Seed dispersal and population genetic variation in the context of fire

A case study on two plant species in fire-prone mallee in South Australia

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ABSTRACT

Fire is and has been a common occurrence in many vegetation types across the world, impacting on the structure and function of those fire-prone ecosystems. Human influence and a changing climate are causing changes in fire regimes, particularly intervals between fires and the seasonal timing of fires. The frequencies of fires are increasing and fire seasons are tending to shift from hot and dry seasons (natural fires) to cooler and wetter seasons (prescribed burns). Clearing for farmland or urban settlements, in which fire-prone vegetation occurs demands new knowledge to understand the interactions between fire regimes and biodiversity.

In this study I examined the effects of fire on two plant species, *Triodia irritans,* and *Callitris verrucosa*. Both species are important features of mallee vegetation and have primarily wind mediated seed dispersal but show different life history traits. The study was carries out at Hincks Wilderness Protection Area on the Eyre Peninsula, South Australia, aiming to assess seed dispersal and population genetic variation under different fire regimes, with a view to making inferences about their resilience to changing fire regimes.

I collected empirical data on primary seed dispersal by physically tracking seeds and measured wind conditions to simulate primary seed dispersal with a trajectory model. Both methods were repeated during different seasons and at different sites. For the genetic assessment I collected plant material from *T. irritans* and *C. verrucosa* at sites with different fire histories to determine population genetic diversity, genetic structure and the distance seeds are dispersed.

Primary seed dispersal by wind for *T. irritans* was found to cover distances of less than 10 m under strong wind conditions. However, historical gene flow between sites was high and rare seed dispersal events of up to 3 km were detected which suggests that secondary seed dispersal and possibly pollen dispersal play an important role in the overall genetic connectivity of *T. irritans* populations. *Triodia irritans* population genetic diversity and structure was similar under various fire regimes. I found no evidence for clonality within this species but high levels of polyploidy.

Estimates of primary seed dispersal by wind for *Callitris verrucosa* were up to 40 m under strong wind conditions while rare dispersal events of up to 8 km were identified. Population genetic diversity and structure in *C. verrucosa* seems to be independent of fire frequency and the species appeared to be panmictic. Population genetic diversity was very low at all sites. Fire season was found to influence seed dispersal distance in *C. verrucosa* with longer seed dispersal distances in the natural fire season of summer compared to spring and autumn (prescribed burning).

Findings of this study suggest that both species are well adapted to current fire regimes, focusing on fire frequencies and size of area burned. However *C. verrucosa* is likely to profit from longer intervals without fire to increase its seed dispersal distances. This information is intended to improve present management of mallee vegetation on the Eyre Peninsula and can potentially be applied to other Mediterranean type ecosystems.

CERTIFICATION

I, Bianca Dunker, certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Bianca Dunker

28 March 2014

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PREFACE

This thesis consists of four main chapters written in article form prepared for publication but not yet submitted to journals. The introduction chapter and the conclusion explain the relationships between the main chapters and put my work into a broader context. As a result of this structure a certain degree of repetition can be expected. References are only presented at the end of each chapter.

I designed my research outline in consultation with my supervisors Michael Bull and Andrew Lowe and my co-supervisor Don Driscoll and David Keith. I did all the field work and the majority of the lab work require for collecting the data presented in the thesis. I conducted statistical analysis and interpretation of the results in consultation with my supervisors and some support by others which have been listed either as co-authors or in the acknowledgements at the end of each chapter.

Assigned contributions for each of the main chapters

Chapter 2

Bianca Dunker, David Keith, C. Michael Bull, Martin Breed, Andrew J. Lowe, Don Driscoll; *A comprehensive approach to assess seed dispersal of two plant species in mallee*

75% me; 5% David Keith, 2% C. Michael Bull, 5% Martin Breed, 5% Andrew J. Lowe, 8% Don Driscoll

Chapter 3

Bianca Dunker, Mike Gardner, Don Driscoll, David Keith, C. Michael Bull, Andrew J. Lowe; *Exploring population genetic diversity and structure of Triodia irritans under various fire regimes*

75% me, 7% Mike Gardner, 3% Don Driscoll, 3% David Keith, 5% C. Michael Bull, 7% Andrew J. Lowe

Chapter 4

Bianca Dunker, Eleanor Dormontt, C. Michael Bull1, David Keith, Don Driscoll, Andrew J. Lowe; *Low genetic diversity of Callitris verrucosa in mallee vegetation*

75% me, 6% Eleanor Dormontt, 4% C. Michael Bull, 4% David Keith, 4% Don Driscoll, 7% Andrew J. Lowe

Chapter 5

Bianca Dunker, C. Michael Bull, David A. Keith, Don. Driscoll; *Fire season influences seed dispersal by wind*

76% me, 7% C. Michael Bull, 7% David A. Keith, 10% Don Driscoll

We shall see but little way if we are required to understand what we see. How few things can a man measure with the tape of his understanding! How many greater things might be seeing in the meanwhile?

-HENRY DAVID THOREAU

CHAPTER ONE

Introduction - Fire and the importance of seed dispersal for population connectivity

Long unburned mallee at Hincks Wilderness Protection Area, SA (Photo B. Dunker)

1.1 Fire in the Australian ecosystem as a natural occurrence

For millions of years fire has been a common occurrence in many vegetation types across Australia (Gill et al., 2001), impacting the structure and function of ecosystems by influencing plant communities and wildlife (Bond and Keeley, 2005). Each fire occurrence is the result of complex interactions between climate, current weather conditions, topographic features, vegetation type and fuel availability (Bowman et al., 2009, Gibson, 2013, Bond and Keeley, 2005, Gill et al., 2001). A fire regime, which is the general pattern in which fires occur in a particular ecosystem, is defined by its frequency of occurrence, season, fuel type and severity (Bowman et al., 2012, Gill et al., 2001, Bond and Keeley, 2005). Modifications of present fire regimes can be caused by changes in rainfall, temperatures, wind patterns and fuel characteristics. It is expected that the worlds changing climate will alter fire regimes by weather patterns and vegetation cover resulting in reduction of the intervals between fires (Driscoll et al., 2010, Bowman et al., 2012, Gibson, 2013). Changes in fire frequency can affect the composition of faunal and floral species in those ecosystems that show long fire histories (Bond and Keeley, 2005). Predicting the effects of changes in fire frequency will be important to sustain the biodiversity of fire-prone ecosystems in the future. In this study I examine past and contemporary fire effects on seed dispersal and genetic variation for two species in a fire-prone ecosystem, with a view to making suggestions about their resilience to changing fire regimes.

1.2 Fire in Australian ecosystems as a management tool

Besides changing climate conditions, human activity, in the form of landscape management and alterations of landscape structure, is another factor that causes changes to fire regimes (Bowman et al., 2009, Pyne, 2010, Bowman et al., 2012). In Australia fire has been used by Aboriginal people as a tool to manage wildlife and plants for tens of thousands of years, while today common applications of fire are: the minimisation of fuel hazard; stimulation of forest and pasture regeneration; maintenance of habitat quality, and conservation of biodiversity (Gill et al., 2001). “Fire and its management is increasingly recognised as an important factor in biodiversity conservation and natural resource management” (Driscoll et al., 2010).

However, fire used in an inappropriate way can reduce biodiversity. Fire regimes that could cause a reduction in biodiversity include: high fire frequency: complete fire exclusion; unseasonal fires, or fires of inappropriate intensity (Bradstock et al., 2006, Bond and van Wilgen, 1996, Céspedes et al., 2012, Smith et al., 2013, Knox and Clarke, 2006, Taylor et al., 2013). The response of biota to fire regimes and potential changes in fire regimes may be strongly influenced by their life-history characteristics (Keith, 2012, Pausas et al., 2004, Noble and Slatyer, 1980, Noble and Gitay, 1996). This means that fire regimes that cause an interruption of life cycles or alter habitat will potentially threaten species (Bond and van Wilgen, 1996, Whelan, 1995).

Many effects of fire regimes on ecosystems are poorly understood which makes prediction of potential impacts difficult and might lead to poor management decisions (Haslem et al., 2011, Driscoll et al., 2010). The goal of fire management, which is to maintain as many native species as possible and to preserve ecosystem function, can only be achieved if knowledge of the complex interactions between species and their environment improves (Driscoll et al., 2010). Within this context, Driscoll et al. (2010) emphasised the need for a species-level approach for measuring biodiversity responses to fire which facilitates the mechanistic understanding required for making predictions and simulations.

1.3 Fire regime changes and their potential effect on biotas

The effect of fire on plant populations can differ due to the variablity in length of time between fire events, the intensity of the fire in each event and differences among plant species of existing life-history characteristics (Bond and van Wilgen, 1996, Sousa, 1984). In fire-prone semi-arid environments the plant life history characteristics important for recovery after fire include: vegetative sprouting; fire-stimulated recruitment; persistent seed banks, and a primary juvenile period shorter than the time interval before the next fire (Pausas et al., 2004, Allen, 2008, Bond and van Wilgen, 1996). Each of these characteristics comes with the need for a particular fire regime. Vegetative sprouting (re-sprouting) species generally do well under fire regimes that keep competition for space to a low level (Keith et al., 2007, Bond and Midgley, 2001, Pausas and Lavorel, 2003), while for non-re-sprouting species, where adults are killed by fire, fire frequencies must exceed the primary juvenile period of a species for plants to be able to build up seed banks (Bond and van Wilgen, 1996, Bradstock and O'Connell, 1988, Gill and McMahon, 1986, Cowling et al.). Hence, a change in fire intervals can cause a threat to certain species and lead to a reduction in their population density (Haslem et al., 2011) which will impact on population genetic diversity and structure (Lowe et al., 2004). Fire intervals imposed under habitat/fire management can be quite different to those of natural wild fires. Fire suppression causes fire intervals to be very long while prescribed burning generally shortens fire intervals aiming to protect surrounding assets and/or to reduce the risk of extensive wild fires (Penman et al., 2011)

Weather conditions around the time of fire are another factor that may influence the survival and response of plants and animals (Knox and Clarke, 2006, Enright and Lamont, 1989). The season of burning generally differs between wild fire and prescribed burns. While wild fires generally happen during the hottest and driest times of the year (Bond and Keeley, 2005, Penman et al., 2011), prescribed burns are often carried out in seasons with cooler and wetter conditions as fires during these periods are easier to control (Van Wilgen et al., 2010). A change in fire season (e.g. caused by prescribed burning) could lead to prevailing weather conditions unfavourable for seedling establishment (Thomas et al., 2010) or insufficient pollination as the flowering season may be over before adult plants recover from the impact of fire (Bond and van Wilgen, 1996). Additionally, wetter weather conditions during prescribed burns often cause a reduction in fire intensity (Penman et al., 2011). Plant species that show fire-stimulated recruitment often require a particularly high fire intensity to trigger the release of seeds, or germination could be reduced as they depend on heat generated by fire for re-establishment (Bradstock, 2008, Enright and Lamont, 1989, Lamont et al., 1993, Nathan et al., 1999). Furthermore, the dispersal of seeds by wind might be adversely influenced by changes in fire season as wind conditions can vary at different times of the year (Figure 1.1). Unfavourable wind conditions could affect particularly serotinous obligate seeding species, that release their seeds after a fire event, reducing seed dispersal distances which could limit their ability to recolonise sites or colonise new suitable habitat (Keith, 2012)..

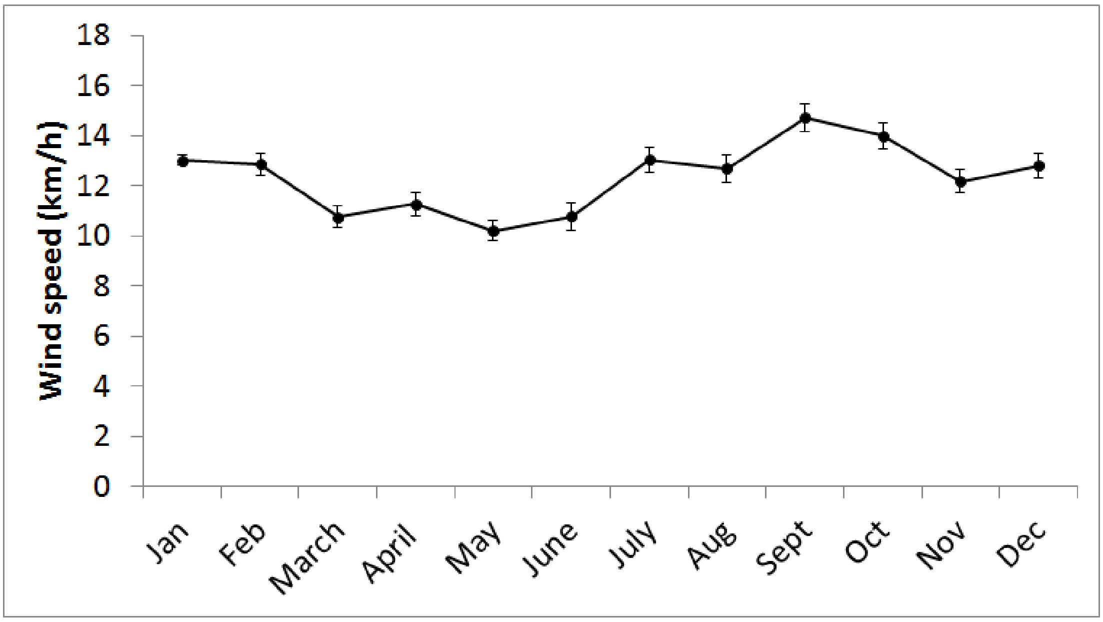

Figure 1.1: Average wind speeds measured at Cleve, SA weather station during the years 2005 – 2009. Error bars represent standard error (The information on wind speeds were sourced from http://www.bom.gov.au/ managed by the Australian Governments Bureau of Meteorology, accessed January 2012)

1.4 Importance of dispersal

The dispersal of seeds and pollen is fundamental to the ecology and evolutionary biology of plants. It influences the genetic diversity and spatial genetic structure within and between plant populations, and represents an essential mechanism for sessile plants to overcome environmental variability (Broquet and Petit, 2009, Harper, 1977, Werner, 1975). In outcrossing species pollen dispersal is generally the main vector for gene flow, whereas seed-mediated gene flow is the primary mode of colonisation (Bacles et al., 2006, Kremer et al., 2012). In the event of an unfavourable fire regime it can be crucial for plant species to have the ability to recolonise an area via dispersal of seeds. However this ability is restricted by the distance seeds are able to travel and the distance between suitable sites. The ability of seeds to disperse over long distances becomes a key trait determining how well species colonise new habitat (Clark et al., 2001, Soons et al., 2004, Nathan et al., 2011) or recolonise previously occupied sites (Bacles et al., 2006, Cain et al., 2000, Groeneveld et al., 2008).

1.5 Parameters that influence seed dispersal distances by wind

The present study will focus on the dispersal of seeds by wind. Seed morphological attributes that promote this type of dispersal are hairs, wings and the small size of diaspores. They are the result of the evolutionary adaptation of the species and affect the motion capacity of the seed (Nathan et al., 2011, Howe and Smallwood, 1982). The dispersal of diaspores via wind is driven by several factors. These factors include:

1. **Seed terminal velocity** (determined by seed size and weight)
2. **Seed release height**
3. **Height of surrounding vegetation and topography**
4. **Wind conditions**

5. **Seed abscission** (detachment of diaspores from the branch, fruit or seed pod)

Thomson et al. (2011) has shown that seed release height plays a more important role than seed terminal falling velocity in determining dispersal distances. The higher the release height of seeds the further the distance seeds may travel away from their source. The difference between release height and canopy height is another important factor. The uplift of seeds above the canopy is necessary for long distance dispersal (Nathan et al., 2011). Other factors that affect seed dispersal indirectly by controlling local air flow/wind conditions include: local topography (Katul and Poggi, 2012); surrounding canopy structure, and ground surface roughness (the structure of the surface over which diaspores travel). Rough surfaces and a topography, which creates turbulent air flow, favour long dispersal distances of seeds while obstacles such as high sand dunes or vegetation can act as a dispersal barrier (Nathan et al., 2011). The ground surface roughness affects the airflow near the ground (Nathan et al., 2001, Andersen, 1991). Potential influencing variables related to wind conditions include: temperature near the ground; general humidity; wind speed, and the variation of wind speed during flight (Andersen, 1991). Conditions that are linked to long distance dispersal are extreme horizontal wind speeds and auto correlated turbulences as well as thermal turbulence and convective updrafts in combination with low to moderate vertical wind speeds (Bullock and Clarke, 2000, Nathan et al., 2011, Soons et al., 2004, Tackenberg, 2003, Okubo and Levin, 1989). These wind conditions can be found, above the canopy top or in the form of turbulence caused by heating of the ground surface, the latter is known as buoyancy-driven turbulences. These turbulences increase the fluctuation of vertical velocity and uplift components in the wind. This effect can be enhanced by land surface heterogeneity and patterns of land use (Nathan et al., 2011). Another factor in the process of seed dispersal is abscission. The conditions required to mediate seed abscission influence the wind conditions diaspores face at and after release. Wind conditions that promote seed abscission are gusts, specifically relatively high horizontal and vertical wind speeds including turbulences, (Green, 2005, Soons and Bullock, 2008). Wind conditions that promote seed abscission are similar to those that promote long distance dispersal, hence it can be assumed that biased seed abscission (i.e. wind speed-dependent) can increase long distance dispersal events (Soons and Bullock, 2008). Surprisingly, only a few studies have examined seed dispersal linked to fire (e.g. Hammill et al., 1998, Whelan, 1986). Since fire causes a change in wind patterns and reduced vegetation cover, conditions after a fire are found to be favourable for long distance dispersal events (Whelan, 1986), which could be advantageous for obligate seeder species. Additionally, seed dispersal is a critical life history component for species spatial distribution, colonisation ability and in fire-prone vegetation for recolonisation or genetic rescue of populations substantially reduced in size. Hence, I suggest that seed dispersal distribution requires more research in the context of species resilience to changing fire regimes.

1.6 Population genetics in fire-prone vegetation

In fire-prone environments population genetic diversity and structure is often influenced by seed dispersal distance and plant life history traits related to re-establishment after fire (England et al., 2002). Long distance seed dispersal is an important parameter for colonisation of new suitable habitat, recolonisation of sites after local population extinction and for genetic connectivity between populations (Soons and Bullock, 2008, He et al., 2004). While the first two situations generally lead to populations showing low genetic diversity and strong spatial genetic structure, the latter generally causes the opposite effect (Banks et al., 2013). Depending on the re-establishment strategy expressed by a particular plant species we expect to find genetic diversity and structure to respond differently to different fire regimes, particularly fire frequency. Earlier studies of re-sprouting species found population genetic diversity and structure to be unaffected by fire (Premoli and Steinke, 2008). While in serotinous, obligate seeding species we would expect to find less diversity and stronger spatial genetic structure with increasing fire frequency.

1.7 Aim of this study

The aim of this study is to determine seed dispersal distributions and population genetic diversity and structure in two native plant species, *Triodia irritans* and *Callitris verrucosa*, with contrasting life histories in the context of fire and fire management. One great concern for species in fire-prone ecosystems is the expected changes in fire regimes, particularly frequency and season of fire occurrences. In this context, I explore the impact of different fire regimes, and how to best manage for biodiversity outcomes for the future.

1.8 Study system and species

1.8.1 Study system and its past and present fire regimes

Mallee is a flammable ecosystem and fire is considered one of the forces that maintain its structure and function (Bond and Keeley, 2005). Mallee vegetation occurs in the semi-arid, Mediterranean and temperate areas of southern South Australia, south-western New South Wales, north-western Victoria and the southwest of Western Australia (Bennett et al., 2010, National Land and Water Resources Audit, 2001). My study system is the mallee of the Eyre Peninsula, SA where I collected my data at Hincks Wilderness Protection Area (Figure 1.2) (33°45' S, 136°03' E; 66658 ha). The region is semi-arid, with an average annual rainfall of 340 mm. The main topographic features are white sand dunes interspersed by hard, reddish-brown swales. Mallee vegetation on the Eyre Peninsula in South Australia is described as Mallee heath, woodlands and shrublands (Joe Tilley, Ranger Hincks Conservation Park, on published data 31/01/2014). The vegetation is dominated by mallee eucalypts: *Eucalyptus incrassata; E. socialis,* and *E. peninsularis* with a multi-stemmed habit. Other common plant genera are: *Melaleuca*: *Acacia*: *Hakea,* and *Callitris*. The understorey is dominated by hummock grasses (*Triodia* species). The composition of vegetation often depends on factors such as rainfall, soil composition as well as fire frequency and intensity (National Land and Water Resources Audit, 2001).

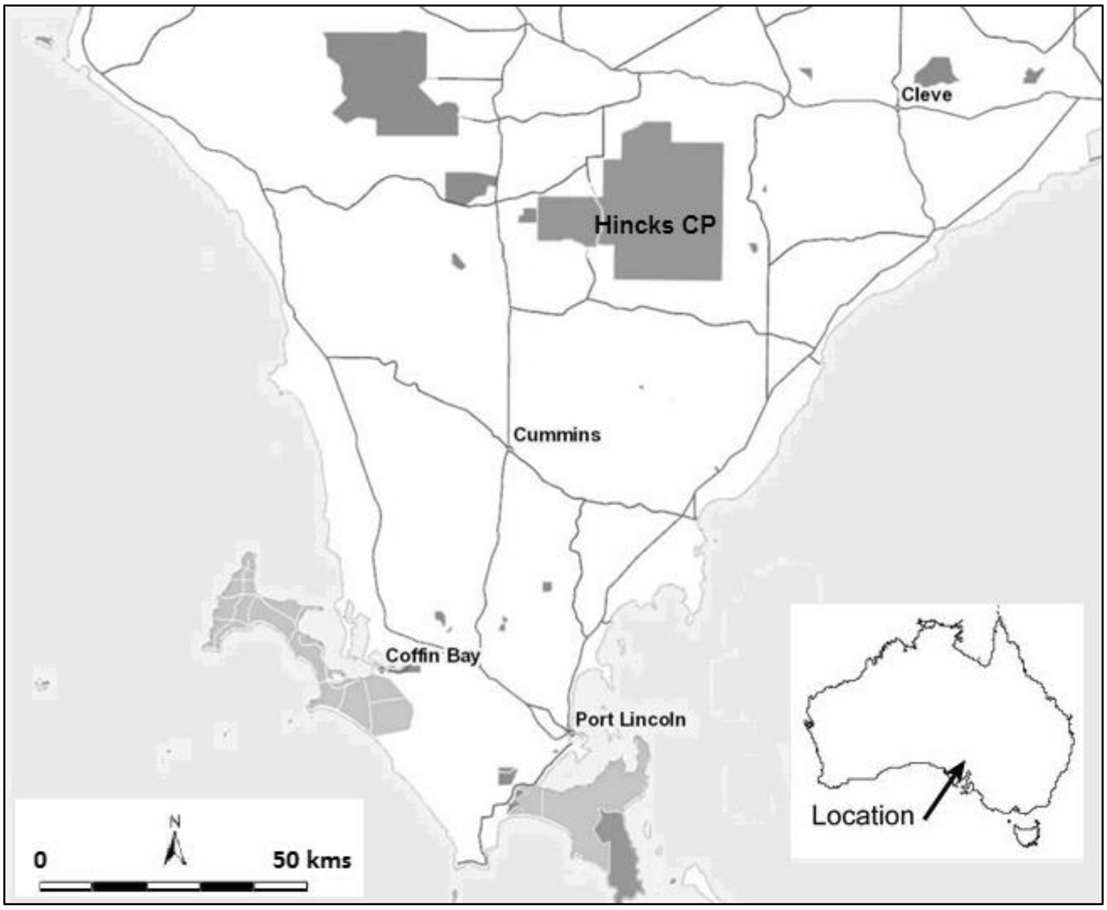

Figure 1.2: Map showing location of Hincks Wilderness Protection Area on the Eyre Peninsula, SA.

