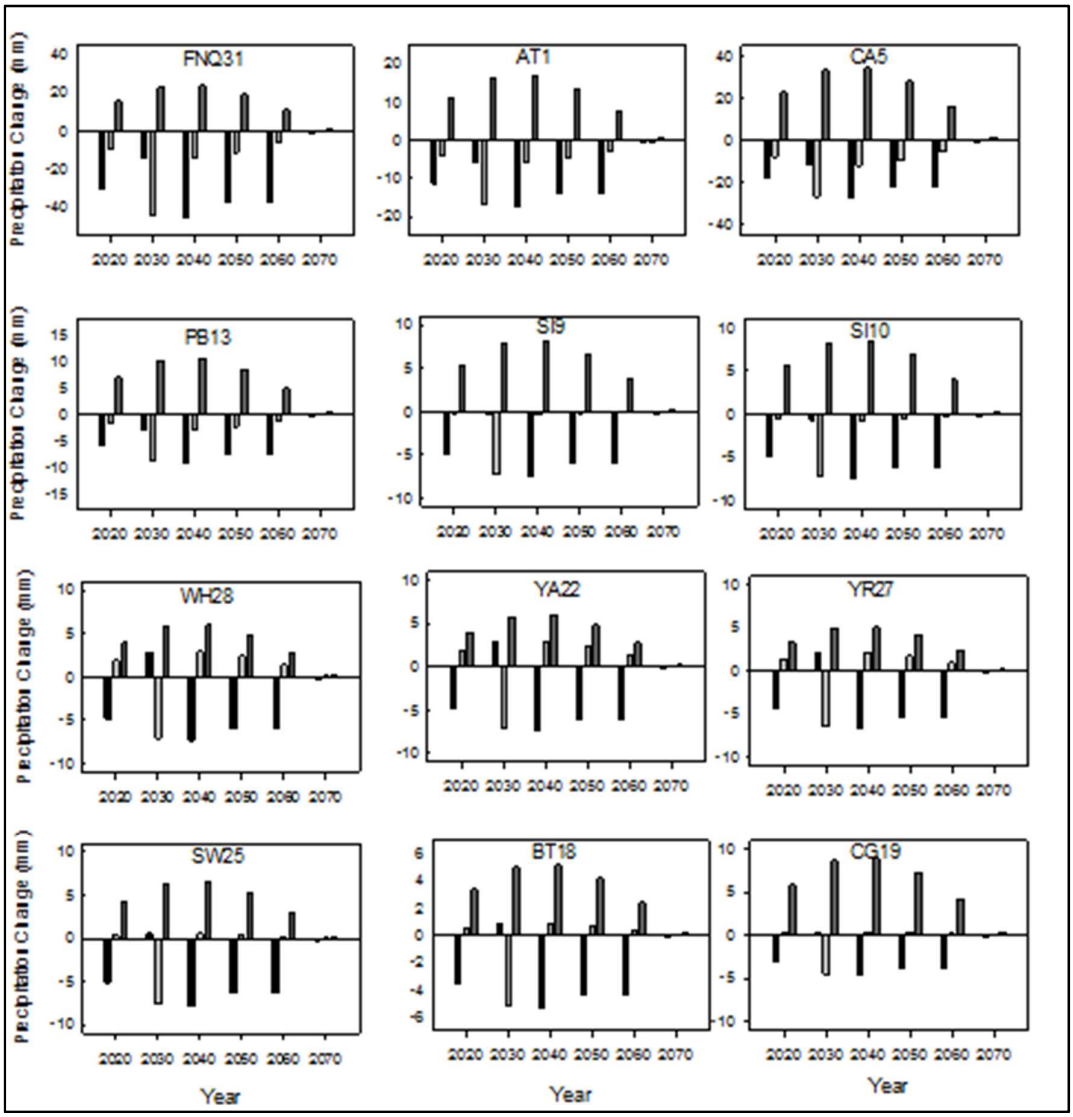
| | | S0 | Ss0 | S1 | Ss1 | S2 | Fec0 | Fec1 | Fec2 | Fec3 |
|---|---|---|---|---|---|---|---|---|---|---|
| FNQ31 | Current | 0.00003 | 0.148 | 0.296 | 0.181 | 0.544 | 15997 | 17798 | 15580 | 30745 |
| | 2020 | 0.00003 | 0.149 | 0.298 | 0.149 | 0.547 | 15902 | 17691 | 15486 | 30561 |
| | 2030 | 0.00003 | 0.147 | 0.295 | 0.180 | 0.541 | 15861 | 17646 | 15447 | 30483 |
| | 2040 | 0.00003 | 0.145 | 0.290 | 0.177 | 0.531 | 15816 | 17596 | 15403 | 30396 |
| | 2050 | 0.00003 | 0.141 | 0.282 | 0.173 | 0.518 | 15768 | 17543 | 15356 | 30304 |
| | 2060 | 0.00003 | 0.137 | 0.274 | 0.168 | 0.503 | 15712 | 17480 | 15301 | 30196 |
| | 2070 | 0.00003 | 0.132 | 0.265 | 0.162 | 0.485 | 15653 | 17415 | 15244 | 30083 |
| AT1 | Current | 0.00006 | 0.148 | 0.296 | 0.181 | 0.544 | 14977 | 16663 | 14586 | 28784 |
| | 2020 | 0.00006 | 0.150 | 0.300 | 0.184 | 0.551 | 14908 | 16586 | 14519 | 28652 |
| | 2030 | 0.00006 | 0.151 | 0.302 | 0.185 | 0.554 | 14865 | 16538 | 14477 | 28569 |
| | 2040 | 0.00006 | 0.150 | 0.300 | 0.184 | 0.551 | 14818 | 16485 | 14431 | 28478 |
| | 2050 | 0.00006 | 0.148 | 0.297 | 0.182 | 0.545 | 14766 | 16428 | 14381 | 28379 |
| | 2060 | 0.00006 | 0.151 | 0.302 | 0.183 | 0.549 | 14711 | 16367 | 14327 | 28274 |
| | 2070 | 0.00006 | 0.143 | 0.286 | 0.181 | 0.544 | 14653 | 16302 | 14270 | 28161 |
| CA5 | Current | 0.00002 | 0.148 | 0.296 | 0.181 | 0.544 | 7076 | 7873 | 6892 | 13600 |
| | 2020 | 0.00002 | 0.154 | 0.308 | 0.189 | 0.566 | 6933 | 7714 | 6752 | 13325 |
| | 2030 | 0.00002 | 0.142 | 0.284 | 0.174 | 0.521 | 6821 | 7589 | 6643 | 13109 |
| | 2040 | 0.00002 | 0.132 | 0.263 | 0.161 | 0.483 | 6699 | 7453 | 6524 | 12875 |
| | 2050 | 0.00002 | 0.121 | 0.241 | 0.148 | 0.443 | 6569 | 7308 | 6397 | 12624 |
| | 2060 | 0.00001 | 0.109 | 0.217 | 0.133 | 0.399 | 6430 | 7154 | 6262 | 12358 |
| | 2070 | 0.00001 | 0.096 | 0.192 | 0.118 | 0.353 | 6284 | 6992 | 6120 | 12078 |
| PB13 | Current | 0.000001 | 0.148 | 0.296 | 0.181 | 0.544 | 89386 | 99447 | 87052 | 171791 |
| | 2020 | 0.000001 | 0.154 | 0.309 | 0.189 | 0.566 | 89750 | 99853 | 87406 | 172491 |
| | 2030 | 0.000001 | 0.161 | 0.322 | 0.197 | 0.592 | 90237 | 100395 | 87881 | 173427 |
| | 2040 | 0.000001 | 0.169 | 0.337 | 0.206 | 0.619 | 90769 | 100986 | 88398 | 174448 |
| | 2050 | 0.000001 | 0.177 | 0.354 | 0.216 | 0.649 | 91341 | 101622 | 88956 | 175548 |
| | 2060 | 0.000001 | 0.185 | 0.371 | 0.227 | 0.680 | 91952 | 102302 | 89550 | 176722 |
| | 2070 | 0.000001 | 0.194 | 0.388 | 0.237 | 0.712 | 92598 | 103021 | 90180 | 177965 |
| SI9 | Current | 0.00011 | 0.148 | 0.296 | 0.181 | 0.544 | 10010 | 11137 | 9749 | 19238 |
| | 2020 | 0.00011 | 0.154 | 0.308 | 0.188 | 0.565 | 10068 | 11201 | 9805 | 19349 |
| | 2030 | 0.00012 | 0.160 | 0.320 | 0.196 | 0.588 | 10134 | 11274 | 9869 | 19476 |
| | 2040 | 0.00012 | 0.167 | 0.334 | 0.205 | 0.614 | 10206 | 11355 | 9939 | 19615 |
| | 2050 | 0.00013 | 0.175 | 0.349 | 0.214 | 0.641 | 10284 | 11442 | 10015 | 19765 |
| | 2060 | 0.00014 | 0.183 | 0.365 | 0.224 | 0.671 | 10368 | 11534 | 10097 | 19925 |
| | 2070 | 0.00014 | 0.191 | 0.383 | 0.234 | 0.702 | 10456 | 11633 | 10183 | 20096 |
| SI10 | Current | 0.00011 | 0.148 | 0.296 | 0.154 | 0.308 | 5290 | 5886 | 5152 | 10167 |