Natural fire regimes in mallee are a result of fuel availability and ignition by lightning in combination with severe weather and dry vegetation, which generally occurs during dry summer storms (Bradstock and Cohn, 2002b, Sullivan et al., 2012). Hence, natural fire season for mallee in South Australia would be summer to autumn (Enright et al., 2012). Fires in mallee occur as crown-fires (Cary, 2002, Sullivan et al., 2012) and can cover areas of up to 100 000 ha depending on fuel connectivity (Bradstock and Cohn, 2002b). After such events, mallee is generally unlikely to burn again for about 10-20 years (Bradstock and Cohn, 2001). In semi-arid mallee woodlands exceptions are wet years that trigger extensive growth of ephemerals (Gibson, 2013) that then provide a high fuel load and enough fuel connectivity in the following dry summer. Around 30 years after fire, primary fuel availability generally reaches its peak. From that point on, the chance of wild fires associated with fuel age does not increase as it is only the loose bark of the eucalypts that continues to accumulate while tree density decreases (Bradstock and Cohn, 2001). The maximum tree height is reached at around 60 years. However, habitat quality for hollow nesting animals still improves for more than a century (Haslem et al., 2011, Bennett et al., 2010). Maximum length for natural fire intervals in semi-arid mallee woodlands found on the Eyre Peninsula was suggested to be more than 200 years (Gibson, 2013). Other authors suggest that typical fire intervals for semi-arid mallee range between decades or centuries due to the rare occurrence of adequate rainfall followed by lightning ignition during dry thunderstorms (Enright et al., 2012).

Common management application for mallee is prescribed burning with two objectives: the reduction of fuel to protect assets, and the conservation of biodiversity (Management Plan - Mallee Parks of the Central Eyre Peninsula, 2007). Combining these two aims is challenging. The burning of mallee at an early age will cause loss of valuable habitat due to the absence of old growth mallee (Haslem et al., 2011). For example, the malleefowl (*Leipoa ocellata),* an endangered bird species, is most abundant in long unburnt mallee, which is its breeding habitat and preferred foraging site (Bradstock and Cohn, 2002b, Clarke, 2005, Department of the Environment, 2014). Additionally, alteration of the fire season due to prescribed burning may affect plant species composition, vegetation structure as well as animal populations, hence overall biodiversity which is one of the key values of the remaining mallee woodlands and shrublands. Mallee serves as habitat for a wide range of species and is one of the most widespread vegetation types in Australia (National Land and Water Resources Audit, 2001). Considering the fragmented nature of mallee vegetation in most areas (Bradstock and Cohn, 2001) and the distinct fire regime requirements of various plants and animals, it is most important to develop suitable fire management plans in order to maintain its biodiversity (Bradstock and Cohn, 2001). Two species that are common and important features of mallee vegetation on the Eyre Peninsula are *Triodia irritans* and *Callitris verrucosa*. They represent two different plant life forms with different re-establishment strategies and hence are expected to express differences in their requirements for a particular fire regime.

1.8.2 Ecology and dispersal features *Triodia*

Triodia R. Br. is known under the common name spinifex grass and is an important feature of mallee vegetation. Its spikelets are 3-20-flowered with upper lemmas being male or sterile. Selfing is common in grasses and it is likely that T. irritans shows a certain degree of polyploidy (Keeler, 1998). Species in this genus show a hemispherical, pyramidal, and annular shape which is caused by the death of older, central culms (Burbidge, 1952). This sclerophyllous above-ground tissue accumulates over years as fuel without decomposition following each episode of biomass production (Bogusiak et al., 1990, Noble and Vines, 1993). This fuel load increases with cumulative rainfall since the last fire (Bogusiak et al., 1990). Many *Triodia* species recover quickly from fire occurrence showing a high growth rate during the first 20 to 30 years following a fire event. After that time, *Triodia* individuals stagnate in growth (Haslem et al., 2011) and slowly start to die back. The highest cover of a *Triodia* individual is generally found between 20 to 50 years after fire (Bennett et al., 2010).

Some *Triodia* species are classified as resprouters, while others are seeders (Rice and Westoby, 1999). Re-sprouting means that species have the ability to regrow after a fire has destroyed the above ground part of the plant, from parts that lay protected under ground (Bowman et al., 2012). This can be advantageous in comparison to seeders which rely on seed supply for regeneration to replace adult plants killed by fire (Bond and van Wilgen, 1996). This variation in re-establishment strategy occurs not only between species but also within species and between locations (Bradstock, 1989, Bogusiak et al., 1990, Rice and Westoby, 1999). In *T. irritans*, both re-establishment strategies have been observed in the field (http://www.landmanager.org.au/fire-responses-triodia-irritans, accessed 28-07-14).

Flowering season for Triodia is generally during summer when seed and pollen are expected to be wind dispersed (Bonney et al., 1994). The released diaspores are stored in the soil and germination happens following adequate rain (Bogusiak et al., 1990, Noble and Vines, 1993, Bradstock, 1989). However, soil seed bank longevity was found to be limited to less than 3 years in *Triodia basedowii* (Westoby et al., 1988). Age of first flowering has not been recorded for *Triodia irritans*, but observations in the field suggest an approximate minimum of around 4 years, which is consistent with recordings for *T. pungens* after fire-kill (Rice and Westoby, 1999).

Considering age of first flowering after fire and limited longevity of soil seed banks, it is possible that there will be times when no seed bank is present. For seeding species this will mean that a fire during those times could cause local extinction. Additionally, re-sprouting, even though it is a very common and resilient strategy for survival in fire-prone environments (Bond and Midgley, 2001) could be inadequate after a combination of fire followed by intensive grazing on regrowth. This could cause a reduction of population size, or even local extinction for re-sprouting *Triodia* species (Kutt and Woinarski, 2007, Rice and Westoby, 1999). A study on the regeneration of *Triodia* after fire found that seedlings tended to be less abundant or even completely absent in predominantly re-sprouting populations (Rice and Westoby, 1999).

It is important to understand *Triodia's* resilience to fire and fire regime changes (Rice and Westoby, 1999, Whelan, 1995, Bond and van Wilgen, 1996) because *Triodia* hummocks provide shelter and are used for foraging by many species (Bonney et al., 1994). These grasses also provide fuel connectivity to help sustain fire occurrences in these ecosystems (Bradstock and Cohn, 2002b, Bradstock and Gill, 1993).

1.8.3 Ecology and dispersal features *Callitris verrucosa*

Callitris verrucosa (A. Cunn. Ex Endl.) F. Muell., is a conifer known under the common name “mallee cypress pine”. It is endemic to the semi-arid zones of Australia and occupies mediterranean type environments. Climatic conditions are dry summers, commonly with unpredictable summer rainfall but with most rainfall during winter (Hobbs et al., 1995). *Callitris verrucosa* grows as a shrub or small tree, either single or multi-stemmed. Its maximum height is 5 - 6 m (Bonney et al., 1994). Flowers are monoecious and pollinated by wind. Seeds have two small wings and are therefore considered to be primarily wind disperesed (Black and Eichler, 1960-1965, Jessop et al., 1986). *Callitris verrucosa* is classified as an obligate seeder, accumulating its seeds in the canopy. It is serotinous, which means that it releases seed from canopy storage (cones) after fire (Bond and van Wilgen, 1996). Hence, its canopy seed banks are usually exhausted following a crown fire (Pausas et al., 2004, Lamont et al., 1991), although it is assumed that a small number of seeds can be released independently of fire events (Bradstock et al., 2006, Bonney et al., 1994). Therefore it seems uncertain whether *C. verrucosa* can be regarded as weakly or strongly serotinous (Adams, 1999, Bradstock and Cohn, 2002a, Bonney et al., 1994).

Callitris verrucosa does not develop appreciable soil seed banks due to lack of seed dormancy mechanisms. Hence, the species relies on dispersal of seeds from its canopy seed banks after a fire. Seeds germinate directly on the soil surface. The germination of seeds on the soil surface can increase the exposure of seeds to unfavourable weather conditions, such as high temperatures and low water potential over an extended period of time (Adams, 1999). Seeds of *C. verrucosa* germinate most rapidly at 18 ºC but germination is inhibited above 20 ºC and below 10 ºC. This relatively narrow range of favourable germination temperatures occurs generally during autumn and spring, hence germination is likely to be restricted to these seasons. It has also been observed that for germination under limiting soil moisture conditions, *Callitris* seeds show the capacity to absorb small amounts of soil water during cycles of light rainfall followed by soil drying. The process of utilizing soil moisture from light showers speeds up germination after sufficient rain in autumn (Adams, 1999). Considering these factors that promote germination, it is clear that seed release that occurs in summer is potentially the most favourable for seedling establishment.

Due to its serotinous character, fire occurrences are essential to sustain population dynamics and, in particular, seedling establishment of *C. verrucosa*. However, *C. verrucosa* appears to exhibit different responses to fire as it can retard or promote fire, depending on fire intensity. It is suppressive of low-moderate intensity fires, but susceptible/fuel providing to high intensity fires (Bradstock and Cohn, 2001, Bonney et al., 1994). Plants need at least 15 years to reach maturity, although it has been observed that small saplings of less than 15 years can produce a small number of cones (Zimmer et al., 2010). Assuming *Callitris* is killed by fire, frequent fire occurrences (at intervals of less than 15 years) are likely to result in reduced population sizes and/or local potential extinction (Bradstock and Cohn, 2002a, Adams, 1999, Armstrong, 2011). In such an event, *C. verrucosa* would have to depend on seed input from surrounding populations to re-establish. Hence, factors that could threaten population persistence are an increase in fire frequency due to changing climate conditions and fire management (Bowman and Harris, 1995). Bradstock and Cohn (2001) assumed that the maximum dispersal distance of seeds is around 50 m, but verification is lacking.

The fact that most dispersal occurs shortly after fire suggests that seeds will experience favourable conditions for long distance dispersal, which could lead to dispersal over greater distances than assumed by Bradstock and Cohn (2001). Based on present literature, primary factors that might promote long distance dispersal are thermal turbulences and updrafts as well as reduced vegetation cover (Tackenberg, 2003, Soons et al., 2004), all of which are characteristic of the post-fire environment.

An understanding of the seed dispersal kernel, the probability density function that describes the pattern of primary seed dispersal distances away from the source (Hardy, 2009, Nathan, 2001), is desirable to help assess the risk of severe population reduction or local extinction for *C. verrucosa*, and its potential for population re-establishment. It can be assumed that a change in the abundance of *C. verrucosa* will have a significant influence on vegetation and habitat structure. This in turn affects cohabiting plant species and animals, particularly for bird species where long unburned stands are considered a key habitat element (Bradstock, 1989, Bradstock and Cohn, 2002a, Bonney et al., 1994).

1.9 Thesis outline

In this thesis I will address the following questions which are outlined as individual chapters and written in form of publications:

1. How far do seeds travel? Determining seed dispersal distributions of the two study species (*Triodia irritans* and *Callitris verrucosa)* by incorporating three complementary methods from different methodological backgrounds (empirical, mechanistic and genetic).

2. Is there a difference in genetic diversity and spatial genetic structure of *Triodia irritans* amongst sites with different fire regimes? Does *T. irritans* show evidence of inbreeding and polyploidy, genetic characteristics often observed in grasses?

3. Does *Callitris verrucosa* show lower genetic diversity in sites that experienced short fire frequencies (less than 15 years)? Can we find evidence for meta-population structures based on the population genetic data?

4. Do changes in fire season affect dispersal distances for diaspores in *Callitris verrucosa*?

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CHAPTER TWO

A comprehensive approach to assess seed dispersal of two plant species in mallee

Ultrasonic anemometer in a recently burned site at Hincks Wilderness Protection Area, SA (Photo B. Dunker)

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2.1 Abstract

Dispersal and subsequent distribution of seeds are a central factor in the population genetics, population dynamics and community ecology of plants. Its assessment is therefore essential to current efforts for understanding ecological processes and can be used for simulations and modelling studies. For studying wind-mediated seed dispersal, numerous methods are established which fall into three main categories: (i) direct tracking studies; (ii) mechanistic models; and (iii) genetic methods. Generally it can be said that each method covers only certain components of a seed dispersal kernel.

In this study, we aimed to attain a more comprehensive understanding of seed dispersal by using a combination of different methods. We chose one method out of each main category: (i) visual tracking; (ii) a trajectory model; and (iii) indirect genetic measures, to assess seed dispersal and maximum gene flow of two species that differed in their growth form as well as their diaspore characteristics.

The direct tracking method provided great insight into dispersal of seeds over short distances and some insight into secondary dispersal of seeds on the ground. Average seed dispersal distance of *Callitris verrucosa* was 1.7 m (±0.07) and there was no evidence that secondary seed dispersal on the ground is an important factor in overall gene flow. *Triodia irritans* average seed dispersal distance was only 0.8 m (±0.02) but seeds demonstrated further secondary movement on the ground. The mechanistic modelling approach, useful for generating a realistic dispersal kernel including seed dispersal over greater distances, estimated maximum seed dispersal distance of *C. verrucosa* to be 39.5 m and for *T. irritans* 9.7 m. The genetic method detected some long distance seed dispersal events of up to about 8 km for *C. verrucosa* and about 3 km for T. irritans.

Synthesis: The approach of applying complementary methods each within its range of feasibility can finally allow a more complete understanding of seed dispersal and its distributions.

2.2 Introduction

Gene flow and particularly seed dispersal is a central factor in the population genetics, population dynamics and community ecology of plants because it maintains connectivity within and between populations (Broquet and Petit, 2009). Assessment of seed dispersal distances provides an insight into recruitment limitation, population recovery following ecological disturbance and the ability of plants to colonise new habitat (Moran and Clark, 2011, Schupp et al., 2010, Bullock and Clarke, 2000, Nathan and Muller-Landau, 2000, Banks et al., 2013). Knowledge of short distance dispersal patterns provides insights into local processes, such as recruitment patterns and species coexistence/competition dynamics (Bolker and Pacala, 1999, Nathan and Muller-Landau, 2000). The dispersal of seeds, particularly over longer distances, defines the potential of a species to colonise new habitat (Nathan and Muller-Landau, 2000, Nathan, 2006, Trakhtenbrot et al., 2005, Bacles et al., 2006) and also allows individual adaptation to changes in environmental conditions through tracking a shifting environmental niche driven by climate change (Trakhtenbrot et al., 2005, Kawecki and Ebert, 2004, Breed et al., 2011, Aitken et al., 2008). Seed dispersal is therefore critical to countering the negative impacts that landscape fragmentation, loss of native vegetation disturbance regimes and climate change are expected to have on plant population viability (Lowe et al., 2005). Accurate measures of dispersal and the development of accurate dispersal kernels are therefore essential to current efforts for understanding ecological processes and their application in simulations and modelling studies (Bullock and Clarke, 2000, Hardy, 2009, Kremer et al., 2012).

There are numerous methods for measuring seed dispersal and developing a species- specific dispersal kernel, the probability density function that describes the pattern of primary seed dispersal distances away from the source (Hardy, 2009, Nathan, 2001). However, a shared problem faced by studies on seed dispersal is that common established methods cover only certain components of the dispersal kernel (SkarpaasShea et al., 2011, Hirsch et al., 2011, Bullock et al., 2006) (Table 2.1).

The most commonly established methods used for studying wind driven seed dispersal in plants fall into three main categories (Nathan, 2001): (i) direct tracking; (ii) mechanistic models, and (iii) genetic methods. The objective of our study was to use the complementary aspects of dispersal knowledge provided by each method with the aim of achieving a more accurate and holistic understanding of seed dispersal distributions of two plant species in fire-prone vegetation.

Table 2.1: Summary of contributions by the different methods to understanding different components of diaspore dispersal

Methodology	Primary dispersal	Secondary dispersal	Seedling establishment
Empirical	X (underestimated)	X/- (to some extent)	-
Mechanistic	X	-	-
Indirect genetics*	X	X	X

*Results represent a cumulative total of all three components which cannot be separated into its parts

Direct tracking methods produce knowledge through direct observations. They provide the data for fitting functions that describe dispersal kernels and offer the possibility to test the fit of mechanistic models (Bullock et al., 2006, Tackenberg, 2003). We included an approach called visual tracking (tracking of seeds by eye). This method is commonly used to gain data on seed dispersal by wind in the field, particularly when aiming for information on dispersal within continuous stands. A common limitation of the direct tracking methods is that they often miss long distance dispersal events (Bullock et al., 2006). Additionally, we used night-time visual tracking to study secondary dispersal on the ground.

Mechanistic models are based on an understanding of dispersal processes which are translated into mathematical equations by making appropriate assumptions. The mathematical parameters of such models can be estimated from empirical observations. Once verified for a system, they can be good predictors of seed dispersal by wind (Bullock et al., 2006). Models based on real wind data allow estimation of dispersal at various locations and under different wind conditions. As a result they can improve our understanding of the variables (biological and physical) that affect the magnitude of seed dispersal by wind (Nathan et al., 2011). A great advantage is that the measurement of wind conditions can be done with little effort. We used a trajectory model (PAPPUS), because it has been shown to provide good estimates of wind mediated seed dispersal including long distance dispersal events (Tackenberg, 2003).

Indirect genetic methods estimate gene flow between groups of individuals in different locations (Broquet and Petit, 2009). They are generally used when sampling individuals in continuous stands, and where inclusion of exhaustive population samples is impossible (Ouborg et al., 1999). They are based on the fitting of theoretical population models to observed genetic structure (Vekemans and Hardy, 2004, Hamrick and Trapnell, 2011, Hardy et al., 2006). These methods quantify the consequences of dispersal (realized seedling establishment) rather than the frequencies of the dispersal events themselves. They therefore quantify the cumulative impact of historical gene flow (Ouborg et al., 1999, Schupp et al., 2010, Broquet and Petit, 2009). Nathan et al. (2011) suggested that this could be of advantage for measuring seed dispersal by wind, as this form of seed dispersal is very complex and highly variable. Depending on the spatial distance covered by a sampling design, genetic methods can be a useful tool for inferring long distance dispersal events (Nathan et al., 2003, Nathan, 2005, Jones and Muller-Landau, 2008, Hardy et al., 2006).

In this paper, all three methods were used to explore seed dispersal patterns of the two plant species, *Triodia irritans* and *Callitris verrucosa,* in fire-prone mallee woodlands in southern Australia. The study species differ in their growth form as well as their diaspore characteristics. Both species can act as important factors influencing fire regimes (Bradstock and Cohn, 2002b, Bradstock and Gill, 1993, Noble and Vines, 1993). Knowledge of variables which play an important role in species distribution such as seed dispersal is essential for accurate fire simulation studies. Hence, our aim is to use the information on seed dispersal provided by each method to provide a comprehensive understanding of seed dispersal distributions for the two study species.

2.3 Material and methods

2.3.1 Study region

The study was carried out at Hincks Wilderness Protection Area (33°45' S, 136°03' E; 66658 ha) on the Eyre Peninsula, South Australia. The region is semi-arid, with an average annual rainfall of 340 mm. The main topographic features are white sand dunes interspersed by hard, reddish-brown swales. The dominant vegetation type in the reserve is mallee woodland, characterised by multi-stemmed eucalypts. Common trees are *Eucalyptus incrassata, E. socialis, E. peninsularis* and *C. verrucosa*. Common understory species include the shrub *Melaleuca uncinata* (Smith, 2012, Joe Tilley, Ranger Hincks Conservation Park, on published data 31/01/2014), and the hummock grass *T. irritans* (Driscoll and Henderson, 2008).

2.3.2 Ecology of study species

Triodia R. Br., spinifex, is a highly flammable grass occurring in hummock grasslands (Suijdendorp, 1981) and mallee woodlands (Noble, 1989). In mallee woodlands, *Triodia* can provide fuel connectivity to sustain fire across landscapes (Bradstock and Cohn, 2002b, Bradstock and Gill, 1993, Noble and Vines, 1993), while unburnt it offers valuable habitat for lizards and other animal species, serving as shelter and foraging grounds (Bradstock and Cohn, 2002b, Kelly et al., 2011, Driscoll et al., 2012). *Triodia* is a hermaphrodite with the potential for selfing (Keeler, 1998). The species is generally classified as a resprouter with seeds stored in the soil (Burbidge, 1952) but both re-establishment strategies, seeding and resprouting, have been observed in the field (http://www.landmanager.org.au/fire-responses-triodia-irritans, accessed 28-07-14). It is believed that soil seed banks are not long lived, possibly less than 3 years (Rice and Westoby, 1999, Westoby et al., 1988). The number of inflorescences per plant can vary between 0 and 50 with release heights ranging between 0.3 and 1.4 m among plants (personal observ.). Reproductive maturity of individuals is generally within 3 to 7 years following fire or germination (Bowman et al., 2008, Rice and Westoby, 1999, http://www.landmanager.org.au/fire-responses-triodia-irritans). The size of *T. irritans* diaspores ranges from 4 to 11 mm and diaspore mass from 0.6 to 2.5 mg (personal observ.). Seed and pollen are mainly dispersed by wind. After dispersal, seeds are most commonly found at hummock edges, where they get caught, and this determines where most seedlings occur (Westoby et al., 1988).

Callitris verrucosa (A. Cunn. Ex Endl.) F. Muell., from the family Cupressaceae, is a canopy dominant of mallee vegetation and a serotinous obligate seeder (Bond and van Wilgen, 1996). It grows as a small stunted tree often with several trunks and rarely 5 to 6 m high (Earle, 2011, Bonney et al., 1994). The foliage is glaucous or dark green and consists of scale-like evergreen leaves. The plant is monoecious; however it produces few viable seeds from selfing (ESA Conference, 2011). Trees reach maturity at an age of 10 to 15 years and can live for a maximum of approximately 250 years (Bradstock and Cohn, 2002a, Zimmer et al., 2010). Female cones are about 3 cm in diameter, densely warted and contain 5 to 25 seeds, depending on the age of the tree (Bradstock and Cohn, 2002a). Each seed has two wings which generally make up more than 70% of the total width with the actual seed located in the middle. Diaspore width which includes seed and wings, ranges from 2 to 6 mm, and mass from 2 to 30 mg. Cones occur solitarily or clustered on short lateral branches and increase in number with tree age (Bradstock and Cohn, 2002a). Cones mature in 18 to 20 months, then may remain closed on the trees for many years until opened by fire (Earle, 2011). Adult and juvenile plants are normally killed by fire if their leaf canopy is completely scorched. Fire initiates the release of seeds, as cones open after exposure to high temperatures (pers. obs.). Seeds remain inside open cones until released and dispersed by wind. Maximum seed dispersal distance was speculated to be around 50 m (Bradstock and Cohn, 2002b).

2.3.3 Direct tracking of seed dispersal

We measured primary seed dispersal for each species using daylight visual tracking and secondary on-ground seed dispersal using night-time visual tracking. Both measurements were carried out during the summer of 2011 and 2012 since summer is the natural season of seed release for both species. For each species we chose six different release sites, three in each of two recently burned locations (see below for dates of burns) which were about 6 km apart. Different locations for each species were chosen with different times since the last fire, based on the plant life history traits related to age of reproduction expressed by each of the species. We were trying to represent the conditions in which their seeds would normally disperse. For *T. irritans* we chose locations that were burned during 2006 (5 years previously) as this species needs a minimum of 3 to 7 years to reach maturity. The seed releases for *C. verrucosa* were carried out in locations that had more recently undergone a planned burn, one in spring 2009 and the other in autumn 2011. This choice was related to *C. verrucosa* being serotinous. As we found limited regrowth of the vegetation after these two fires, conditions were assumed to be similar to the immediate post-fire conditions under which *C. verrucosa* normally releases its seeds.

At each burned location we carried out seed release experiments at three release sites positioned 800 m apart from each other along a transect that was placed parallel to and 100 m from the burn edge (Figure 2.1). We maintained this distance within the burnt location to avoid bias in dispersal distances due to potential edge effects

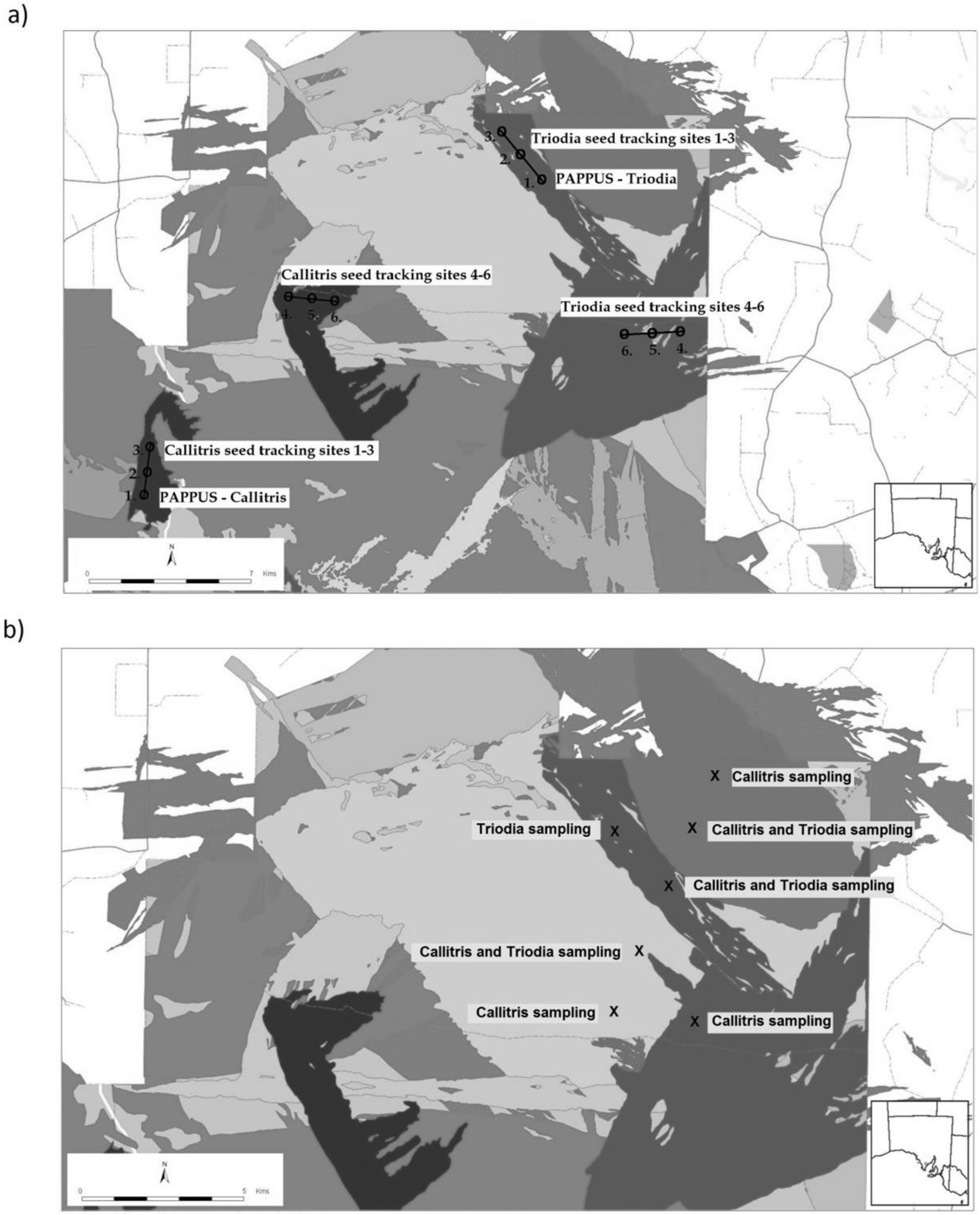

Figure 2.1: Location of sampling sites within the Hincks Wilderness Protection Area on the Eyre Peninsula, South Australia (dark grey = fire in 2006; medium dark grey = fire in 1999; light grey = fire in 1977) (The information on fire history since 1959 was sourced from www.naturemaps.sa.gov.au managed by the South Australian Department of Environment, Water & Natural Resources, accessed November 2011)

Seeds used for our experiments were collected from other populations within the reserve. At each release site, 20 randomly chosen diaspores were released from each of four different heights giving a total of 480 seeds for *T. irritans*. For *C. verrucosa* seeds were released from each of three different heights, giving a total of 360 seeds. The different heights for each species represented common heights observed for either flower heads (*T. irritans*) or cones (*C. verrucosa*) respectively. In the case of *T. irritans,* chosen heights were 0.3, 0.5, 0.8 and 1.1 m while for *C. verrucosa* we chose 1, 2 and 3 m (Table 2.1). The seeds were marked with fluorescent dye to make relocation easier.

To determine the terminal falling velocity, we dropped 100 randomly chosen seeds with and without dye from 10 m height under calm conditions inside a building. Falling duration was timed with an electronic watch (compare Hammill et al. (1998)). *Callitris* seeds with dye (8.3±4.3 mg; 3.0±.05 m/s) showed no significant difference in the falling time (t_{198} =0.943; P =0.346) compared to seeds without dye (9.8 SE±4.9 mg; 3.1 SE±.07 m/s). The same assessment was done for *Triodia* comparing one hundred seeds with dye (1.9 SE±0.4 mg; 2.6 SE±0.7 m/s) with one hundred seeds without dye (1.6 SE±0.3 mg; 2.2 SE±0.6 m/s). In this case we found seeds with dye fell significantly faster (t_{198} =4.62; P <0.001) which means that we are likely to have underestimated seed dispersal distances for *Triodia* in the subsequent field trials.

In the field trials, the 20 seeds were released at each height individually by placing them on a small platform on a mast, and allowing them to be carried away by the wind. The released seeds were followed by eye and the linear distance from the release point to where the seed first landed was measured to the nearest cm using a tape measure. Seed release experiments across all locations and sites were conducted over 1-2 days per site with release of the 20 seeds for each height taking 60 – 240 min depending on the wind conditions. Seed release was restricted to conditions when there was a horizontal wind speed range of 8 - 25 km/h. At lower wind speeds seeds would not take-off and at higher wind speeds seeds could not be relocated. This is a limitation of the method and will cause a bias towards shorter dispersal distances.

For the night-time visual tracking we established groups of 10 florescent coloured seeds on the ground within 10 m of each of the six seed daylight visual tracking sites. The treatment of seeds with fluorescent coloured dye was done to make their relocation easier. After four days a UV light was used to search for the seeds at night, and to record the distance moved since release on the ground of the seeds that could be relocated, and number of missing seeds. The distance for each relocated seed was measured to the nearest cm using a tape measure.

Table 1.2: Distribution of seed cones and plant heights for *Callitris verrucosa* (n = 133) and flower heights for *Triodia irritans* observed in the study area

	Callitris verrucosa		Triodia irritans
Percentile	Cone height (m)	Plant height (m)	Flower height (m)
Min.	0.04	0.3	0.2
25th quartile	0.3	0.5	0.5
Average	0.6 ± 0.4	0.9±0.5	0.7±0.02
75th quartile	0.8	1.3	0.8
Max.	3.4	3.5	1.4

2.3.4 Mechanistic seed dispersal

Wind measurements that were required in the PAPPUS model were recorded at one site for each of the species respectively (Figure 2.1). In both cases the position was close to one of the seed release sites used for the direct tracking. For *C. verrucosa* the more recently burned location was chosen as this location would be more representative of actual dispersal conditions. Measurements continued for two weeks including the time during which seed release experiments were conducted. An ultrasonic anemometer (Model WindMaster (Part 1590-PK-020), Gill Instruments Ltd, Lymington, UK) measured horizontal wind speed, horizontal wind direction, and vertical wind speed every 0.1 s, for 24 h on each of 14 days, producing a dynamic, three dimensional wind speed vector. The measured values were used to simulate the course of the wind vector in PAPPUS. The anemometer measured wind at a height of 0.9 m for *T. irritans* (minimum possible height for the device) and 2 m for *C. verrucosa*. PAPPUS then generated estimated wind profiles for the other heights that were used during seed release. Additionally for *C. verrucosa* estimates for maximum tree heights (5 m) were included to assess maximum dispersal distance. Generated values from all heights were used in the model to estimate seed dispersal distances.

PAPPUS derives predictions of seed dispersal distances taking into account wind profile, landscape slope (hilly, sloping upwards/downwards, even), surface roughness (**Z**ero **P**lane **D**isplacement (ZPD) which is influenced by vegetation height and density), and terminal falling velocity of the seeds. We used the pre-set landscape feature “rolling hills” in PAPPUS that we considered was comparable to the sand dunes found in the study area. The topography factor was set at 0.8 for hilly ground. ZPD, a parameter ruling the logarithmic wind profile was calculated as 0.7 multiplied by the average height of the surrounding vegetation (pers. comm. O. Tackenberg) measured at the site of the instrument. This resulted in a value of 0.6 ZDP for *T. irritans* and 1 ZDP for *C. verrucosa*. To determine the terminal falling velocity, we dropped 100 randomly chosen coloured seeds from 10 m height under calm conditions inside a building. Falling duration was timed with an electronic watch (compare Hammill et al. (1998)). The overall average seed falling rate (*C. verrucosa* 3.0 m/sec and *T. irritans* 2.5 m/sec) was then used as the falling velocity in PAPPUS (Tackenberg, 2003). Remaining settings were kept at default (pers. comm. O. Tackenberg). Based on all these parameters, PAPPUS calculates the movement of a single diaspore during small time periods (0.1s). Those short periods are then summed together to reveal the flight path (Tackenberg, 2003).

We set PAPPUS to simulate 10,000 random seed releases and related dispersal distances per 24 h. This was repeated for each height based on the windprofiles generated over the two-week anemometer recording period. We expected that our measurements showed average wind conditions because a comparison with wind conditons that occurred during the rest of the month, based on daily measurements of wind speed at Cleve weather station (Commonwealth of Australia 2014, Bureau of Meteorology), did not show any significant differences from our measurements (ANOVA, P>0.05). Furthermore we did not detect any significant differences among summer months when comparing observed wind conditons at Cleve for January, February and December among the years 2010, 2011 and 2012 (ANOVA, P>0.1).

2.3.5 Indirect genetic estimate of dispersal

For the indirect genetic method we used two different neutral molecular markers, microsatellites for *T. irritans*, which are co-dominant and AFLP's (amplified fragment polymorphism) for *C. verrucosa*, which are dominant. Co-dominant markers are considered to be more informative because the allelic variations of one locus can be distinguished, while dominant markers only provide information on the presence or absence of one allele without being able to distinguish between phenotypes of heterozygotes and homozygotes (Lowe et al., 2004). However, both markers are commonly used for assessing spatial genetic structures (Dewoody, 2006, Sampson and Byrne, 2012, Hardy et al., 2006).

Plant material collection for DNA extraction was carried out during the summer month of 2010, 2011 and 2012 at seven sites located in Hincks Wilderness Protection Area (Figure 2.1). *Triodia irritans* was collected from four sites and *C. verrucosa* from six sites of which three sites were identical for both species. The size of the sampling sites was roughly 500 m x 500 m and distance between sites about 2 km. At each site we collected 50 individuals that were at least 20 m apart from each other. This sampling was designed to provide good insight into the different scales of dispersal (at small scale within sites and at large scale amongst sites). This is necessary for detecting patterns of gene flow including gene flow over long distances (Hardy et al., 2006). Plant leaf material was collected for both species and flower heads for *T. irritans* if present. All sampled individuals were GPS-located.

Plant material was stored in silica gel until DNA extraction. Genomic DNA extractions were performed on the dried plant material at the Australian Genome Research Facility (AGRF) using Machery Nagel kits (www.agrf.org.au). For *T. irritans* eight microsatellites were developed while for *C. verrucosa* amplified fragment length polymorphism (AFLP's) was used because the development of suitable polymorphic microsatellite loci failed. For details on the methods applied for each of the species see chapter 2 and 3.

2.4. Analysis

Seed dispersal distributions for all release heights revealed by daylight visual tracking and the PAPPUS model for both species were described by minimum, maximum, median and mean dispersal distances as well as 1st and 3rd quartile. For *Callitris* the data from the maximum release height (5m) used in the model was kept separately as most trees within the study sites did not reach heights greater than 3 m but we still wanted to capture the potential for long distance seed dispersal. To identify the dispersal kernel that described our daylight visual tracking and modelled data best, different probability density functions were fitted to the frequency distributions of seed dispersal distances. This was done using the default goodness-of-fit distance in the maximum-likelihood function fitdisrt() in the 'fitdistrplus' library in R (R Development Core Team, 2008). We used Akaike weights to determine the best fitting model (Quinn et al., 2011). In our analysis we compared five distributions (Weibull, log-normal, Gaussian (normal), exponential and Gamma) which are all commonly used to describe dispersal distributions of wind dispersed species (Quinn et al., 2011, Schurr et al., 2008).

Using the genetic data set (microsatellites in *T. irritans* and AFLP's in *C. verrucosa*) genetic differentiation (Nei's Gst) were calculated in Genalex version 6.5 (Peakall and Smouse, 2012), which was used to estimate number of migrants between sites contributing alleles per generation (Nm). Population assignment of individuals was carried out in GenoDive version 2.0b23 (Meirmans and Van Tienderen, 2004). For both species we used a data set that included all sites resulting in n=243 samples for *T. irritans* and n=289 samples for *C. verrucosa*.

Triodia irritans genotype data set showed evidence for polyploidy (more than two alleles per individual across loci and populations). Hence, maximum polyploidy level in GenoDive version 2.0b23 (Meirmans and Van Tienderen, 2004) was set for hexaploidy when Nei's Gst-values was calculated for each site. These values were used in the formula Nm = ((1/ Nei's Gst) – 1 / 4) to estimate number of migrants per generation. For population assignment of individual we used the following settings; Allele frequencies were calculated from marker data, significance threshold was set to 0.05, number of permutations was 1000 and selfing rate was set to 0.18 (calculated in Genodive version 2.0b23 (Meirmans and Van Tienderen, 2004)). All other settings were kept at default.

For *Callitris verrucosa* which we assume to be diploid, we followed the same procedure as for *T. irritans*, with minor variations. For assignment of individual to populations we used selfing rate set to 0, as this species is known as outcrossing.

2.5 Results

2.5.1 *Triodia irritans*

The daylight visual tracking showed seed dispersal distances of *T. irritans* range between 0.1 - 3.8 m with a mean dispersal distance of 0.8 ± 0.023 m. The majority of seeds (75%) landed within 1 m of the source (Table 2.2). The shape of the seed dispersal distribution for primary dispersal of diaspores by wind was best described with a gamma density function (Figure 2.2). In measurements of secondary dispersal of diaspores on the ground, nearly two thirds of the diaspores (65%) were lost, and for the remaining diaspores a maximum on-ground dispersal distance of 1.48 m was recorded (Table 2.2). The mechanistic model estimated a wider range of seed dispersal distances for *T. irritans* ranging from 0 up to 9.7 m. The mean dispersal distance was 1.3 ± 0.003 m. The majority of seeds (75%) landed within 1.8 m of the source (Table 2.2). Estimated dispersal distances best fit a gamma density function (Figure 2.2). Based on the genetic data, Nei's Gst-values showed that genetic differentiation between sites increased with increasing distance, ranging from 0.004 to 0.221. Hence, higher numbers of migrants contributing alleles per generation were found in sites that were in closer distance to each other (Table 2.3). The same trend was found in the results from the population assignment. Seed dispersal decreased with increasing distances, detecting a maximum dispersal distance of 3 km (Figure 2.3; Table 2.2).

Table 2.2: Dispersal distances [m] and standard deviations for *Triodia irritans* and *Callitris verrucosa* derived from the three different methods (visual tracking (daytime and night-time), mechanistic modelling (PAPPUS), and genetics) applied.

Species	Method	n	Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
Triodia irritans	Visual tracking	480	0.1	0.4	0.7	0.8 (0.02)	1.0	3.8
	Visual tracking 2nd	44	0.0	0.1	0.4	0.4 (0.1)	0.5	1.5
	PAPPUS	160000	0.0	0.5	1.0	1.3 (0.003)	1.8	9.7
	Genetics							3000
Callitris verrucosa	Visual tracking	360	0.2	0.8	1.3	1.7 (0.07)	2.3	10.6
	Visual tracking. 2nd	107	0.0	0.0	0.0	0.1 (0.03)	0.1	1.4
	PA 1-3m	120000	0.1	2.0	3.6	4.6 (0.01)	6.6	20.2
	PA 5m	40000	1.1	7.6	6.9	13.1 (0.05)	17.5	39.5
	Genetics							8000

Qu. =Quartile

Visual tracking 2nd = Visual night-time tracking to measure on ground dispersal

PA = Mechanistic model PAPPUS with different seed release heights

( ) = Standard error

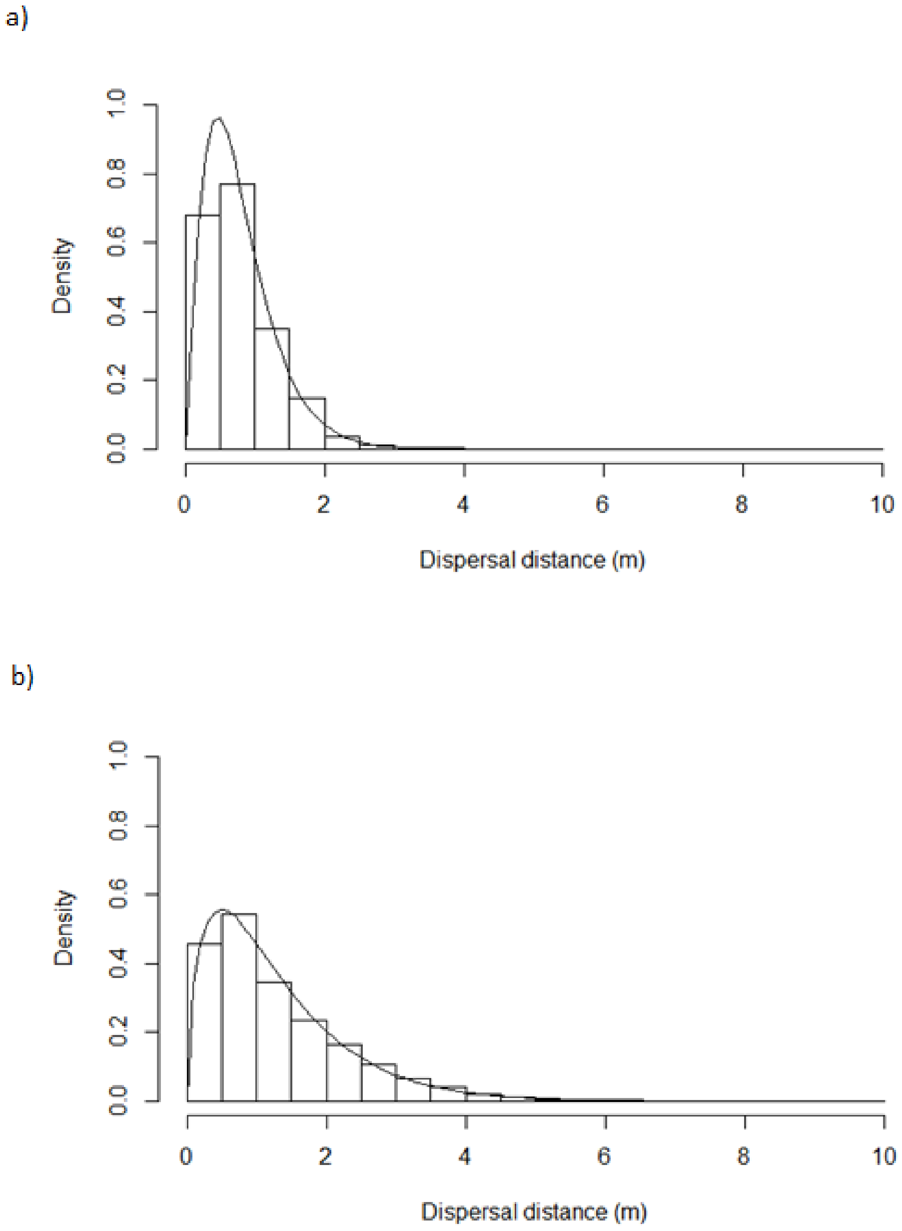

Figure 2.2: Dispersal kernel for *Triodia irritans* based on seed dispersal distance from a) daylight visual tracking and b) mechanistic modelling; fitted probability density functions a) gamma function, b) gamma function

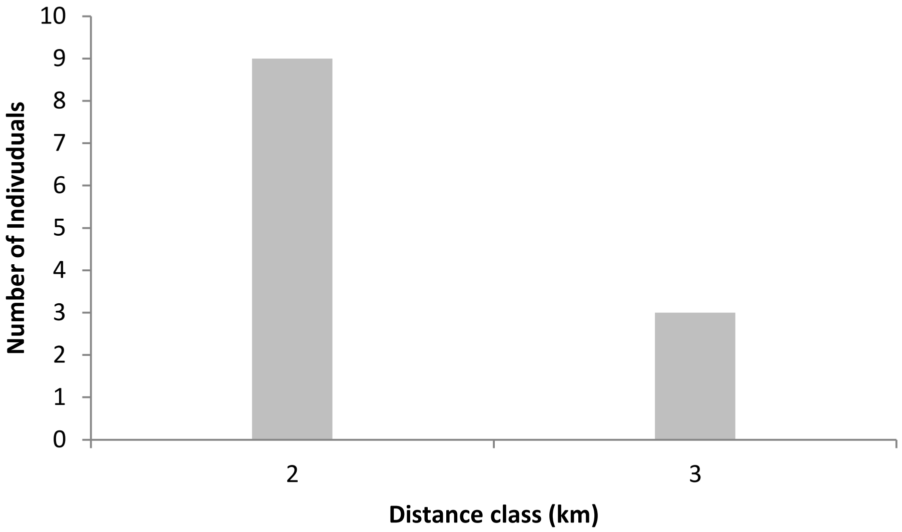

Figure 2.3: Seed dispersal distances for *Triodia irritans* based on results of population assignment using microsatellite data from 4 different sampling sites.