| | 2020 | 0.00011 | 0.154 | 0.308 | 0.188 | 0.565 | 5334 | 5934 | 5194 | 10251 |
| | 2030 | 0.00012 | 0.160 | 0.320 | 0.196 | 0.588 | 5384 | 5990 | 5243 | 10347 |
| | 2040 | 0.00012 | 0.167 | 0.334 | 0.205 | 0.614 | 5439 | 6051 | 5297 | 10452 |
| | 2050 | 0.00013 | 0.175 | 0.349 | 0.214 | 0.641 | 5498 | 6116 | 5354 | 10566 |
| | 2060 | 0.00014 | 0.183 | 0.366 | 0.224 | 0.671 | 5561 | 6187 | 5416 | 10688 |
| | 2070 | 0.00014 | 0.191 | 0.383 | 0.234 | 0.703 | 5628 | 6262 | 5481 | 10817 |

| | | S0 | Ss0 | S1 | Ss1 | S2 | Fec0 | Fec1 | Fec2 | Fec3 |
|---|---|---|---|---|---|---|---|---|---|---|
| WH28 | Current | 0.00004 | 0.154 | 0.308 | 0.188 | 0.564 | 3766 | 4190 | 3667 | 7237 |
| | 2020 | 0.00004 | 0.160 | 0.320 | 0.196 | 0.587 | 3820 | 4250 | 3720 | 7342 |
| | 2030 | 0.00004 | 0.167 | 0.334 | 0.204 | 0.612 | 3874 | 4310 | 3772 | 7445 |
| | 2040 | 0.00005 | 0.174 | 0.348 | 0.213 | 0.639 | 3933 | 4375 | 3830 | 7558 |
| | 2050 | 0.00005 | 0.182 | 0.364 | 0.223 | 0.668 | 3997 | 4447 | 3893 | 7682 |
| | 2060 | 0.00005 | 0.191 | 0.381 | 0.233 | 0.699 | 4066 | 4524 | 3960 | 7815 |
| | 2070 | 0.00005 | 0.148 | 0.296 | 0.181 | 0.544 | 4140 | 4606 | 4032 | 7957 |
| YA22 | Current | 0.00001 | 0.154 | 0.308 | 0.188 | 0.564 | 34032 | 37862 | 33143 | 65406 |
| | 2020 | 0.00001 | 0.160 | 0.320 | 0.196 | 0.587 | 34436 | 38312 | 33536 | 66182 |
| | 2030 | 0.00001 | 0.167 | 0.334 | 0.204 | 0.612 | 34837 | 38758 | 33927 | 66953 |
| | 2040 | 0.00001 | 0.147 | 0.348 | 0.213 | 0.639 | 35277 | 39248 | 34356 | 67800 |
| | 2050 | 0.00001 | 0.182 | 0.365 | 0.223 | 0.670 | 35756 | 39781 | 34822 | 68719 |