Table 2.3: Pairwise Nei's Gst-values (above diagonal) and related number of migrants contributing alleles per generation between different sampling sites (below diagonal) for *Triodia irritans*

	Site1	Site2	Site3	Site4
Site1		0.004	0.019	0.221
Site2	62.3		0.017	0.201
Site3	12.9	14.5		0.221
Site4	0.9	1.0	0.9	

2.5.2 *Callitris verrucosa*

For *C. verrucosa*, daylight visual tracking measured seed dispersal distances ranged between 0.2 and 10.6 m with a mean dispersal distance of 1.7 ± 0.7 m. The majority of seeds (75%) landed within 2.3 m from the source (Table 2.2). Distribution of primary diaspore dispersal by wind was best described by a lognormal density function (Figure 2.4, Table S2.1). Secondary dispersal of diaspore on the ground was found to be limited. Average dispersal distance on the ground was less than 20 cm and only 12 % of the diaspores went missing (Table 2.2). PAPPUS calculated seed dispersal distances from release heights up to 3 m for *C. verrucosa* between 0.1 and 20.2 m with a mean dispersal distance of 4.6 ± 0.009 m. The majority of seeds (75%) landed within 6.6 m of the source (Table 2.2). For a release height of five meters a maximum dispersal distance of 39.5 m was calculated (Table 2.2). The seed dispersal distribution was best described by a gamma density function (Figure 2.4). Nei's Gst-values showed genetic differentiation between sites was quite similar cross all sites ranging from 0.01 to 0.03. Numbers of migrants contributing alleles per generation for the different sites ranged from 7 to 19 (Table 2.4). Population assignment showed seed dispersal to decrease with increasing distances, detecting a maximum dispersal distance of 8 km (Figure 2.5; Table 2.2).

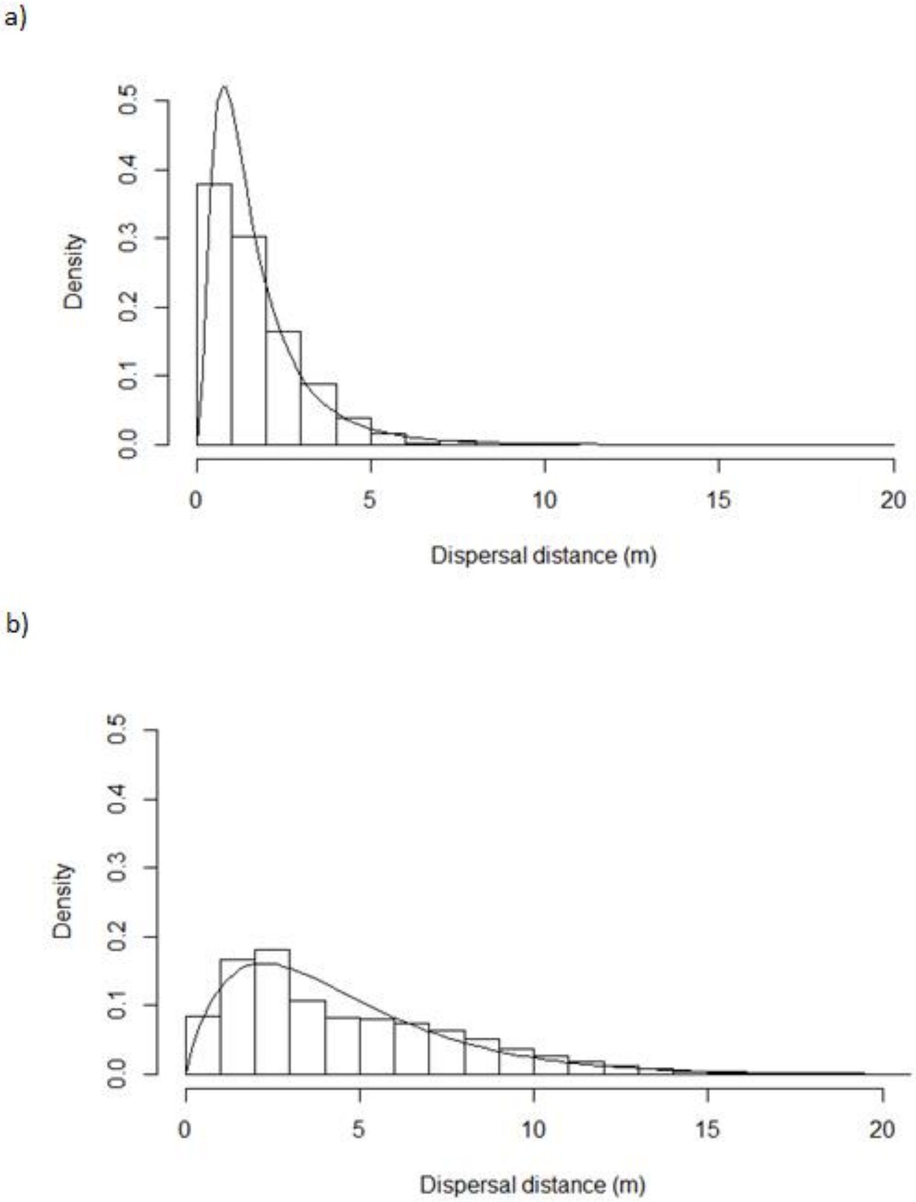

Figure 2.4: Dispersal kernel for *Callitris verrucosa* based on seed dispersal distance from a) daylight visual tracking and b) mechanistic modelling; fitted probability density functions a) lognormal, b) gamma function

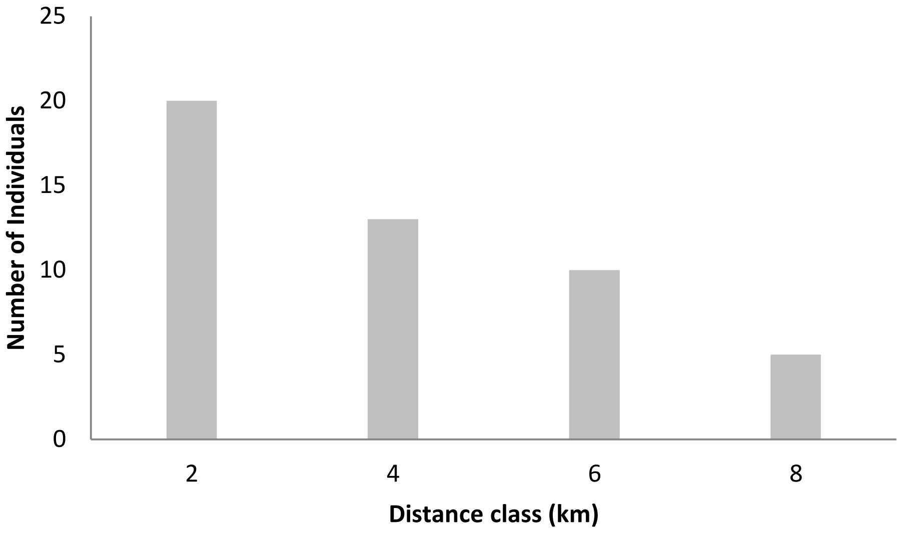

Figure 2.5: Seed dispersal distances for *Callitris verrucosa* based on results of population assignment using AFLP data from 6 different sampling sites.

Table 2.4: Pairwise Nei's Gst-values (above diagonal) and related Number of migrants contributing alleles per generation between different sampling sites (below diagonal) for *Callitris verrucosa.*

	Site2	**Site3**	**Site4**	**Site5**	**Site6**	**Site7**
Site2		0.02	0.04	0.03	0.04	0.03
Site3	15		0.01	0.01	0.02	0.02
Site4	7	19		0.02	0.03	0.03
Site5	10	19	12		0.02	0.01
Site6	7	11	10	15		0.02
Site7	7	11	7	21	14	

2.6 Discussion

Estimations of seed dispersal distances using the three different methodologies provided good insights into seed dispersal distributions for both species. We were able to identify seed dispersal distributions including long distance dispersal and indications of the importance of secondary seed dispersal for overall seed dispersal. This information will be useful in further population, simulations and modelling studies.

2.6.1 *Triodia irritans*

Based on our results from the daylight visual tracking and mechanistic modelling, primary seed dispersal by wind was found to be very limited in *T. irritans* (< 10 m). Dispersal distances are comparable with findings in other grasses with similar seed weights and release heights. *Enneapogon cenchroides*, a wind dispersed grass with hairy seeds showed a maximum dispersal distance of 13 m (Cheplick, 1998), *Festuca paradoxa* (propagule weight 0.967 mg) and *Setaria geniculata* (propagule weight 1.76 mg) dispersed less than 5m under calm wind conditions (Rabinowitz and Rapp, 1981). Low genetic differentiation between sites and subsequent high numbers of migrants, contributing alleles per generation for each site suggesting high gene flow (pollen and seed) amongst neighbouring sites which is likely linked to more extensive pollen dispersal. This result is coherent with findings from a study on *Cynosurus cristatus* an outcrossing, wind pollinated, perennial grass found as a common component of pastures of moderate fertility in Britain. This species too showed very low genetic differentiation amongst adult subpopulations (Gst = 1.14%) and even lower genetic differentiation when comparing pollen pool of subpopulations (Gst =0.28%per cent) (Ennos, 1985). We further detected low numbers of seed dispersal events amongst sites (between 1 and 4%) with highest numbers between neighbouring sites at 2 km distance. Since primary diaspore dispersal by wind was identified as being limited by both the daylight visual tracking and the model, long distance seed dispersal detected by the genetic approach is likely to be linked to secondary dispersal on the ground or other dispersal vectors that have not been considered in our study such as seed dispersal by animals. Results from the night-time visual tracking found that nearly half of the diaspores went missing. This could have been a result of bird or ant predation (Bonney et al., 1994) or more extensive secondary dispersal of diaspores on the ground, which is possible on sandy surfaces (Fort and Richards, 1998) considering their light weight.

2.6.2 *Callitris verrucosa*

Maximum distances for primary dispersal of diaspores for *C. verrucosa* for release heights up to 3 m estimated by PAPPUS were 20 m. However based on model estimations for maximum release height, seeds are likely to cover distances up to 40 m which was close to the 50 m for seed dispersal distances suggested by Bradstock and Cohn (2002b). The difference in maximum dispersal distances between release heights shows that seed dispersal distances are dependent on release height, strongly increasing with increasing height, which was also found in other studies (SkarpaasSilverman et al., 2011, Thomson et al., 2011). Results from both methods (direct tracking and mechanistic modelling) found the majority of seeds (75%) to land within a short distance (< 2 m) from the adult plant, a general pattern found in other seed dispersal studies (Nathan, 2006, Howe and Smallwood, 1982, Willson, 1993), forming a leptokurtic dispersal kernel, another common observation particularly in wind dispersed species (Nathan and Muller-Landau, 2000). Secondary dispersal of seeds via on ground travel seemed to have little impact on the overall dispersal of diaspores as the majority of diaspores (88%) where found close to (< 1.4 m) the start location. However, the genetic method found evidence for long distance seed dispersal covering up to 8 km. What has caused seeds to travel such great distances is unknown but could be potentially linked to either extreme weather conditions in combination with little vegetation cover after fire (Whelan, 1986) or other dispersal vectors such as birds (pers. comm. David Keith, 2014) which haven't been considered in this study. Genetic differentiation between sites was low, suggesting high gene flow (seed and pollen) amongst sites related to extensive seed dispersal found over distances of 2 km and extensive pollen dispersal amongst sites. Dispersal of seeds over extensive distances of several kilometres can be caused by wind conditions such as willy willies which lift seeds up above the canopy (Horn et al., 2001) or other dispersal vectors such as birds (Higgins et al., 2003).

2.6.3 Direct tracking

For both of our study species, data derived from visual tracking during daytime was limited to short distance dispersal events (maximum distance 10.6 m). This limitation is not uncommon as direct tracking often tends to underestimate dispersal due to either diaspore characteristics and difficulties of relocating seeds under certain wind conditions (i.e. smaller, further dispersing diaspores are more difficult to be followed by eye during their flight) (Bullock et al., 2006). Based on literature (e.g. Hirsch et al. (2011)), the fact that seed losses were greater under stronger winds and the limitation of seed dispersal measure to wind speeds below 25 km/h we inferred that the daylight visual tracking is biased towards shorter distances. We recorded a 5 % loss of released seeds in *C. verrucosa* particularly during strong winds. Additionally, non-consideration of wind velocity threshold required for natural seed abscission (Soons et al., 2004) and/or the limited diaspore release height as dispersal distance increases with height (McEvoy and Cox, 1987, Thomson et al., 2011) can cause a bias towards shorted distances. However, the direct observations do provide good insights into dispersal processes in the field e.g. where seeds land and the key dispersal barriers. During field work we observed that for *T. irritans,* released diaspores were often trapped under established *Triodia* hummocks. Additionally, empirically observed experiments also provide insight into different dispersal mechanisms. Night-time visual tracking showed that in the case of *T. irritans*, secondary dispersal must play a more important role in the overall dispersal of diaspores compared to *C. verrucosa*. This information is useful for understanding dispersal and subsequent competition processes within sites, and can be most usefully used to understand the success rate of local seedling establishment and formation of soil seed banks.

2.6.4 Mechanistic model

We believe that mechanistic modelling provides an effective method for exploring variation in seed dispersal from different release heights, across different environments and under different weather conditions and across a broader range of ambient wind speeds. For conservation considerations and for estimates of gene flow, the assessment of maximum dispersal distances is important as it represents a relevant variable.

Potential sources of error in our study that could have caused a bias in estimated dispersal distances are the effect of seed abscission (minimum wind velocity required to trigger seed release) which has not been incorporated because necessary data was not available. The collection of wind data during a limited time frame (in this case 14 days) can result in weather extremes being missed. As a control, the wind data derived in our study was verified to be consistent with the overall mean wind conditions during the months of natural seed release, but this does not exclude the possibility of undetected weather extremes that would have supported longer distance dispersal of diaspores. Additionally, wind recordings for the PAPPUS model were collected at one site only. In our case this site was particularly chosen to detect long distance dispersal events as it was more exposed than other sites.

2.6.5 Genetics

Indirect genetic methods provide information on the cumulative historical effective gene flow and long distance dispersal events (Schupp et al., 2010). This information can help to understand gene flow patterns, population spatial genetic structures and connectedness of populations over multiple generations as well as potential colonisation ability (Ouborg et al., 1999, Schupp et al., 2010, Broquet and Petit, 2009). Estimates of gene flow using indirect population genetics includes all possible dispersal vectors including secondary dispersal, outcomes of seed abscission and seedling establishment, which were not identified or measured in this study. Additionally, absence of drift-dispersal equilibrium (a required assumption of the underlying genetic models), which can be the case particularly when working with constantly changing ecosystems such as fire-prone vegetation, could limit the accuracy of results based on indirect genetic methods (Ouborg et al., 1999, Bullock and Clarke, 2000, Hardy et al., 2006). By studying the consequences of dispersal rather than the frequencies of the dispersal events themselves (Bullock and Clarke, 2000, Ouborg et al., 1999), indirect genetic methods only display dispersal events that lead to successful establishment of individuals. Therefore, dispersal estimates are locally and temporally specific as conditions for seedling establishment change. However, indirect genetic method can provide a reliable estimate of the cumulative historical impact of maximum dispersal distances.

2.6.6 Conclusion

We obtained a good understanding on the dispersal of diaspores for our two study species by allowing each method to provide complementary information.

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2.9 Supplementary Materials:

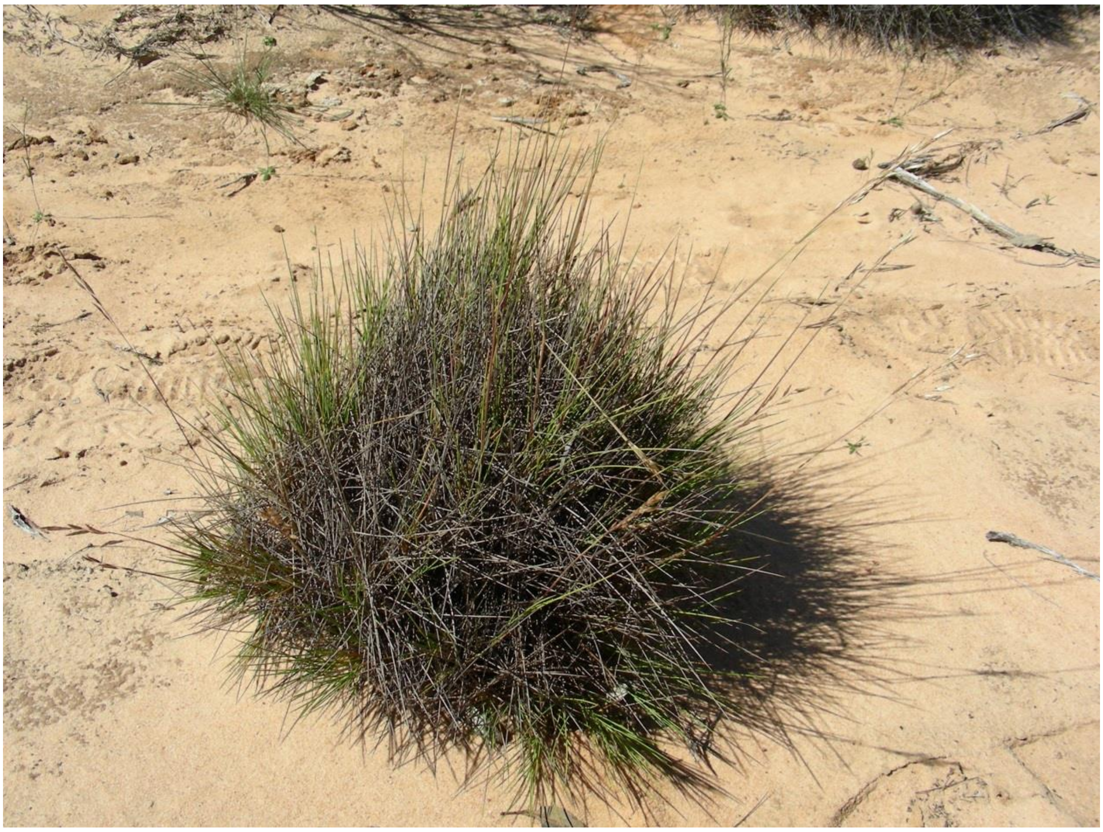
Table S2.1: Likelihood for best fit of different probability density functions fitted to seed dispersal distances from the direct tracking method and the mechanistic model

Species	Function	Method	Likelihood	AIC*	Akaike weights
Triodia irritans	Weibull	Empirical	-280	564	0.00
	log normal	Empirical	-269	543	0.15
	normal	Empirical	-354	712	0.00
	exponential	Empirical	-369	739	0.00
	gamma	Empirical	-268	539	0.85
	Weibull	PAPPUS	-192333	384671	0.00
	log normal	PAPPUS	-194443	388889	0.00
	normal	PAPPUS	-230756	461517	0.00
	exponential	PAPPUS	-202216	404434	0.00
	gamma	PAPPUS	-190896	381796	1.00
Callitris verrucosa	Weibull	Empirical	-530	1064	0.00
	log normal	Empirical	-512	1029	1.00
	normal	Empirical	-622	1248	0.00
	exponential	Empirical	-563	1128	0.00
	gamma	Empirical	-520	1045	0.00
Callitris verrucosa 1-3 m	Weibull	PAPPUS	-290622	581247	0.00
	log normal	PAPPUS	-293501	587005	0.00
	normal	PAPPUS	-311439	622883	0.00
	exponential	PAPPUS	-301971	603944	0.00
	gamma	PAPPUS	-290174	580353	1.00

*Akaike information criterion

CHAPTER THREE

Exploring population genetic diversity and structure of *Triodia irritans* under various fire regimes



Triodia irritans hummock at Hincks Wilderness Protection Area, SA

(Photo B. Dunker)

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3.1 Abstract

Triodia spp. are important components of fire-prone mallee vegetation in Australia, providing shelter and foraging grounds for animals and lizards as well as fuel connectivity to sustain fire occurrences. *Triodia* spp. display great variation in their recovery strategies after burning. Two strategies, resprouting and obligate seeding, have been observed. Which strategy is adopted can vary among *Triodia* species and within species at different sites. The re-establishment strategy expressed by individual plant species is one of the factors influencing how genetic diversity and spatial genetic structure of individual plant populations are affected by different fire regimes.

In this study we documented the genetic diversity and spatial genetic structure of *Triodia irritans* in four sites that were at different stages of recovery from fire, from five to more than 30 years, and had experienced different fire frequencies. This species has been observed to express both re-establishment strategies.

Genetic diversity at eight polymorphic microsatellite loci was high and showed no significant difference among the four sites. We found no evidence of clonality within the species but potential for high levels of inbreeding (G_{is}= 0.16). Spatial genetic structure was very weak in all of the sites.

Synthesis: Genetic diversity within populations of *Triodia irritans* was high and found to be similar under various fire regimes. Based on the observed spatial genetic structure, gene flow within and between sites appears to be high which suggests good potential for rapid restoration of genetic diversity and structure after fire.

3.2 Introduction

Disturbances such as fire can lead to changes in species population genetic diversity and spatial genetic structure. Patterns of spatial genetic structure and diversity, expressed in a plant population after a fire event depend on the plants’ life history traits linked to population recovery and the nature of the disturbance particularly its frequency, intensity and season of occurrence as these factors can cause reduction in population sizes (Banks et al., 2013). Understanding the demographic and evolutionary responses of natural plant populations to disturbance is important as this information aids predicting how species will cope with changes in disturbance regimes caused by human activity.

One way that disturbance influences genetic diversity and structure within populations is through reduction of population size, which often leads to a decrease of genetic diversity within a population (Lowe et al., 2004). Additionally, small populations are more prone to genetic drift so this reduces genetic diversity within populations even further while increasing genetic divergence among populations (Ellstrand and Elam, 1993). In semi-arid ecosystems, obligate seeding and vegetative sprouting are alternative plant life history traits linked to population recovery after burning (Keeley, 1986, Premoli and Steinke, 2008, Noble and Bradstock, 1989). In these environments small populations are likely to be linked to either low survival rates in resprouting species, low establishment success for obligate seeder, or local extinction which can be followed by re-establishment of a few individuals (Ellstrand and Elam, 1993, Ayre et al., 2009, Krauss, 1997) in which case the effect of a bottleneck is expressed as a result of a severe decline in population size. The spatial genetic structure within populations has been found to differ for those various scenarios of population decline (Banks et al., 2013).

In this study we ask whether the genetic diversity and structure of an Australian plant species inhabiting a fire-prone ecosystem differs among sites that have been exposed to a range of fire regimes.

Our expectations of how genetic diversity and structure for resprouter species will be affected by different fire regimes are based on observations from previous studies. Premoli and Steinke (2008) suggested that fire which had burnt all above ground parts of adult plants had little effect on the genetic diversity and spatial genetic structure for resprouter species because genetically identical individuals would reappear via resprouting. As long as resprouting capacity is not compromised by too frequent burning, resprouter species should therefore show little change in genetic diversity or structure under different fire regimes (Bond and Midgley, 2001, Wright and Clarke, 2007). However, if fires are too infrequent, the establishment of new individuals of a resprouter species may be inhibited by increasing competition from dominant seeding plant species (Bond and van Wilgen, 1996). At the same time, if older individuals of resprouter species senesce and die, populations will become smaller, reducing genetic diversity and disrupting established genetic structure.