| | 2060 | 0.00001 | 0.192 | 0.383 | 0.234 | 0.703 | 36271 | 40354 | 35324 | 69709 |
| | 2070 | 0.00001 | 0.148 | 0.296 | 0.181 | 0.544 | 36821 | 40966 | 35859 | 70766 |
| YR27 | Current | 0.00003 | 0.154 | 0.308 | 0.188 | 0.564 | 23865 | 26551 | 23242 | 45866 |
| | 2020 | 0.00003 | 0.160 | 0.320 | 0.196 | 0.587 | 24043 | 26749 | 23415 | 46208 |
| | 2030 | 0.00003 | 0.167 | 0.334 | 0.204 | 0.612 | 24216 | 26942 | 23584 | 46542 |
| | 2040 | 0.00003 | 0.174 | 0.348 | 0.213 | 0.639 | 24407 | 27154 | 23770 | 46908 |
| | 2050 | 0.00004 | 0.182 | 0.364 | 0.223 | 0.668 | 24613 | 27384 | 23970 | 47304 |
| | 2060 | 0.00004 | 0.191 | 0.381 | 0.233 | 0.669 | 24836 | 27631 | 24187 | 47731 |
| | 2070 | 0.00004 | 0.148 | 0.296 | 0.181 | 0.544 | 25072 | 27895 | 24418 | 48187 |
| SW25 | Current | 0.00001 | 0.154 | 0.308 | 0.188 | 0.565 | 11432 | 12719 | 11133 | 21971 |
| | 2020 | 0.00001 | 0.160 | 0.320 | 0.196 | 0.588 | 11552 | 12853 | 11250 | 22202 |
| | 2030 | 0.00001 | 0.167 | 0.334 | 0.204 | 0.613 | 11682 | 12997 | 11377 | 22451 |
| | 2040 | 0.00001 | 0.175 | 0.349 | 0.214 | 0.641 | 11824 | 13155 | 11515 | 22724 |
| | 2050 | 0.00001 | 0.183 | 0.365 | 0.223 | 0.670 | 11977 | 13326 | 11665 | 23019 |
| | 2060 | 0.00001 | 0.191 | 0.382 | 0.234 | 0.702 | 12142 | 13509 | 11825 | 23336 |
| | 2070 | 0.00001 | 0.148 | 0.296 | 0.181 | 0.544 | 12318 | 13705 | 11996 | 23674 |
| BT18 | Current | 0.00007 | 0.150 | 0.300 | 0.184 | 0.551 | 35689 | 39706 | 34757 | 68590 |
| | 2020 | 0.00008 | 0.141 | 0.283 | 0.173 | 0.591 | 35811 | 39842 | 34876 | 68825 |