Plants that are obligate seeders re-establish populations from dormant soil seed banks mostly within the first year after fire (Keeley, 1986). For these species the pre-fire population genetic diversity and spatial genetic structure is often preserved in the seed banks (Shimono et al., 2006, Llorens, 2003, Mandak et al., 2012). However, compared to resprouter species, populations of obligate seeder species have a greater chance for an increase in genetic diversity and a reduction in spatial heterogeneity of genetic structure. This theory is based on the observation that seeders generally produce more seeds and show greater re-establishment success from seeds than resprouters (Segarra-Moragues et al., 2013, Hansen et al., 1991). Hence, there is a greater opportunity for seeds from other sites to contribute to local seed banks. On the other hand, seeder species are more susceptible to short fire frequencies (Bradstock et al., 1998) which can deplete seed banks before the next fire. Fire regimes with fire intervals shorter than the time required for plants to mature or to produce a sufficient amount of seed, can lead to reduced population density or even local extinction of obligate seeder species (Noble, 1989). In that case most, if not all, individuals in a population re-establishing after fire would have to come from seeds from surrounding populations. As a result, we expect that frequently burned populations of an obligate seeder species would exhibit founder effects; with the establishment of patches of closely related individuals expressing strong spatial genetic structure and reduced genetic diversity, resulting from limited seed sources (Davies et al., 2010, Premoli and Kitzberger, 2005, Lowe et al., 2004, Banks et al., 2013). Over time spatial genetic structure is likely to become more random while population genetic diversity should increase during a period without fires if there is sufficient gene flow from surrounding areas.

Spinifex grass (*Triodia* spp.) is a distinctive and major structural element of Australian arid and semi-arid environments, and a significant component of the understorey of fire-prone mallee vegetation in southern Australia (Burbidge, 1952, Noble and Bradstock, 1989). Individuals can show a hemispherical, pyramidal or annular shape, the latter shape being caused by the death of the older central culms (Burbidge, 1952). This highly flammable grass provides fuel connectivity that sustains fire occurrences across mallee dominated landscapes (Bradstock and Cohn, 2002, Bradstock and Gill, 1993, Noble and Vines, 1993). When unburnt, *Triodia* also offers valuable habitat for lizards and other small animal species providing, shelter and foraging resources (Bradstock and Cohn, 2002, Kelly et al., 2011, Driscoll et al., 2012).

Despite the importance of *Triodia* for mallee and other arid ecosystems, its regeneration biology and its population genetics are poorly understood (Bogusiak et al., 1990, Bradstock, 1989, Noble, 1989). After fire has burned spinifex some species in this genus re-establish by seeding recruitment, others by vegetative resprouting while a few species can use both strategies (Rice and Westoby, 1999, Bradstock, 1989, Bogusiak et al., 1990). Factors that determine mechanisms of persistence and regeneration within individual *Triodia* species, or individual populations of a *Triodia* species, are still unknown (Rice and Westoby, 1999). The re-establishment strategy is a major factor affecting how species persist under different fire regimes, how their genetic diversity and structure is shaped by fire regimes, and how they would respond to changes in fire regimes (Rice and Westoby, 1999, Whelan, 1995, Bond and van Wilgen, 1996). Resprouting grass species are recognized as the most fire-resistant component of fire-prone plant communities (Bond and van Wilgen, 1996). Their resprouter strategy is based on continuous leaf growth from intercalary meristems and the growth of new tillers from protected buds. The time when these resprouting grasses are most vulnerable to damage is when fire occurs during their active growth period (Bond and van Wilgen, 1996). Obligate seeding *Triodia* species which are killed in fire and regenerate from seedlings, are most vulnerable after fire until they have flowered profusely enough to ensure some new input into soil seed banks. In *T. pungens,* which re-establishes from seed, this can take between 4 and 7 years after a fire (Craig, 1994).

For *Triodia irritans*, a species common in mallee on the South Australia’s Eyre Peninsula, both re-establishment strategies after fire, resprouting and obligate seeding, have been observed (http://www.landmanager.org.au/fire-responses-triodia-irritans). Since we expect genetic diversity and structure to be affected differently by particular fire regimes depending on the re-establishment strategy expressed, we conducted a pilot genetic study. In this study we explore genetic diversity and spatial genetic structure of *T. irritans* populations from four sites which have experienced different fire regimes. Fire regimes included in this study expressed different times since fire and different inter-fire intervals. Based on our understanding from other studies we would predict genetic diversity and structure to be similar under a wider range of fire regimes as *T. irritans* did show potential for resprouting. However, fire regimes that include repeated burns within short time frames may cause loss of individuals for plants expressing either strategy. Hence, we hypothesise that increased fire intervals (< 4-7 years between fires) can result in less genetic diversity and structure. Additionally we expect to find less genetic diversity and structure in more recently burned sites (< 4-7 years after fires) as well older sites (> 30 years after fire) as populations that age showed strong dieback of old individuals and little evidence for seedling establishment. Additionally, we assess *T. irritans* level of inbreeding, often found in grasses, and whether this species shows evidence for clonality as a result of re-establishment via resprouting and/or separation of annular shape culums in older individuals, which is caused by the death of the older, central culms. Although the *Triodia* genome has not been extensively investigated it can be expected to display polyploidy as this is a very common feature of grasses in the family Poaceae (Levy and Feldman, 2002).

The aim of this study is to improve our knowledge of *T. irritans;* focusing on its re-establishment after fire and our understanding of its susceptibility to variations in fire regimes to provide information necessary to improve managed burning programs.

3.3 Methods

3.3.1 Study region

The study was carried out at Hincks Wilderness Protection Area (33°45' S, 136°03' E; 66658 ha) on the Eyre Peninsula, South Australia. The region is semi-arid, with an average annual rainfall of 340 mm. The main topographic features are white sand dunes interspersed by hard, reddish-brown swales. The dominant vegetation type in the reserve is mallee woodland, characterised by multi-stemmed eucalypts. Common trees are *Eucalyptus incrassata, E. socialis, E. peninsularis* and *C.*

verrucosa. Common understorey species include the shrub *Melaleuca uncinata* (Smith, 2012, Joe Tilley, Ranger Hincks Conservation Park, on published data 31/01/2014), and the hummock grass *T. irritans* (Driscoll and Henderson, 2008). Individuals of *T. irritans* appeared to be continuously distributed inside Hincks Wilderness Protection Area at the time of this study.

3.3.2 The study species

Most species of the genus *Triodia R. Br.* (spinifex) occur on well-drained nutrient poor soils in the arid zone of Australia (Winkworth, 1967). *Triodia* species are generally found either as a central component of hummock grasslands (Suijdendorp, 1981) or in the understorey of mallee woodlands (Noble, 1989). Their combined range occupies about 22% of Australia's landmass (Rice and Westoby, 1999, Griffin, 1984). Individual plants grow fastest during the first 20 to 30 years following a fire, and then stagnate in growth (Haslem et al., 2011) before slowly starting to die back. So the highest ground cover of *Triodia* is generally found in areas 20 to 50 years after fire (Bennett et al., 2010). Reproductive maturity of individuals is reached after 4 to 7 years following either resprouting after fire or germination of new seedlings (Bowman et al., 2008, Craig, 1994). Flowering time of *Triodia* is during summer or in response to rainfall at other times of the year. Seed production appears to be erratic, and plants very rarely generate viable seeds (Jacobs, 1973). Seed germination of *T. irritans* appears to be responsive to heat shock because seed germination after rain events was highest in natural populations following hotter fires (Bogusiak et al., 1990, Noble and Vines, 1993, Bradstock, 1989). Also, in laboratory tests, germination rate was higher after exposing seeds to 80-100˚C, compared to cooler temperature exposure, suggesting that fire promotes germination (Bogusiak et al., 1990). In sites that have not been burned for more than 20 years seedling establishment seems to be negligible (Bradstock, 1989). Seed and pollen are mainly dispersed by wind (Armstrong, 2011), with dispersed seeds commonly found at hummock edges. This is why many seedlings were observed to occur adjacent to other *Triodia* hummocks (Westoby et al., 1988). Although we do not know seed survival times for most *Triodia* species, seeds of *T. basedowii* persist in soil seed banks for less than 3 years (Rice and Westoby, 1999, Westoby et al., 1988).

3.3.3 Data collection

Plant material of *T. irritans* was collected for DNA extraction in October 2010 and April 2011 at four 25 ha sites (approximately 500 x 500 m) within the reserve, spaced about 2 km apart. Sites 1 and 2 were mainly flat with few sand dunes running across; while site 3 was located in a swale between dunes and 4 was on a sand dune slope, facing southwest. The four sites represented a range of times since fire and fire histories (www.naturemaps.sa.gov.au). Sites 1 and 2 were the most recently burned (5 years after fire), site 3 was a medium aged burn (12 years after fire), and site 4 represented a long unburned stand for this reserve (34 years after fire) (Figure 3.1). Since 1959 the sites had been burned between one and four times. Site 2 experienced two fires within 7 years while the other sites had experienced no fires less than 11 years apart (Table 3.1). If the genetic diversity and genetic structure of *Triodia* populations were affected by the time since a fire or the frequency of fires, we would expect to find differences between the sites.

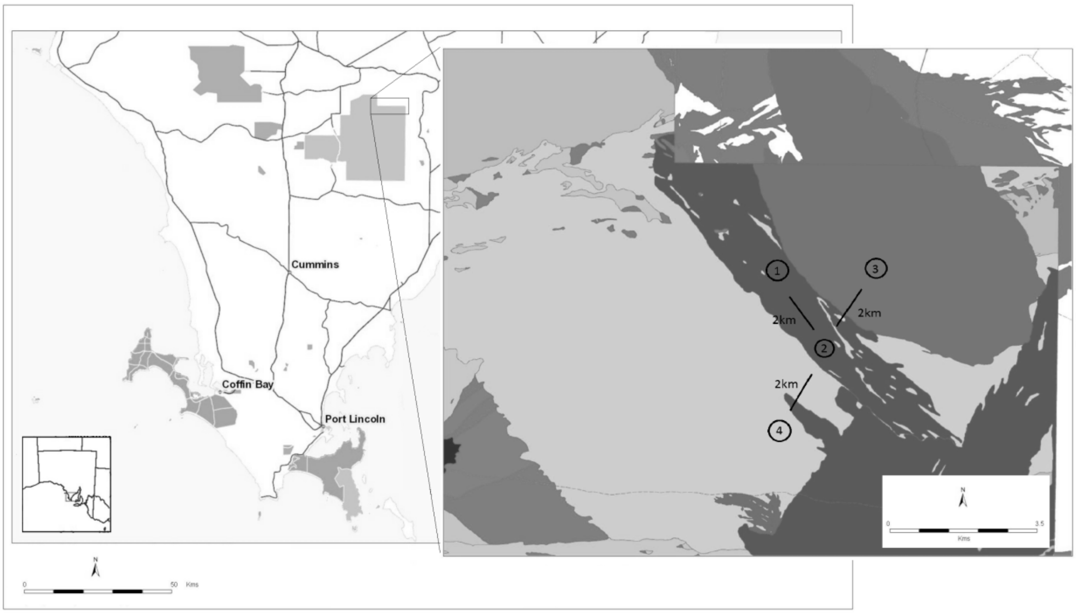


Figure 3.1: Location of *Triodia irritans* sampling sites within the Hincks Wilderness Protection Area on the Eyre Peninsula, South Australia (dark grey = fire in 2006; medium dark grey = fire in 1999; light grey = fire in 1977) (The information on fire history since 1959 was sourced from www.naturemaps.sa.gov.au managed by the South Australian Department of Environment, Water & Natural Resources, accessed November 2011)

Table 3.1: Characteristics of fire regimes in sampling sites for *Triodia irritans* at the time of the study with fire records dating back to 1950

Field site	Type of burn	Month	Date	Years to last fire	Burn intervals
1	Prescribed burn	April	2006	4	
	Bushfire	December	1977		29
	Bushfire	December	1965		12
2	Prescribed burn	April	2006	4	
	Bushfire	December	1977		29
	Bushfire	November	1966		11
	Bushfire	November	1959	7	
3	Bushfire	January	1999	12	
	Bushfire	December	1977		22
	Bushfire	November	1966		11
4	Bushfire	December	1977	34	

At each site we collected inflorescences or leaf samples from 50 individuals that were at least 20 m apart from each other, and from 20 adjacent individuals within a 5 m radius circular area. Locations of all sampled individuals in the broad scale sampling were recorded using a GPS-device. For the exhaustive sampling only the centre point was GPS-located and degree and distance from the centre point was recorded for each sampled individual. This sampling design provided two spatial scales for analysis of genetic structure at each site as well as investigations on clonality within this species. For a randomly chosen subset of the sampled plants in site 2, 3 and 4 (site 1 was not included due to missing data) we measured the diameter of the plant assuming that it would be linked to plant age. We also counted the number of inflorescences for a randomly chosen subset of the sampled plants in all sites to identify its reproductive stage and therefore its potential contribution to local seed input.

3.3.4 Microsatellites

The collected plant material was stored in silica gel until DNA extraction. Genomic DNA extractions were performed on dried spikelet or leaf material at the Australian Genome Research Facility (AGRF; www.agrf.org.au) using Machery Nagel kits following manufacturer’s instructions.

A microsatellite library was compiled for *T. irritans* using next-generation sequencing. For this purpose genomic DNA (12 µg) pooled from 16 individuals was sequenced on a Roche 454 GS-FLX system at AGRF following Gardner et al. (2011).

We used the program iQDD 0.9 (Meglécz et al., 2010) to identify sequences with a minimum of eight di-, tetra- or penta-base repeats and to remove redundant sequences. The program produced 160 individual sequences for potential microsatellites for *T. irritans*. Out of those we chose 50 design primers with PCR product lengths between 80 and 480 base pairs. Those primers were tested for amplification and polymorphism using Polymerase Chain Reactions (PCR) followed by poly-acrylamide gel electrophoresis. Eight suitable polymorphic microsatellite loci were identified. These loci were amplified in each of 280 field collected samples (70 per site) using fluorescently labelled forward primer and an unlabelled reversed primer (Table 3.2).

Table 3.2: Characteristics of microsatellite loci for *Triodia irritans*

Loci	Primer sequence (5'-3') (F, forward; R, reverse)	Repeat motif	N_A	No. of bands*	Size range (bp)
Ti16	F-TGGTGAAGACCTTGTGAAGAGA	$(AT)_{10}$	18	1-4	180-230
	R-TTTTGCTTAACCTTCACCGC	-	-	-	-
Ti22	F-TGTTTCTCAGTGTGGGTTTCA	$(TTG)_{10}$	23	1-6	210-300
	R-CTTCGAACATGCAGGGAGTT	-	-	-	-
Ti24	F-GACACCATGAAGGAAATCGG	$(TA)_{11}$	27	1-5	230-300
	R-GCAGGAGCAAACTGTACTGAAG	-	-	-	-
Ti25	F-GTTCGAGGCGAACAGAAAGT	$(CT)_{10}$	26	1-4	260-350
	R-CGCTGTCAAGCATTTCAAGA	-	-	-	-
Ti37	F-TGTTGGCTGCTGCACTAATC	$(AT)_{10}$	11	1-4	100-170
	R-TCTGAGTTGTCTTTTGCTAAGCTC	-	-	-	-
Ti38	F-CAAGCAGAAGCATGCCATAG	$(TC)_9$	15	1-4	110-180
	R-GAAGAAAATTACATGCCTCCAAA	-	-	-	-
Ti39	F-TGGGACAGGAGAACGACTTC	$(ACG)_8$	2	1-2	130-170
	R-CGTGATGACGTAGTCGAACG	-	-	-	-
Ti40	F-TCCATTTCTGTGATGGAGCTA	$(TA)_9$	7	1-4	110-180
	R-AGCAACAATAGTCCGAAGGG	-	-	-	-

*per individual

PCR reactions were performed in a total volume of 10 µl containing 2-10 ng of template DNA, 4.56 µl of water, 1µl of 10x PCR Gold Buffer, 2 mM $MgCl_2$, 0.8 mM dNTP, 0.2 µM of each of the labelled forward primer and the unlabelled reverse primer, 1.2 mM of BovineSerumAlbmin, 5 M of Betaine and 0.2 Units of TaqGold Enzyme. Amplifications were carried out in a Corbett Research Palm cycler Model CG1-96 with the following cycling parameters: 94˚C for 9 min, followed by 34 cycles of 94˚C for 45 s, 45 s at an annealing temperature of 50˚C (Ti_8), 52˚C (Ti_4, Ti_5, Ti_7 and Ti_9) or 54˚C (Ti_1, Ti_2, Ti_3 and Ti_6), 72˚C for 1 min, and a final extension at 72˚C for 30 min and 25˚C for 30 s).

The amplicons for each sample were pooled and sent to AGRF for fragment separation on an AB3730 DNA Analyser (Applied Biosystems) with the size standard GS500 (-250) LIZ.

3.3.5 Plant characteristics

We used the Kruskal-Wallis Test to assess differences in mean diameter sizes of hummocks and average number of inflorescences between sites, rather than a parametric ANOVA because sample sizes were small, mean variances among size categories were not homogeneous and individuals within the different size categories were non-normally distributed.

3.3.6 Microsatellite loci and estimation of ploidy

Alleles were scored using GeneMapper 4.0 (Applied Biosystems) with manual checking. The final data set was reduced in size for most sites due to problems with sample amplification and consisted of 61 individuals for site 1, 64 individuals for site 2, 70 individuals for site 3, and 48 individuals for site 4. The likely polyploidy of *Triodia irritans* (see results) could possibly explain the problems with sample amplification that we experienced (Dufresne et al., 2014). Partial heterozygotes could not be reliably detected from microsatellite data as a result of polyploidy (Hanson et al., 2007, Dufresne et al., 2014). We therefore described multilocus phenotypes for each individual using presence or absent of all alleles at each locus turning co-dominant marker into dominant marker, following Clarke et al. (2012). Those phenotypes are not equal to genotypes as the number of copies of each allele at a locus is ambiguous unless individuals are homozygous or fully heterozygous (Clarke et al., 2012, Dufresne et al., 2014). To simplify data analysis, we assumed that the populations had a uniform level of ploidy and, because some individuals showed up to six different alleles at individual loci, we assumed all individuals were hexaploid.

3.3.7 Genetic Diversity and clonality

Allelic diversity, calculated as the number of alleles per locus averaged over all eight loci (Na), observed gametic heterozygosity (*H*o), expected heterozygosity (Hs) and inbreeding coefficient (Gis) were assessed for each sampled site using the program GenoDive version 2.0b19 (Meirmans and Van Tienderen, 2004). To identify existing clonality, the observed number of single locus phenotypes averaged over loci (*N*'p) was calculated by using the multilocus 'matches' function with GENALEX version 6.5 (Peakall and Smouse, 2012). To explore population genetic diversity in *T. irritans* under different fire regimes we compared genetic diversity measures among the four sites. Pairwise genetic distances between individuals averaged over loci was calculated in POLYSAT (Clark and Jasieniuk, 2011) using a method developed by Bruvo et al. (2004). The Bruvo distance is a genetic distance measure that is similar to band-sharing indices used with dominant data but takes into account mutational distances between alleles. This method appeared most suitable as it can be used for calculations from microsatellite genotypes irrespective of ploidy level (Bruvo et al., 2004).

3.3.8 Spatial genetic structure

To assess spatial genetic structures amongst sites we tested for relatedness between individuals from different sites using the genetics data from all sites applied to a Principal Components Analysis (PCoA) in GenoDive Version 2.0b23. The estimated genetic variance within and among sites was partitioned applying a hierarchical AMOVA and pairwise φ_{PT} (an analogue of F_{ST}) between sites based on the Bruvo genetic distance matrix in GENALEX version 6.5 (Peakall and Smouse, 2012).

To assess spatial genetic structure within each site, the Pairwise KINSHIP coefficients (F_{ij}) over all loci (Loiselle et al., 1995) were calculated at two different scales using SPAGeDi software (Hardy and Vekemans, 2002). First, we used all genotyped individuals in each site, including the exhaustive samples (20 individuals within a 5 m radius) with distances between pairs of individuals displayed in metres. In a second approach we used only individuals from the exhaustive sampling, 5 m radius area within each site with distances between pairs of individuals displayed in cm. To make the polyploid dataset compatible with the software, we followed the recommendations of O. Hardy (pers. comm. 09/03/2013). Hence, we set the program to assume hexaploidy for all genotypes and replaced missing data with zeroes for loci with fewer than six alleles. The number of distance intervals was adjusted to the highest possible number that maintained a CV partic value of less than 1 for each distance interval. The tests were run with 500 permutations. For each site we calculated *Sp*-values which can be used to identify the strength of the spatial genetic structure and which are suitable for comparison of spatial genetic structure between populations and species (Vekemans and Hardy, 2004). The *Sp*-value mostly depends upon the rate of decrease of pairwise kinship coefficients between individuals with the logarithm of the distance in two dimensions described by the formula ($Sp = - b/ (1-F_{(1)})$). In this formula - b is the regression slope of the mean pairwise kinship coefficient (F_{ij}) on the natural logarithm of distance interval (r_{ij}), and $F_{(1)}$ is the mean F_{ij} between individuals in the first distance interval (Vekemans and Hardy, 2004, Hardy et al., 2006).

3.4 Results

3.4.1 Plant characteristics

The size distributions of *T. irritans* plants varied among the sites (Kruskal-Wallis 1-way ANOVA $P< 0.001$; Figure 3.2) with more large-diameter plants at site 4 (the long unburned site), but more smaller plants (some with diameters less than 10 cm) at site 3 (the intermediate time since fire site). The widest distribution of sizes was observed in site 4 with sizes ranging from less than 10 cm to about 80 cm diameter. There was also a significant difference in the number of flowering plants, and the number of flowers per plant among the subsets of individual plants assessed at each site (Table 3.3). Sites 1 and 2, the most recently burned sites, had significantly less flowering than sites 3 and 4.

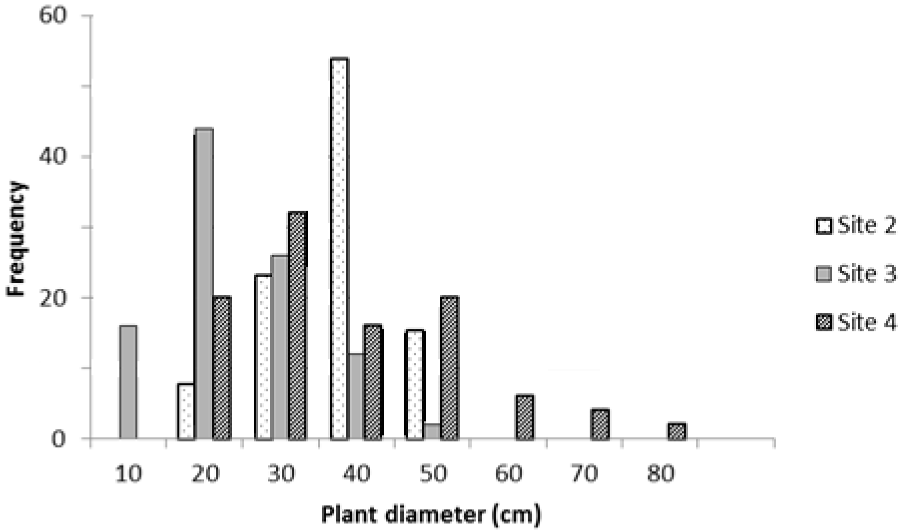


Figure 3.2: The percentage of individuals in different size classes for *Triodia irritans* found at sites with different times since fire (Site 2 was burned in 2006, site 3 in 1999 and site 4 in 1977). Kruskal-Wallis 1-way ANOVA p< 0.001

Table 3.3: Average number of inflorescences calculated for a subset of sampled individuals and percent of flowering individuals in that sampled subset for *Triodia irritans* found at sites with different times since fire (Site 1 and 2 were burned in 2006, site 3 in 1999 and site 4 in 1977).