| | 2030 | 0.00007 | 0.132 | 0.263 | 0.161 | 0.483 | 35933 | 39978 | 34995 | 69060 |
| | 2040 | 0.00007 | 0.121 | 0.242 | 0.148 | 0.445 | 36067 | 40127 | 35126 | 69318 |
| | 2050 | 0.00006 | 0.110 | 0.220 | 0.135 | 0.404 | 36213 | 40289 | 35267 | 69597 |
| | 2060 | 0.00006 | 0.098 | 0.196 | 0.120 | 0.360 | 36368 | 40462 | 35419 | 69896 |
| | 2070 | 0.00005 | 0.296 | 0.148 | 0.544 | 0.181 | 36534 | 40647 | 35580 | 70215 |
| CG19 | Current | 0.00002 | 0.288 | 0.144 | 0.529 | 0.176 | 27679 | 30794 | 26956 | 53195 |
| | 2020 | 0.00002 | 0.274 | 0.135 | 0.497 | 0.166 | 27777 | 30903 | 27051 | 53384 |
| | 2030 | 0.00002 | 0.251 | 0.126 | 0.462 | 0.154 | 27879 | 31018 | 27151 | 53581 |
| | 2040 | 0.00002 | 0.231 | 0.115 | 0.424 | 0.141 | 27992 | 31143 | 27261 | 53798 |
| | 2050 | 0.00002 | 0.231 | 0.115 | 0.424 | 0.141 | 28115 | 31279 | 27380 | 54034 |
| | 2060 | 0.00001 | 0.185 | 0.093 | 0.340 | 0.113 | 28246 | 31425 | 27508 | 54285 |
| | 2070 | 0.00001 | 0.148 | 0.296 | 0.181 | 0.544 | 28385 | 31580 | 27644 | 54553 |

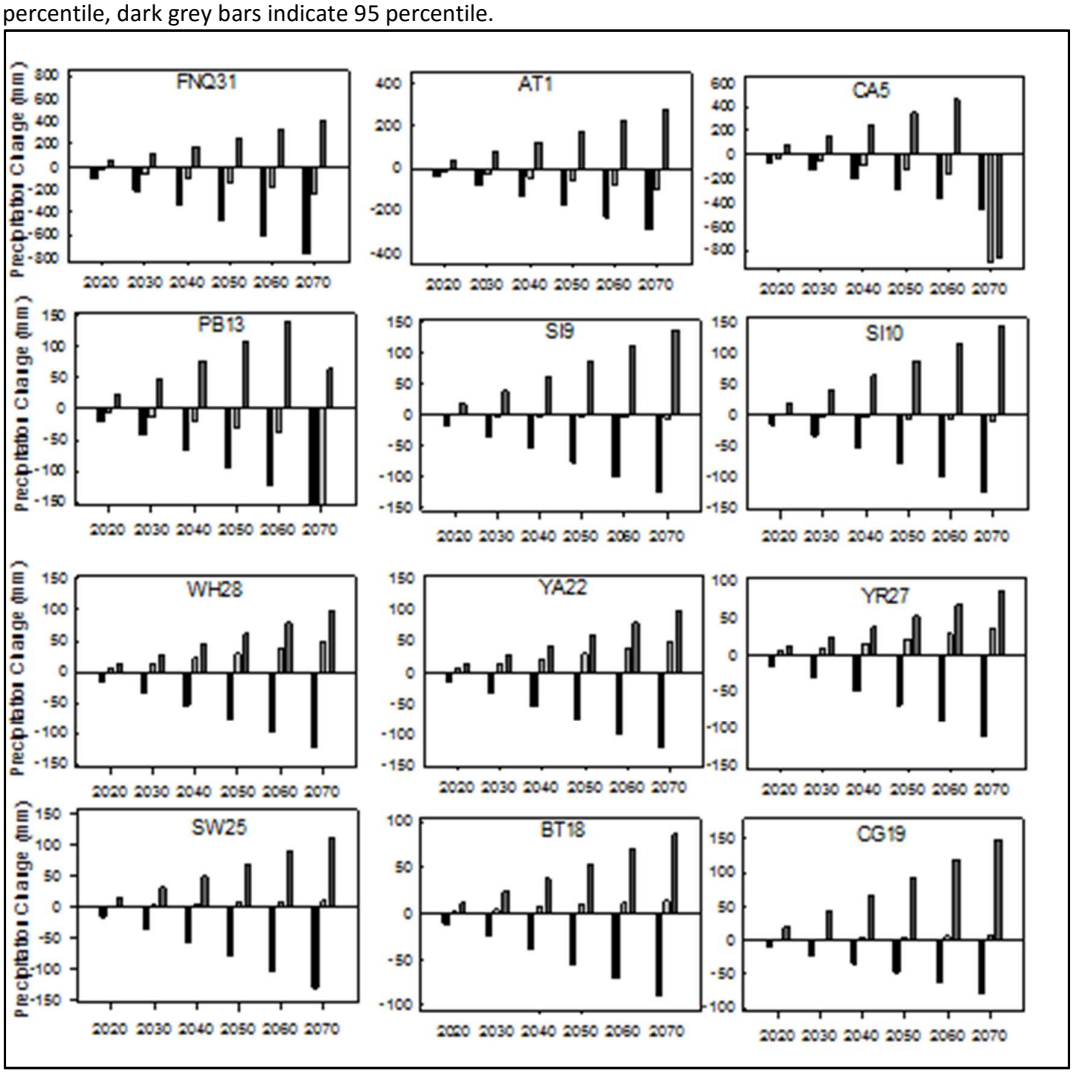
Appendix 7. Cumulative quasi-extinction probability (PQE) for each *P. australis* population at the end of the 100-year PVA model duration for each climate change scenario.

| Region | Population | Baseline | RCP 2.6 | RCP 8.5 |
|---|---|---|---|---|
| North | FNQ31 | 0.000 | 0.000 | 0.000 |
| | AT1 | 0.000 | 0.000 | 0.000 |
| | CA5 | 0.000 | **0.034** | **0.033** |
| Mid | PB13 | 0.000 | 0.000 | 0.000 |
| | SI9 | 0.000 | 0.000 | 0.000 |
| | SI10 | 0.000 | 0.000 | 0.000 |
| South | WH28 | 0.000 | 0.000 | 0.000 |
| | YA22 | **0.002** | 0.000 | 0.000 |
| | YR27 | 0.000 | 0.000 | 0.000 |
| | SW25 | 0.000 | 0.000 | 0.000 |
| Inland | BT18 | 0.000 | 0.000 | 0.000 |
| | CG19 | 0.000 | 0.000 | 0.000 |

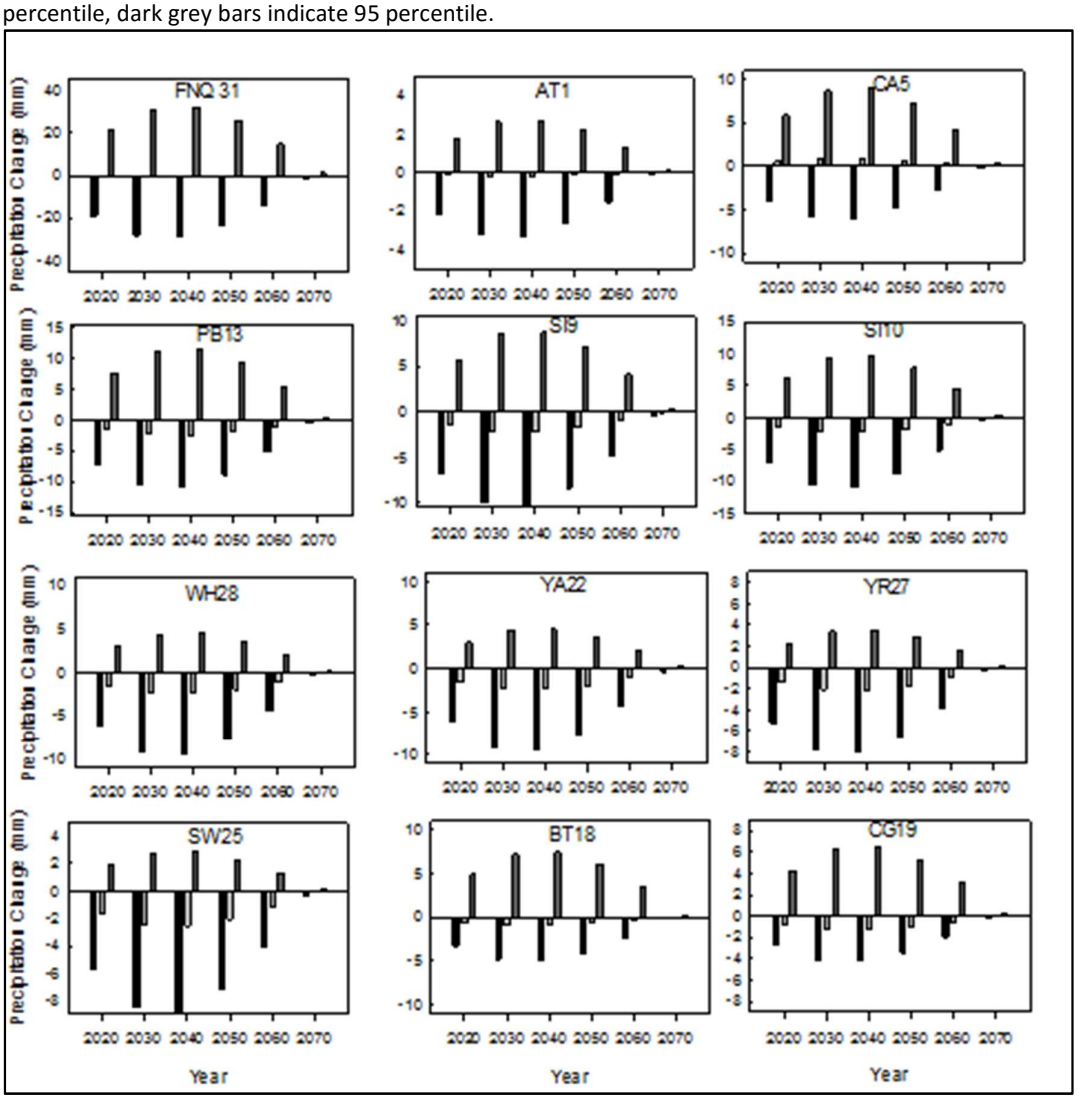
Appendix 8. Predicted change in precipitation (mm) of the wettest months (January, February, March) at *P. australis* sites from 2020 to 2070 for emission scenario RCP 2.6; 5th and 95th percentiles of climate ensemble models given. For all, black bars indicate 5th percentile, light grey bars indicate 50th percentile, dark grey bars indicate 95 percentile.



Appendix 9. Predicted change in precipitation (mm) of the wettest months (January, February, March) at *P. australis* sites from 2020 to 2070 for emission scenario RCP 8.5; 5th and 95th percentiles of climate ensemble models given. For all, black bars indicate 5th percentile, light grey bars indicate 50th percentile, dark grey bars indicate 95 percentile.



Appendix 10. Predicted change in precipitation (mm) of the driest months (June, July, August) at *P. australis* sites from 2020 to 2070 for emission scenario RCP 2.6; 5th and 95th percentiles of climate ensemble models given. For all, black bars indicate 5th percentile, light grey bars indicate 50th percentile, dark grey bars indicate 95 percentile.



Appendix 11. Predicted change in precipitation (mm) of the driest months (June, July, August) at *P. australis* sites from 2020 to 2070 for emission scenario RCP 8.5; 5th and 95th percentiles of climate ensemble models given. For all, black bars indicate 5th percentile, light grey bars indicate 50th percentile, dark grey bars indicate 95 percentile.