Number of inflorescences	Site 1	Site 2	Site 3	Site 4
N	20	20	29	50
Average*	0.3	3	13	11
SE	0.1	0.3	0.5	0.3
Percent of flowering plants	5	35	90	80

*Kruskal-Wallis 1-way ANOVA comparing number of inflorescences amongst the different sampling sites (p< 0.001)

SE = Standard error

3.4.2 Estimation of ploidy

The presence of more than two alleles per locus was detected in individuals from all of the four sites (Table 3.4). The maximum number of alleles observed in an individual at one locus ranged from two (Ti39) to six (Ti22) (Table 3.5). This indicates that *T. irritans* at our study area is likely to be hexaploid, although we cannot confidently eliminate the alternative that individuals of different ploidy levels coexisted at some or all sites.

Table 3.4: Percentage of individuals of *Triodia irritans* with different numbers of alleles detected for different sampling sites

Sites	n	1-2 Alleles	3 Alleles	4 Alleles	5 Alleles	6 Alleles
1	61	64.7	30.9	2.9	1.5	0.0
2	64	57.1	31.4	11.4	0.0	0.0
3	70	42.9	52.9	4.3	0.0	0.0
4	48	11.4	44.3	35.7	7.1	1.4

Table 3.5: Percentage of different allele numbers detected for the different loci amongst sampled individuals of *Triodia irritans*

Loci	n	1-2 Alleles	3 Alleles	4 Alleles	5 Alleles	6 Alleles
Ti16	241	96.1	3.5	0.4	0.0	0.0
Ti22	227	85.8	7.9	4.6	1.3	0.4
Ti24	200	85.1	10.4	4.0	0.5	0.0
Ti25	189	93.8	5.2	1.0	0.0	0.0
Ti37	209	91.6	6.5	1.4	0.5	0.0
Ti38	188	97.4	1.6	1.0	0.0	0.0
Ti39	239	100	0.0	0.0	0.0	0.0
Ti40	206	98.1	1.4	0.5	0.0	0.0

3.4.3 Genetic diversity and clonality

We detected a total of 129 unique alleles across the eight loci. The number of alleles per locus ranged from 2 to 27 resulting in a mean of 16.8±3.4 (SE) (Table 3.2). There was no evidence for clonality as each of the 243 completely genotyped individual possessed a unique multilocus phenotype. None of the parameters describing genetic diversity at each site, mean numbers of alleles per locus (N_A), observed heterozygosity (H_O) expected heterozygosity (H_S), or inbreeding coefficient (Gis) (Table 3.6) seem to differ greatly among sites. Estimated inbreeding coefficient over all sites was 16 %. The hierarchical AMOVA based on the Bruvo genetic distance matrix showed that the greatest proportion of variation (89%) was found within sites. Excluding site 4 we detected an even higher proportion of the total sample variation (99%) within sites.

Table 3.6: Indices of genetic diversity estimated for individuals of *Triodia irritans* at each of the sampled site

Site (fire)	N	N_A	H_O	H_S	G_{is}
1 (recently)	61	11.9	0.589	0.707	0.167
2 (recent, and frequently)	64	12.4	0.55	0.69	0.203
3 (medium burn age)	70	11.1	0.606	0.689	0.121
4 (long unburned)	48	9.3	0.557	0.652	0.146
All sites (SE)	243	16.8 (3.4)	0.575 (0.08)	0.684 (0.07)	0.159 (0.112)

N_A = Number of alleles per locus, averaged over loci,

Ho= Observed Heterozygosity

H_S = Heterozygosity within sites (expected Heterozygosity)

G_{is}= Inbreeding coefficient

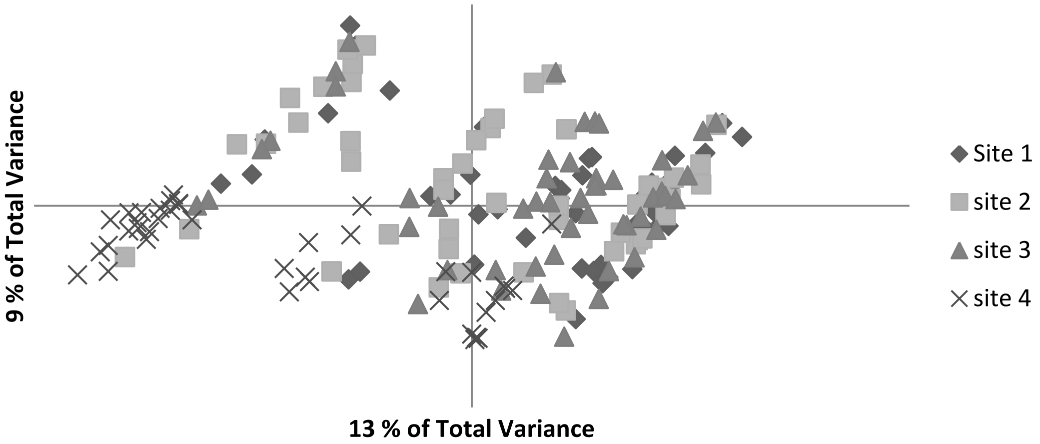


Figure 3.3: Principal Coordinates (PCoA) showing the amount of variance amongst individuals of *Triodia irritans* from the different sampling sites, explained by the first two axes

Table 3.7: Pairwise Population ϕPT Values based on *Triodia irritans* mean distance matrix including all sampled individuals (n= 243). ϕPT Values below diagonal. Probability, p (rand >= data) based on 999 permutations is shown above diagonal.

	Site 1	Site 2	Site 3	Site 4
Site 1		0.127	0.001	0.001
Site 2	0.004		0.001	0.001
Site 3	0.019	0.017		0.001
Site 4	0.221	0.201	0.221	

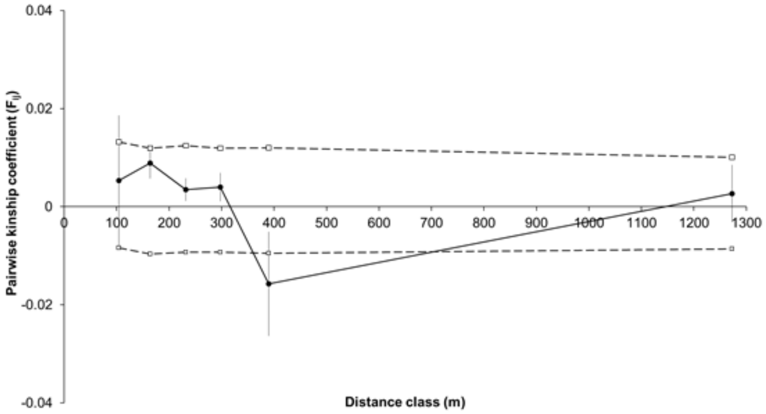
3.4.4 Spatial Genetic Structure

Genetic clustering of individuals visualised by the PCoA (Figure 3.3) and estimated pairwise φ_{PT} among sites (Table 3.7), showed that individuals from site 1, 2 and 3 tended to cluster together while individuals from site 4, the long unburned site, tended to form a slightly separated genetic grouping. For the total sample collected at each site, there was weak evidence for spatial genetic structure in site 3 displaying a significant decrease in relatedness with increasing distance between individuals (Figure 3.4c), while relatedness between individuals from site 1, 2 and 4 showed no significant spatial correlation of relatedness (Figure 3.4a, b, d). The *Sp*-value at each site was low

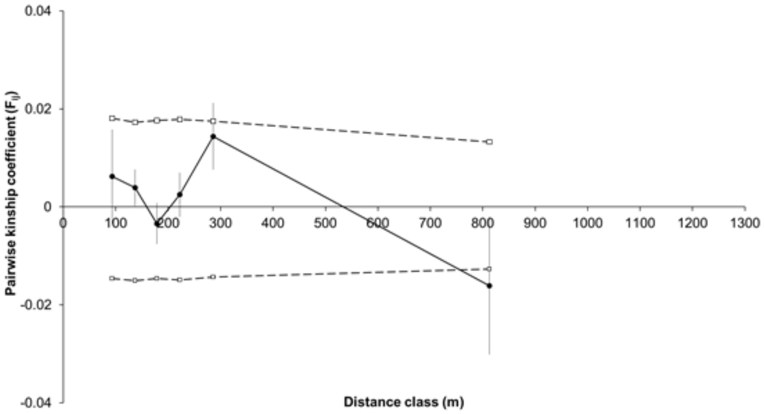
(Figure 3.4) which indicates that, spatial genetic structure was weak. We found even less evidence for significant spatial genetic structure at the finer spatial scale when including only individuals from the exhaustive sampling (Figure 3.5).

Figure 3.4: Average kinship coefficients (F_{ij}) between pairs of individuals of *Triodia irritans* plotted against the geographical distance (m) in each sampling site including all samples; (a) Site 1, (b) Site 2, (c) Site 3, (d) Site 4. Dashed lines represent 95% confidence intervals for pairwise KINSHIP coefficient (F_{ij}) under the null hypothesis that genotypes are randomly distributed. Significant values for the pairwise KINSHIP coefficient (F_{ij}) are found above the upper 95% confidence interval. Vertical lines represent SE-values of pairwise KINSHIP coefficients (F_{ij}). Site 1, *Sp*-value = 0.003; Site 2, *Sp*-value = 0.004; Site 3, *Sp*-value = 0.003; Site 4, *Sp*-value = 0.002

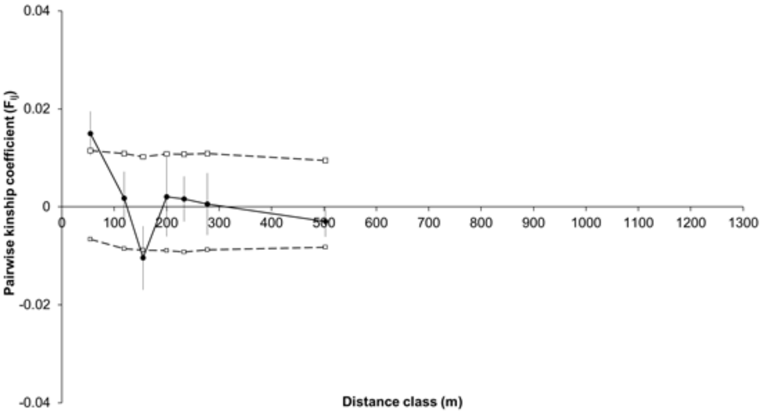
a)


0.04
0.02
0
-0.02
-0.04
Pairwise kinship coefficient (F_{ij})
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
Distance class (m)

b)


0.04
0.02
0
-0.02
-0.04
Pairwise kinship coefficient (F_{ij})
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
Distance class (m)

c)


0.04
0.02
0
-0.02
-0.04
Pairwise kinship coefficient (F_{ij})
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
Distance class (m)

d)

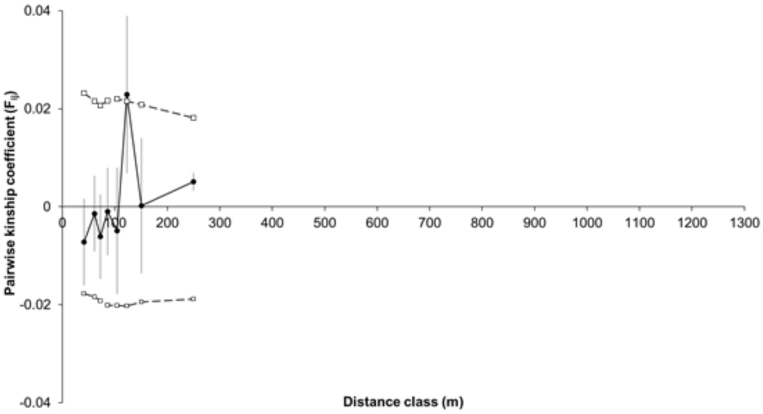
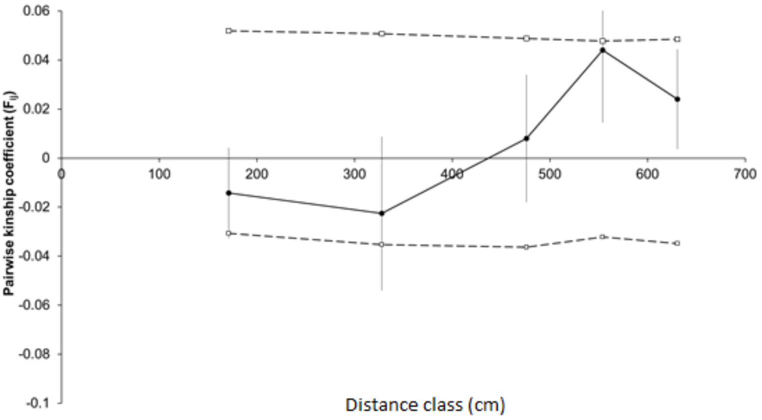

0.04
0.02
0
-0.02
-0.04
Pairwise kinship coefficient (F_{ij})
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
Distance class (m)

Figure 3.5: Average kinship coefficients (F_{ij}) between pairs of individuals of *Triodia irritans* plotted against the geographical distance (cm) in each exhaustive sampling site; (a) Site 1, (b) Site 2, (c) Site 3, (d) Site 4. Dashed lines represent 95% confidence intervals for pairwise KINSHIP coefficient ((F_{ij}) under the null hypothesis that genotypes are randomly distributed. Significant values for the pairwise KINSHIP coefficient (F_{ij}) are found above the upper 95% confidence interval. Vertical lines represent SE-values of pairwise KINSHIP coefficients (F_{ij}). Site 1, *Sp*-value = -0.0159; Site 2, *Sp*-value = -0.0137; Site 3, *Sp*-value = -0.0126;
Site 4, *Sp*-value = -0.0007

a)


Pairwise kinship coefficient (F_{ij})
0.06
0.04
0.02
0
-0.02
-0.04
-0.06
-0.08
-0.1
0
100
200
300
400
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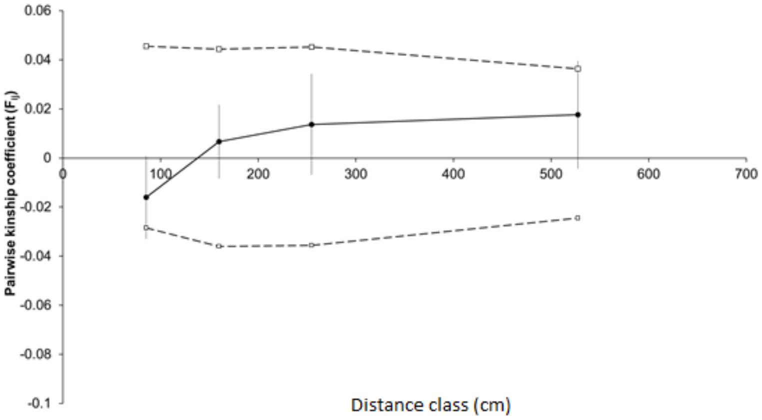
Distance class (cm)

b)


Pairwise kinship coefficient (F_{ij})
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Distance class (cm)

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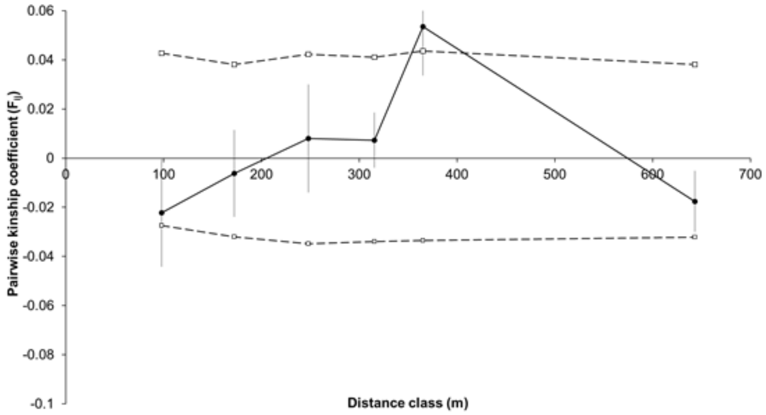
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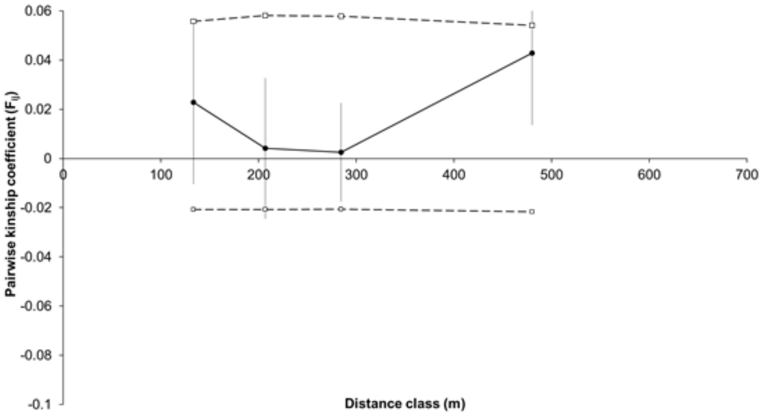
Pairwise kinship coefficient (F_{ij})
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Distance class (m)

3.5 Discussion

The genetic diversity of *T. irritans* was equally high in each of the four sampled sites. Observations of high levels of genetic diversity are common in polyploid species (Lowe et al., 2004). The similarity in diversity among sites despite their different fire histories suggests that the range of past fire regimes at the four sites did not affect genetic diversity within *T. irritans*, and that any regime from within that range could be used without reducing genetic diversity. Both strategies, resprouting as well as obligate seeding can maintain genetic diversity levels under suitable fire regimes. Populations of resprouting species were found to be resilient to various fire regimes due to the survival of most individuals (Premoli and Steinke, 2008), while an obligate seeding re-establishment strategy could produce similar patterns if genetic diversity is maintained consistently in soil seed banks established before fire (Mandak et al., 2012, Llorens, 2003, Hanin et al., 2013). The genetic data showed no evidence for clonality within this species. This would lead to the conclusion that older individuals do not separate into several neighbouring culums. Inbreeding appears to be low in *T. irritans* (G_{is}= 0.1) compared to *Bromus tectorum* an inbreeding annual grass (F_{IS}= 0.9) (Meyer et al., 2013). This is further supported by a study on *Poa hiemata*. This species showed a similar inbreeding coefficient averaged over all sites (F_{IS}= 0.09) as *T. irritans* and was referred to as a species which showed low levels of inbreeding (Byars et al., 2009). Hence, our data would suggest that inbreeding is very rare within *T. irritans*.

The majority of the regional genetic variation of *T. irritans* resided within the sampled sites, and there was little genetic divergence among the sites. Maintaining high levels of genetic diversity within sites, and low divergence among adjacent sites is indicative of strong gene flow between sites (Lowe et al., 2004). Populations at sites 1, 2 and 3 were very similar in genetic composition, while site 4 located to the south-west of the other sites, formed an identifiably separate cluster. The sample size of sites is too small to allow rigorous explanations for this pattern, but this small genetic distinction could result from gene flow barriers due to the crest and swale structures of sand dunes that appeared to be more aggregated around site 4 and were running on a NW to SE gradient (Department of Environment and Heritage, 2005). Additionally, vegetation in site 4 was much older and hence, denser and higher compared to vegetation cover observed in the other three sites. We did not consider that genetic differentiation among populations could result from genetic drift in small isolated populations due to the continuous distribution of *T. irritans* within Hincks Wilderness Protection Area.

There was no consistently significant spatial genetic structure within any of the sites and *Sp*-values (mean *Sp*-value = 0.003 SE±0.0004) were much lower than would be expected for plants with small heights which generally do not disperse very far; estimated *Sp*-values = 0.046 SE±0.0643 (n=24) for herbaceous plants (Vekemans and Hardy, 2004). The absence of spatial genetic structure even over very small scale (less than 10 m) would suggest that seedlings do not grow in close vicinity to the parent plant. This lack of significant spatial genetic structure in our study species is surprising considering that most primary seed dispersal by wind was measured to be no more than 10 m under commonly occurring wind conditions (see Chapter 2) and for both re-establishment strategies (resprouter or seeder) we expected to find strong spatial genetic structure based on findings from former studies of other species re-establishing after fire (Premoli and Steinke, 2008, Shimono et al., 2006). For a resprouter, established spatial genetic structures should be retained as established individuals survive fire events (Premoli and Steinke, 2008) and in seeder populations soil seed banks generally preserve some of the spatial patterns of genetic diversity of the parent population (Shimono et al., 2006, Llorens, 2003, Mandak et al., 2012). There are some common explanations for why studies have not detected spatial genetic structure. One is that the information from the genetic markers used might be insufficient to detect real patterns within populations (Vekemans and Hardy, 2004). However, we consider that the use of eight polymorphic loci and the sampling effort undertaken in this study exceeds the effort of many other studies that have detected spatial genetic structures in populations. Another explanation would be that the spatial scale of the sampling design did not encompass the spatial scale of real genetic structure in the population (Vekemans and Hardy, 2004). Spatial scales included in our study ranged from less than 5 m to more than 500 m; hence, if there is spatial genetic structure it may appear over even greater distances. This was confirmed by an analysis of gene flow in *T. irritans* which including all four sampling sites that showed spatial genetic structure at a distances of around 100 m (Figure S3.1). Additionally, rare long distance dispersal events of seeds have been detected over distances of up to 3 km (see Chapter 2). This provides further evidence for extensive gene flow within spatially restricted sites but limited gene flow among sites spaced further apart.

Plant characteristics recorded in the sampling sites provide some indications of re-establishment patterns. Sites 1 and 2 (unburned for 5 years) had low numbers of flowering plants with few inflorescences per plant, suggesting those populations were made up of mostly immature individuals, which is consistent with previous reports that *Triodia* species take about 4 to7 years to achieve sufficient flowering to produce a viable seed bank (Craig, 1994). If we assume that diameter reflects plant age, then the youngest plants were in site 3 (unburned for 12 years) while the most recently burned site 2 shows the highest number of individuals in the medium size class. Since local seed input (seeds from plants within sites) is only present after plants matured, we expected to find most recruitment in site 3, while site 2 would rely on sporadic seed input from surrounding sites. The site with the longest time since fire (site 4) had some individual plants almost twice the diameter of the largest plants at other sites and a wide range of plant sizes which is suggesting regular recruitment over a period without fires. However, in comparison between site 3 and 4 we found less than half individuals in the medium diameter size class (20 to30 cm) and non in the small diameter size class (1 to10 cm) at site 4 which could support the theory that re-establishment decreases with population age.

Based on our data we suggest that *T. irritans* populations are resilient to variations in fire regimes, displaying no significant differences in population genetic diversity or structure with fire history, indicating that there are high levels of gene flow between sites. From our study, the genetic diversity of *T. irritans* was maintained across sites with a range of fire histories (frequency and time since fire). However further studies of *T. irritans* population genetics are recommended to investigate the dynamics of older sites and replicates of different fire histories.

3.6 Acknowledgements

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3.8 Supplementary Materials:

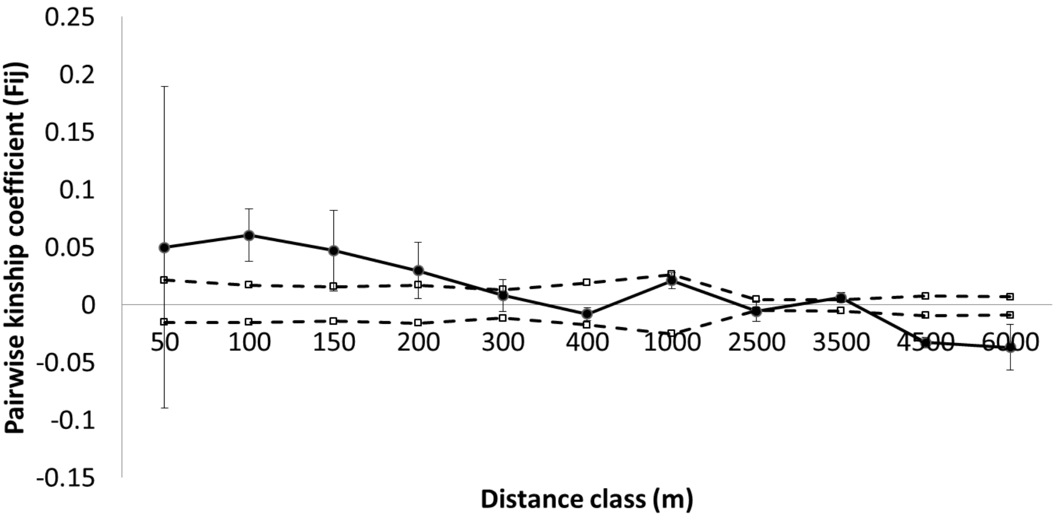

Figure S3.1: Average kinship coefficients (F_{ij}) between pairs of individuals of *Triodia irritans* plotted against the geographical distance (m). Samples were collected at four sampling sites that covered a distance of around 6 km. Dashed lines represent 95% confidence intervals for pairwise KINSHIP coefficient (F_{ij}) under the null hypothesis that genotypes are randomly distributed. Significant values for the pairwise KINSHIP coefficient (F_{ij}) are found above the upper 95% confidence interval. Vertical lines represent SE-values of pairwise KINSHIP coefficients (F_{ij}).

CHAPTER FOUR

Low genetic diversity of *Callitris verrucosa* in mallee vegetation

Mature *Callitris verrucosa* at Hincks Wilderness Protection Area, SA

(Photo B. Dunker)

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4.1 Abstract

How particular plant species are affected by different fire regimes depends largely on their strategies of recovery after fire. Species that rely on fire-stimulated recruitment from persistent seed banks are expected to be sensitive to changes in fire intervals, with short fire intervals (of less than the time for maturity of individual plants) having the potential to cause reduction in population density or local extinction. Reduction in population size can lead to low genetic diversity in plant populations, which could reduce the ability of the populations to persist through environmental changes.

In this study we examined genetic diversity and structure in a serotinous obligate seeder, *Callitris verrucosa*, across sites of variable fire intervals and times since the last fire within fire-prone mallee woodland. Using amplified fragment length polymorphism we aimed to investigate if genetic diversity in a fire-sensitive species is reduced under fire regimes with short fire intervals. Additionally we looked for evidence of metapopulation structures consistent with conditions of local extinction and recolonisation.

We did not identify any significant correlations between genetic diversity and both number of fire intervals and times since the last fire. Genetic diversity at all sampled sites was exceptionally low. However there was a trend towards an increase in genetic diversity with time since fire. There was no evidence for metapopulation structures amongst sites. Instead, finding little genetic distinction between sites, this species appears to be panmictic with strong gene flow within and amongst sites.

4.2 Introduction

In fire-prone vegetation, where crown-fire regimes are common, plant life history attributes such as: fire-stimulated recruitment; persistent seed banks, and time until first reproduction are important traits determining population recovery after burns. Species that rely on fire-stimulated recruitment and persistent seed banks seem to be sensitive to changes in fire regimes (Bond and Midgley, 2001). For example, seedling establishment of serotinous woody species is strongly affected by the burn's season so in population studies the frequency of newly establishing seedlings can be higher following summer compared to winter fires (Wright and Clarke, 2007).

Another potential threat to the persistence of plant populations of serotinous obligate seeders is short fire intervals (Christensen, 1985, Fisher et al., 2009). Generally, recruitment in an obligate seeder after fire would happen from locally produced seeds stored in seed banks (Bond and van Wilgen, 1996). However, a fire interval shorter than the time required for a plant to produce enough seed to guarantee replacement (primarily due to fires during the juvenile period) will cause a reduction in population density or even lead to local extinction (Bradstock et al., 1996, Enright et al., 1998). A simulation study on the effect of increased fire frequency, due to prescribed burning, showed reductions in population size and high risk of extinction for obligate seeders (Bradstock et al., 1998, Bradstock et al., 2006). In this case the species would rely on population re-establishment via seeds coming from other sites which is called the rescue effect (Gotelli, 1991).

Reduction in population size and local extinction followed by subsequent re-colonisation due to fire can reduce genetic diversity and alter the spatial genetic structure of plant populations (Banks et al., 2013, Ayre et al., 2009, Krauss, 1996). A decrease in genetic diversity can reduce the ability of populations to persist through environmental changes (Aguilar et al., 2008). Hence, of the question whether short fire frequencies lead to reduced genetic diversity within plant populations is of importance when considering biodiversity in fire-prone ecosystems.

In fire-prone environments, genetic diversity in plant populations of serotinous obligate seeders is maintained by gene flow through pollen and seed dispersal and stored in seed banks until fire induces seed release (canopy seed banks) or germination (soil seed banks) (Bradstock et al., 1996, Pausas et al., 2004). In serotinous obligate seeding species, the level of genetic diversity within populations after fire should reflect the diversity of adult plants previously at the site that have been killed by fire and potentially incoming propagules from other sites (Davies et al., 2010, Austerlitz et al., 2000, Banks et al., 2013). So when conditions have caused the absence of seed banks after a fire event, potential recruitment via seed from other sites into burned areas is of great importance (He et al., 2004). Spatial genetic structure as a result of local population extinction and plant recruitment via individual seeds coming in from surrounding sites would be expected to display a founder effect; manifesting as the establishment of patches of closely related individuals and low genetic diversity (Davies et al., 2010, Premoli and Kitzberger, 2005). However, over time long distance dispersal of seeds, arriving in occupied patches, can have an homogenizing effect on the overall spatial genetic structure (Bohrer et al., 2005) and an effect of increasing the initially low levels of genetic diversity within founder populations (Pannell and Charlesworth, 2000, Banks et al., 2013).

Rescue and/or founder effects are characteristics generally observed in species that display metapopulation structures (Hanski, 1985). A metapopulation is defined as a group of genetically distinct populations (subpopulations) of the same species, separated by space. Each subpopulation cycles in relative independence of the other populations and eventually goes extinct as a consequence of demographic (Levins, 1969). The dynamics observed in metapopulations is that local populations may go extinct and be subsequently recolonized by immigration from other subpopulations; the fate of such a system of local populations depends on the balance between extinctions and colonisations. In fire-prone environments these dynamics often lead to a reduction in overall population genetic diversity (Pannell and Charlesworth, 2000).

A common tree species in fire-prone mallee vegetation on the Eyre Peninsula of South Australia is *Callitris verrucosa.* This species is a serotinous obligate seeder, with fire stimulated recruitment from an exhaustible canopy seed bank. The trees accumulate seeds within cones that remain on the tree until an intense crown fire, which releases the seed and kills the parent tree. Seed release between fires is negligible. These trees take about 15 years to reach sexual maturity. Under fire regimes with short intervals (less than 15 - 20 years) between crown fires, the accumulation of canopy seed banks will be limited. So if adult trees are killed by the fires, local populations are expected to decrease. This can result in loss of genetic diversity within populations, if not population extinction (Keith et al., 2007, Bradstock and Cohn, 2002). Seed dispersal from surrounding unburnt or less frequently burned areas can potentially mitigate the threats of reduced genetic diversity and local extinction in frequently burned sites. Seed dispersal distances of 8 km have been detected for this species (see chapter 2).

In this study we examined genetic variation of *Callitris verrucosa*, across sites that varied in fire frequency and time since last fire. Based on the literature we were expecting to find lower genetic diversity and strong spatial genetic structure in sites that experienced short fire frequencies (less than 15 years), evidence of rescue effect, compared to sites that have been burned less frequently. With increasing time since the last fire we expected genetic diversity to be higher and spatial genetic structure reduced. Additionally, we assessed genetic distance and differentiation amongst sites to determine whether *Callitris* shows evidence for metapopulation structures. It is hoped that our findings will improve our understanding of the species *C. verrucosa* and aid in evaluating its resilience under current and future fire regimes.

4.3 Material and methods

4.3.1 Study region

This study was carried out at Hincks Wilderness Protection Area (33°45' S, 136°03' E; 66658 ha) on the Eyre Peninsula, South Australia. This area is semi-arid with an average annual rainfall of 340 mm. The main topographic features are white sand dunes interspersed by hard, reddish-brown swales. The dominant vegetation type in the reserve is mallee woodland, characterised by multi-stemmed eucalypts. Common trees are *Eucalyptus incrassata, E. socialis, E. peninsularis* and *C. verrucosa*. Common understory species include the shrub *Melaleuca uncinata* (Smith, 2012, Joe Tilley, Ranger Hincks Conservation Park, on published data 31/01/2014), and the hummock grass *T. irritans* (Driscoll and Henderson, 2008).

4.3.2 Study species

Callitris verrucosa (A. Cunn. Ex Endl.) F. Muell., a native mallee-pine from the family Cupressaceae, is a canopy dominant of mallee vegetation and a serotinous obligate seeder (Bond and van Wilgen, 1996). It grows as a small stunted tree, often with several trunks, usually up to 3 m but can reach 6 m height (Bonney et al., 1994). The foliage is glaucous or dark green and consists of scale-like evergreen leaves. The plant is monoecious, however it has been suggested to produce few viable seeds from selfing (ESA Conference, 2011). Female cones are about 3 cm in diameter and densely warted. Cones contain 5 to 35 propagules (winged seeds) and are solitary or clustered on short lateral branches. The number of seeds and cones increases with tree age (Bradstock and Cohn, 2002). Cones mature at 18-20 months, then remain closed on the trees for many years until opened by fire (Earle, 2011). Seeds are dispersed by wind. Plants generally produce their first fruits at an age of 10-15 years and can live for about 250 years (Bradstock and Cohn, 2002, Zimmer et al., 2010). Essential conditions for seed germination and success of post fire seedling establishment are sufficient rainfall coinciding with cool ambient temperatures in the winter following seed dispersal (Bradstock and Cohn, 2002, Adams, 1999).

In Hincks Wilderness Protection Area, *C. verrucosa* occurs in clusters of various sized individuals as well as scattered and isolated individual trees. Within the reserve we observed that *C. verrucosa* only grows in areas with white sandy soils often associated with elevated dune topography which is consistent with reports for this species at other locations (Harden, 1990, Zimmer et al., 2010).

4.3.3 Sample collection

Leaf material of *Callitris verrucosa* was collected in October 2010, April 2011 and February 2012 from six sites within Hincks Wilderness Protection Area, comprising various fire histories (Table S4.1). Fire history information from the last 50 years was sourced from www.naturemaps.sa.gov.au managed by the South Australian Department of Environment, Water & Natural Resources. This information includes fire season, year of fire occurrence and the number of years between firers (fire frequency). We had one repeat for times since the last fire by which sites 1 and 6 were the most recently burned sites (6 years since the last fire), sites 2 and 4 were intermediate (13 years since the last fire) and sites 3 and 5 were the longest unburned (35 years since the last fire). Sites 1 and 2 showed the highest fire frequency with one interval being shorter than 15 years compared to the other sites (Table S4.1). The size of each sampling site was approximately 500 m x 500 m with distances between sites of about 2 km (Figure 4.1). At each site, we collected leaf material from 50 individuals (seedlings and adults) which were separated from each other by at least 20 m. The location of each sampled individual was GPS-located.

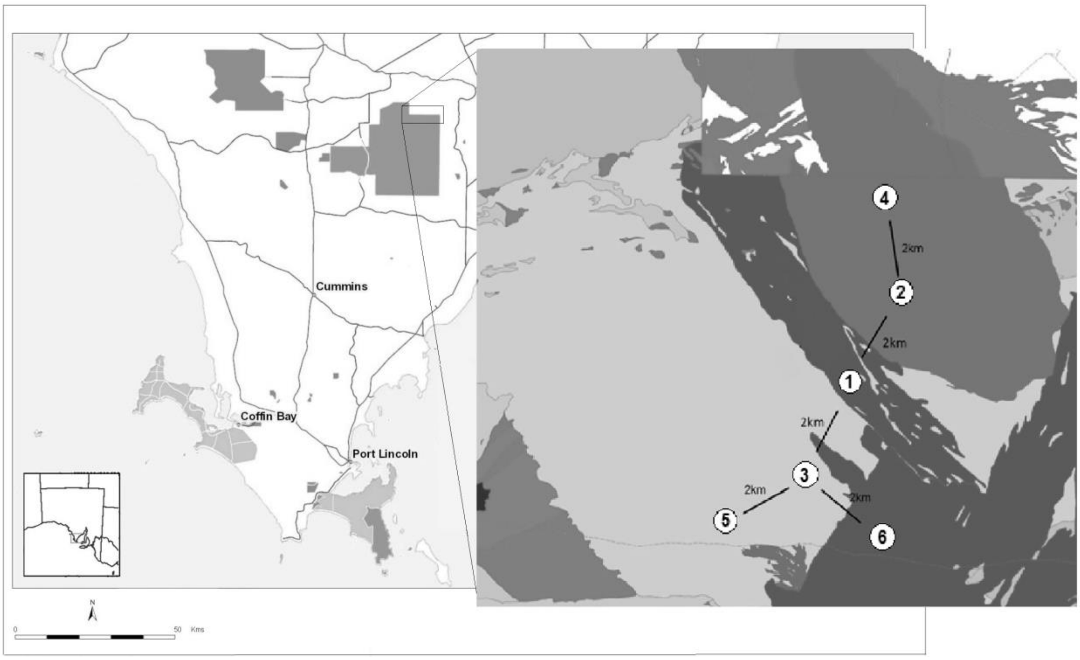


Figure 4.1: Location of *Callitris verrucosa* sampling sites within the Hincks Wilderness Protection Area on the Eyre Peninsula, South Australia (dark grey = fire in 2006; medium dark grey = fire in 1999; light grey = fire in 1977) (The information on fire history since 1959 was sourced from www.naturemaps.sa.gov.au managed by the South Australian Department of Environment, Water & Natural Resources, accessed November 2011)

4.3.4 Genetics

The collected plant material of *C. verrucosa* was stored in silica gel until DNA extraction. Genomic DNA extractions were performed on leaf material at the Australian Genome Research Facility (AGRF) using Machery Nagel kits in single or 96 well format (www.agrf.org.au).

Amplified fragment length polymorphisms (AFLPs) were used as genetic markers for this study. AFLPs are dominant markers with alleles scored as the presence or absence of a band (Lowe et al., 2004). In this study AFLP analysis was carried out according to the method of Vos et al (1995) with some modifications. Restriction digests were performed in 20 µl reactions with ~200 ng of DNA, 1 x restriction digest buffer 2, 10 U MseI (New England Biolabs), 10 U EcoRI (New England Biolabs), and 1 x BSA. Reactions were incubated for 3 hours at 37 °C, followed by 20 minutes at

65 °C to denature the enzymes and 4 °C for 10 minutes to cool down and stop the reaction. Adapters were ligated to the digested DNA fragments in reactions containing 20 µl of digested DNA, 1 x T4 ligase buffer, 0.25 µM EcoRI adapter, 2.5 µM MseI adapter and 3 U of T4 DNA ligase (New England Biolabs). Reactions were incubated overnight at 16 °C. The digested and ligated DNA was diluted to a concentration of 1 in 10.

Pre-selective amplifications contained 2 µl of diluted, digested and ligated DNA, 1 x Optimised DyNAzyme™ EXT buffer (including 1.5 mM Mg^{2+}), 0.2 mM of each dNTP, 0.5µM MseI (+C), 0.5µM EcoRI (+A) primers and 0.25 U DyNAzyme™ EXT DNA polymerase to give a final PCR reaction volume of 25 µL. Reactions involved an initial denaturation step of 75 °C for 2 minutes, then 26 cycles of 94 °C for 30 seconds, 56 °C for 30 seconds, 75 °C for 2 minutes, and a final extension at 60 ºC for 30 minutes and 30 ºC for 10 seconds. PCR products were run on agarose gel to check for successful amplification.

Selective amplifications contained 1 µl of 1 in 30 diluted pre-selective PCR product, 1 x TaqGold buffer (Applied Biosystems), 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.3 µM MseI + 3bp primers, 0.3 µM EcoRI +3 bp primers and 0.75 U TaqGold (Applied Biosystems) in a final PCR reaction volume of 15 µL. Reactions involved an initial denaturation step of 94 °C for 9 minutes, then 9 cycles of 94 °C for 30 seconds, 65-56 °C for 30 seconds (reduce by 1°C per cycle), 72 °C for 2 minutes, then 21 cycles of 94 °C for 30 seconds, 56 °C for 30 seconds, 72 °C for 2 minutes and a final extension at 72 ºC for 30 minutes and 25 ºC for 30 seconds. Twelve selective amplifications were trialled using a range of + 3bp primer combinations on eight individuals of each species. Products were run on 5% acrylamide gels using a Gelscan GS2000 (Corbet Research) and the four most suitable combinations, based on appropriate number and strength of bands, polymorphisms and ease of scoring, were chosen for selective amplification of all samples (Table 4.2). Products were separated using the ABI 3730 DNA analyzer (Applied Biosystems) with the GeneScan™ – 500 LIZ® size standard. Approximately 10% of DNA samples were re-extracted and the AFLP process repeated to allow validation of loci and error rate calculations. A negative control was included throughout the extraction/AFLP process to enable exclusion of non-specific bands.

Table 4.2: Primer sequences and number of amplified fragment length polymorphism (AFLP) loci (n) scored from each primer pair in *Callitris verrucosa*

EcoRI primer	Mse I primer	n
TACTGCGTACCAATTCAGC(FAM)	GACGATGAGTCCTGAGTAACAG	77
TACTGCGTACCAATTCAGC(NED)	GACGATGAGTCCTGAGTAACCG	31
TACTGCGTACCAATTCAGC(PET)	GACGATGAGTCCTGAGTAACGA	68
TACTGCGTACCAATTCACG(VIC)	GACGATGAGTCCTGAGTAACAG	123

Genemapper® Software v4.0 (Applied Biosystems) was used to automatically create a binary bin set that would score presence/absence of bands at each loci and generate a phenotype table. The generated phenotype table was exported into Excel (Mircosoft Excel 2010) and reduced to fragment size range of 130-500 base pairs to reduce co-migration effects. Error rate of the automatic scoring of Genemapper software was assessed by manual scanning for scoring errors using 5% of the individuals in the generated phenotypes table. Major scoring errors were identified and corrected in all individuals so that the final scoring error was reduced to less than 1%. We then used AFLPScore v1.4 (Whitlock et al., 2008) to carry out different error rate analyses on the non-normalised dataset using different locus and phenotype thresholds. A phenotype table was created in AFLPscore v1.4 using a locus threshold of 300 relative frequency units (RFU) and a phenotype threshold of 200 RFU for peak heights which retained 521 loci at an error rate of under 11 %. In Excel we reduced error rates further by manually eliminating loci with high error rates from the phenotype table. We used three error rate thresholds 7% (retained number of loci 443), 5% (retained number of loci 383), and 3% (retained number of loci 299) and compared them in terms of their information value.

4.3.5 Data analyses

The phenotype table was analysed with GenALEx 6.5b3 (Peakall and Smouse, 2012) and STRUCTURE (Pritchard et al., 2000). Results were compared among the datasets with different error rates to identify the phenotype table with the lowest error that still contained enough information to give insights into population genetics of *C. verrucosa.*

We chose the phenotype table with an error rate of 3 % to assess genetic diversity measures for each population over all loci in GENALEX 6.5b3 (Peakall and Smouse, 2012). Those measures included number of effective alleles, Shannon's Information Index, expected heterozygosity and percentage of polymorphic loci. The calculations were based on the assumption that the Hardy-Weinberg-equilibrium was present in the data set. To test for differences in genetic diversity measures among sites with different fire frequencies (frequently burned sites (less than 15 years between past fires) vs less frequently burned sites) and different times since fire we applied the Kruskal-Wallis test for independent samples in SPSS Statistics 20.0. Additionally we included a correlogram to look for differences in genetic diversity with time since fire. An AMOVA was applied to the dataset to assess percentage of genetic variation within and between populations. Clustering of individuals from each site was visualised with a principal coordinate analysis (PCoA) in GENALEX v6.4 (Peakall and Smouse, 2012). Furthermore we calculated the pairwise population PhiPT values, an analogue of F_{st}, a measure for genetic differentiation among populations.

Spatial genetic structure was assessed for each site using the AFLP data in SPAGeDi v1.3 (Hardy and Vekemans, 2002). We used the KINSHIP coefficient for dominant markers in diploids (Hardy, 2003) with different inbreeding coefficients 0.1, 0.2 and 0.3 to include a sensitivity analysis. The number of distance intervals was adjusted to the highest possible number that maintained a CV partic value of less than 1 for each distance interval. The tests were run with 500 permutations. The strength of the spatial genetic structure was assessed with *Sp* statistics, given by $Sp = -b/(1 - F_{(1)})$, where $-b$ is the regression slope of the mean pairwise kinship coefficient (F_{ij}) on the natural logarithm of distance interval (r_{ij}), and $F_{(1)}$ is the mean F_{ij} between individuals in the first distance interval (Vekemans and Hardy, 2004). Spatial genetic structure was considered significant where the observed value fell above the 95% confidence interval for randomly distributed genotypes.

4.4 Results

The comparison of phenotype table with different error rates resulted in a final error rate of 3% across 299 loci. This error rate was chosen as it was the lowest error and still retained the minimum number of 200 loci, suggested for measuring genetic diversity and structure using AFLP data (Bonin et al., 2007).

Genetic diversity as measured by Shannon's index of diversity (I) was very low in all populations (Table 4.3). There were no significant differences amongst sites with different fire frequencies or time since the last fire for various indices of genetic diversity (Number of effective alleles (P =0.53 / P =0.18), Shannon's index of diversity (P =0.8 / P =0.18), expected heterozygosity (P =0.8 / P =0.17), percent of polymorphic loci (P =0.53 / P =0.17)) following Kruskal-Wallis 1-way ANOVA tests. There was a non-significant trend towards increase in genetic diversity (Shannon's index of diversity) with an increase in time since fire (Figure 4.2).

Table 4.3: Indices of genetic diversity per site for *Callitris verrucosa* using AFLP data

		N	Na	Ne	I	He	uHe	%P
Site 1	Mean	50.000	0.943	1.047	0.073	0.037	0.037	47.16%
	SE	0.000	0.058	0.007	0.007	0.004	0.004	
Site 2	Mean	50.000	1.187	1.052	0.087	0.042	0.043	59.20%
	SE	0.000	0.057	0.006	0.007	0.004	0.004	
Site 3	Mean	50.000	1.127	1.049	0.080	0.040	0.040	56.19%
	SE	0.000	0.057	0.006	0.007	0.004	0.004	
Site 4	Mean	49.000	1.191	1.052	0.085	0.042	0.042	59.53%
	SE	0.000	0.057	0.007	0.007	0.004	0.004	
Site 5	Mean	49.000	1.274	1.063	0.101	0.050	0.051	63.55%
	SE	0.000	0.056	0.007	0.007	0.005	0.005	
Site 6	Mean	42.000	1.040	1.048	0.074	0.036	0.037	51.84%
	SE	0.000	0.058	0.008	0.007	0.004	0.004	
Total *	Mean	48.333	1.127	1.052	0.083	0.041	0.042	56.24%
	SE	0.068	0.023	0.003	0.003	0.002	0.002	2.41%

*over Loci and sites

Na = No. of Different Alleles

Ne = No. of Effective Alleles = 1 / (p^2 + q^2)

I = Shannon's Information Index = -1* (p * Ln (p) + q * Ln(q))

He = Expected Heterozygosity = 2 * p * q;

uHe = Unbiased Expected Heterozygosity = (2N / (2N-1)) * He; Where for Diploid Binary data and assuming Hardy-Weinberg Equilibrium, q = (1 - Band Freq.)^0.5 and p = 1 – q

% P = Percent of polymorphic loci

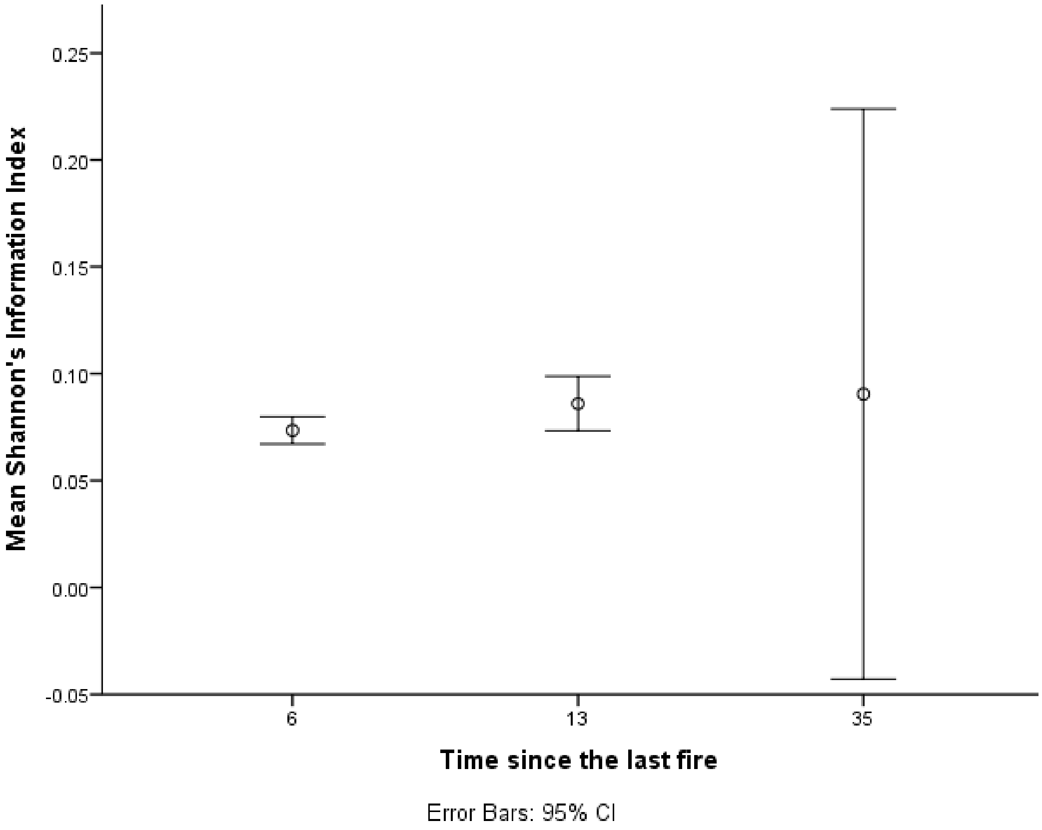


Figure 4.2: Correlation between mean Shannon's index of diversity and time since the last fires for *Callitris verrucosa.*

The AMOVA showed 98% of the genetic variation was expressed within populations. Clustering of individuals from each site visualised with the PCoA showed no differences between sites. Both axes together did explain about 40 % of total variation (Figure 4.3). This was additionally supported by very low PhiPT values between sites (Table 4.4).

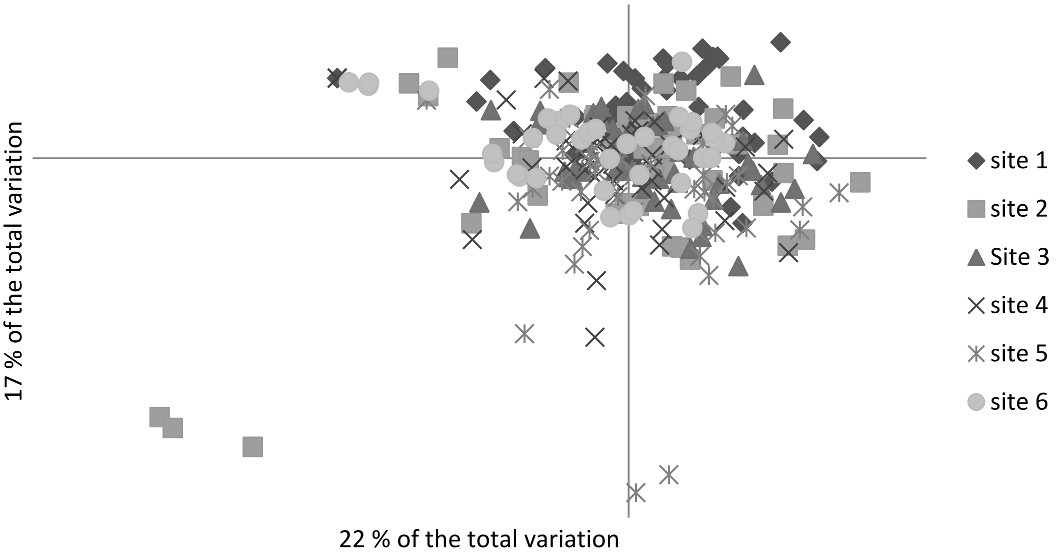


Figure 4.3: Principal Coordinates (PCoA) showing the amount of variance amongst individuals of *Callitris verrucosa* from the different sampling sites, explained by the first two axises using a binary distance matrix in GenAlEx 6.5b3.

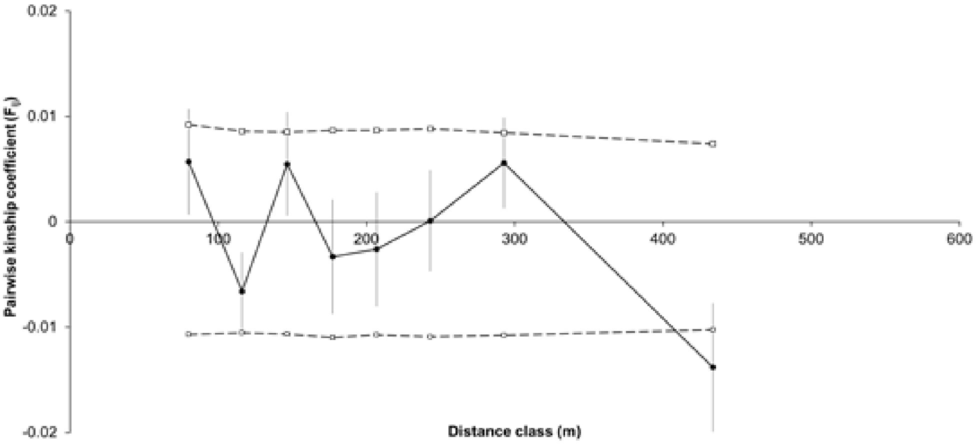
Table 4.4: Pairwise site ϕPT values based on *Callitris verrucosa* AFLP binary table including all sampling sites. ϕPT values are shown below diagonal. Probability, p (rand >= data) based on 999 permutations is shown above diagonal.

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Site 1		0.010	0.010	0.010	0.010	0.010
Site 2	0.016		0.010	0.010	0.010	0.010
Site 3	0.036	0.013		0.010	0.010	0.010
Site 4	0.025	0.013	0.020		0.010	0.020
Site 5	0.036	0.022	0.025	0.016		0.010
Site 6	0.034	0.023	0.033	0.012	0.018	

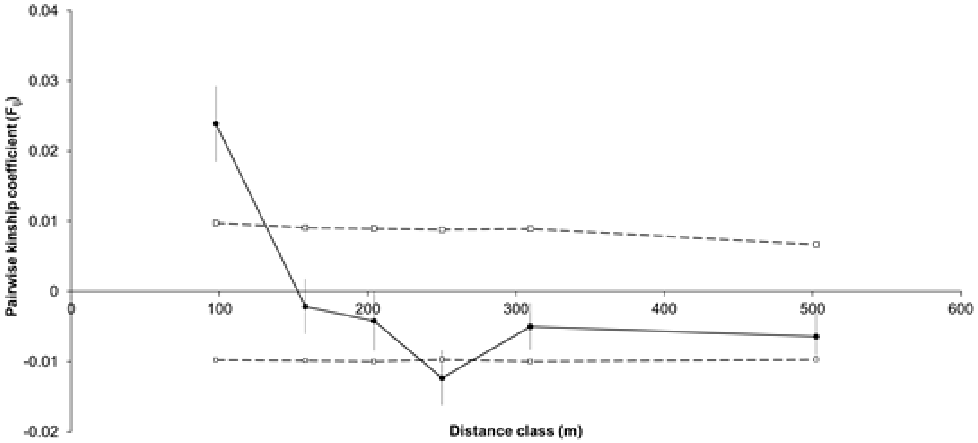
Fine-scale spatial genetic structure was not significant in most populations. The only exceptions were site 2 where significant spatial genetic structure was detected to about 120 m (‘*Sp*’-value = 0.0176) and site 6 where weak evidence for spatial genetic structure was detected to about 100 m (‘*Sp*’-value = 0.0123) (Figure 4.4).

Figure 4.4: Average kinship coefficients (F_{ij}) between pairs of individuals of *Callitris verrucosa* plotted against the geographical distance in each sampling site; (a) Site 1, (b) Site 2, (c) Site 3, (d) Site 4, (e) Site 5, (f) Site 6. The dashed lines represent 95% confidence intervals for average kinship coefficient (F_{ij}) under the null hypothesis that genotypes are randomly distributed. Site 1, *Sp*-value = 0.003; Site 2, *Sp*-value = 0.017; Site 3, *Sp*-value = -4.507E-05; Site 4, *Sp*-value = 0.004; Site 5, *Sp*-value = 0.001 Site 6, *Sp*-value = 0.012

a)


Pairwise kinship coefficient (F_{ij})
0.02
0.01
0
-0.01
-0.02
0
100
200
300
400
500
600
Distance class (m)

b)


Pairwise kinship coefficient (F_{ij})
0.04
0.03
0.02
0.01
0
-0.01
-0.02
0
100
200
300
400
500
600
Distance class (m)

c)

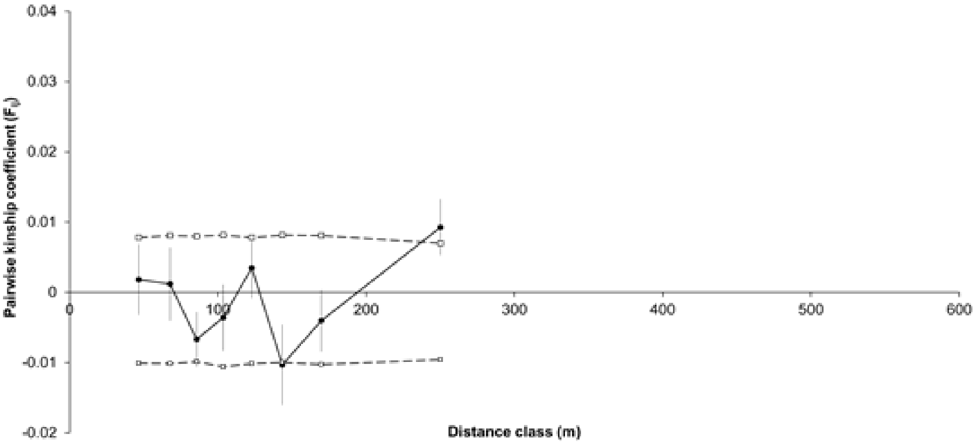
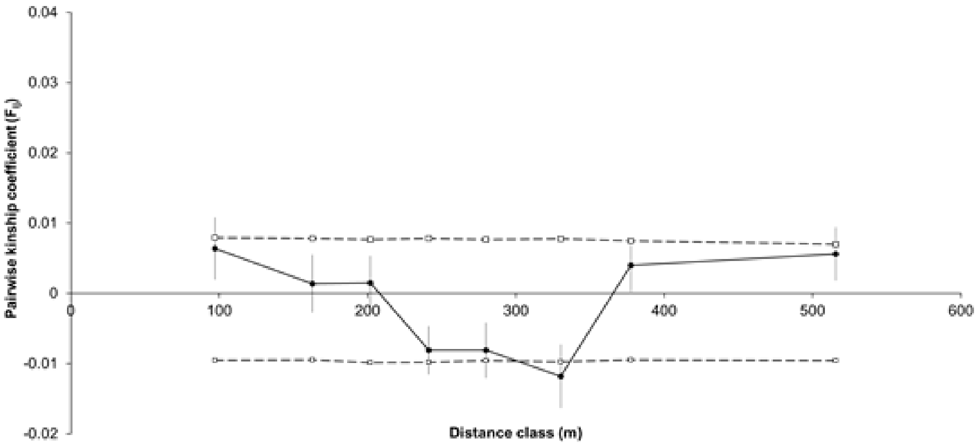

Pairwise kinship coefficient (F_{ij})
0.04
0.03
0.02
0.01
0
-0.01
-0.02
0
100
200
300
400
500
600
Distance class (m)

Figure 4.4: Average kinship coefficients (F_{ij}) between pairs of individuals of *Callitris verrucosa* plotted against the geographical distance in each sampling site; (a) Site 1, (b) Site 2, (c) Site 3, (d) Site 4, (e) Site 5, (f) Site 6. The dashed lines represent 95% confidence intervals for average kinship coefficient (F_{ij}) under the null hypothesis that genotypes are randomly distributed. Site 1, *Sp*-value = 0.003; Site 2, *Sp*-value = 0.017; Site 3, *Sp*-value = -4.507E-05; Site 4, *Sp*-value = 0.004; Site 5, *Sp*-value = 0.001 Site 6, *Sp*-value = 0.012

d)


Pairwise kinship coefficient (F_{ij})
0.04
0.03
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100
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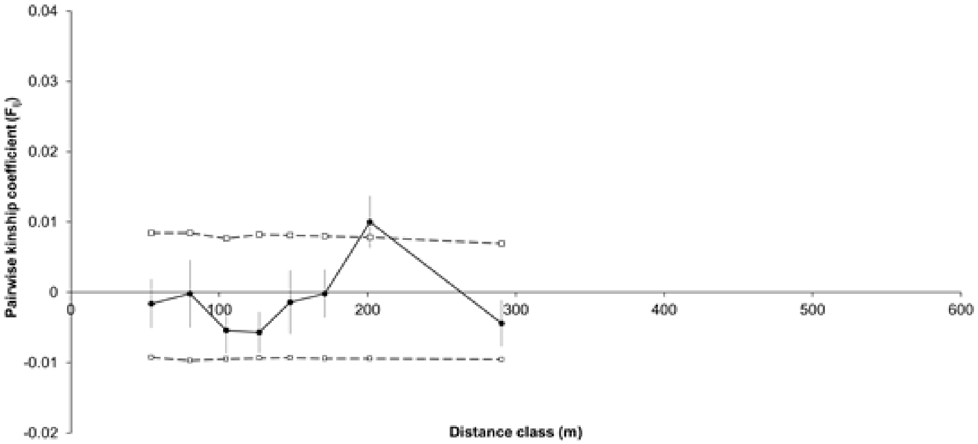
500
600
Distance class (m)

e)


Pairwise kinship coefficient (F_{ij})
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-0.01
-0.02
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100
200
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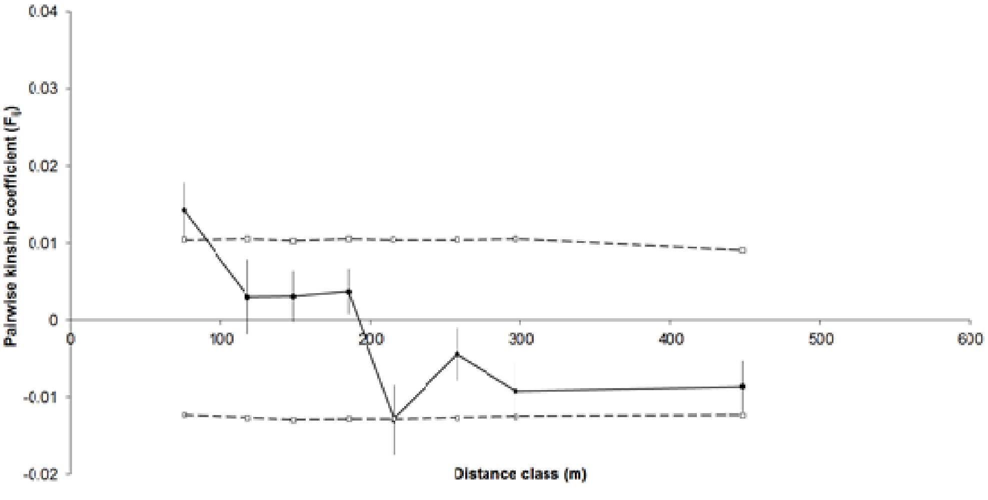
400
500
600
Distance class (m)

f)


Pairwise kinship coefficient (F_{ij})
0.04
0.03
0.02
0.01
0
-0.01
-0.02
0
100
200
300
400
500
600
Distance class (m)

4.5 Discussion

There was no evidence supporting our hypothesis that fire regimes with short fire intervals (less than 15 years) lead to reduced population genetic diversity nor did we find that genetic diversity significantly increased with time since the last fire. This could suggest that *C. verrucosa* is either able to maintain genetic diversity within its seed banks even with fire frequencies of less than 15 years between fires (this is possible as Zimmer (2010), mentioned that small saplings do produce a small number of cones as an insurance against frequent fire). Additionally or alternatively, seed dispersal within the Hincks Wilderness Protection Area could be sufficient to outbalance a loss of genetic diversity within sites. However, there was very low genetic diversity for *C. verrucosa* across all six sites. Despite the fact that low genetic diversity appears to be a feature of fire-sensitive plant species, e.g. *Persoonia mollis* (H =0.3; %P = 85) (Krauss, 2000) and can be found in coniferous trees e.g. *Araucaria cunninghamii* (H_e =0.14±0.04 (SE)) and *Agathis robusta* (H_e =0.19 ±0.05 (SE)) (Peakall et al., 2003), the results presented here for *C. verrucosa* are some of the lowest observed (H_e= 0.041 ±0.002 (SE)). Additionally, we found number of polymorphic marker in *C. verrucosa* to be rather low (%P= 56 ±2 (SE)) compared to *Persoonia mollis* (%P = 85), an outcrossing angiosperm. This could potentially suggest that *C. verrucosa* performs low degrees of self-pollination as selfers/clonal species tend to show less than 50% polymorphic markers (Krauss, 2000).

Our finding of little genetic differentiation between sites for *C. verrucosa* suggests that this species forms a single panmictic population within the larger study area rather than forming a metapopulation structure. Because little genetic differentiation was found between sites, and the majority of genetic variation was observed within sites, we considered historical gene flow to be high. Estimated long distance seed dispersal distance for *C. verrucosa* was 800 m (see Chapter 2). Long distance dispersal of seeds can be common in woody plant species with wind mediated seeds

(Hamrick et al., 1992, Petit and Hampe, 2006, Austerlitz et al., 2000), e .g. *Betula lenta* 777 ± 734 m^2 and *Abies magnifica var. shastensis* 7511 ± 2372 m^2 (Nathan and Muller-Landau, 2000).

The weak correlation between genetic and geographical distance found within all sites is a likely result of extensive seed and pollen dispersal (Hamrick et al., 1992, Petit and Hampe, 2006) and also supports the theory that *C. verrucosa* forms a panmictic population rather than a meta population structure. The only site that was different and did display significant spatial genetic structure was site 2. This finding could possibly be related to the topography of site 2 (same as site 3 in Chapter 3), which was located in a swale. Dispersal at that site was possibly restricted due to reduced wind speeds (it is a sheltered area) and the existence of dispersal barriers in the form of surrounding sand dunes.

To explain our finding of low levels of genetic diversity in all six sites which covered an extensive area within Hincks Wilderness Protection Area, we propose three alternative hypotheses:

1. The whole area might have gone through a severe genetic bottleneck in the past. As Bradstock and Kenny (2003) have suggested, if at least half the fire intervals in a landscape are unfavourable for a species, we can expect a significant population decline. Long periods of localized extinction and subsequent recolonisation can lead to low population genetic diversity in sites (Krauss, 1997) which over time would have become homogenised among sites as gene flow was extensive (Slatkin, 1987). A loss of genetic diversity in the past in combination with habitat fragmentation (preventing gene flow from other populations that might have aided the recovery of genetic diversity levels) could be a likely cause for the present low genetic diversity (Primack and Miao, 1992, Bradstock and Kenny, 2003, Gotelli, 1991). To verify this hypothesis of one or more past population bottlenecks, data on

unfavourable fire regimes (long period of frequent fire occurrences) in the past are required (e.g. palaeo fire records).

2. The initial establishment of *C. verrucosa* in that area resulted from a low number of founder individuals; hence the current population might still show a founder effect (Banks et al., 2013).
3. Low population genetic diversity could be a natural characteristic of *C. verrucosa.*

To verify the last two hypotheses a study investigating population genetic diversity of *C. verrucosa* from other populations occurring in different areas on the Eyre Peninsula and other regions over a much larger scale, could be carried out. Recent human related fragmentation is unlikely to be a cause of the low diversity as a single *Callitris* tree can live for 250 years (Zimmer et al., 2010) which exceeds the time since clearing by European settlers by more than 100 years (Twidale and Smith, 1971, Sumerling, 1987).

Whatever the causes the consequence for *C. verrucosa* may be that it lacks the levels of genetic diversity that a plant population will require to respond to changing environmental conditions. Future management plans should carefully determine the extent to which the genetic characteristics reflect adaptations to local conditions, and how much attention should be given to increasing the degree of diversity. For broader conservation issues, we need to know how typical *C. verrucosa* is in terms of the patterns of genetic diversity, and whether it is unique or more widely representative of obligate seeder species in the mallee vegetation.

4.6 Acknowledgements

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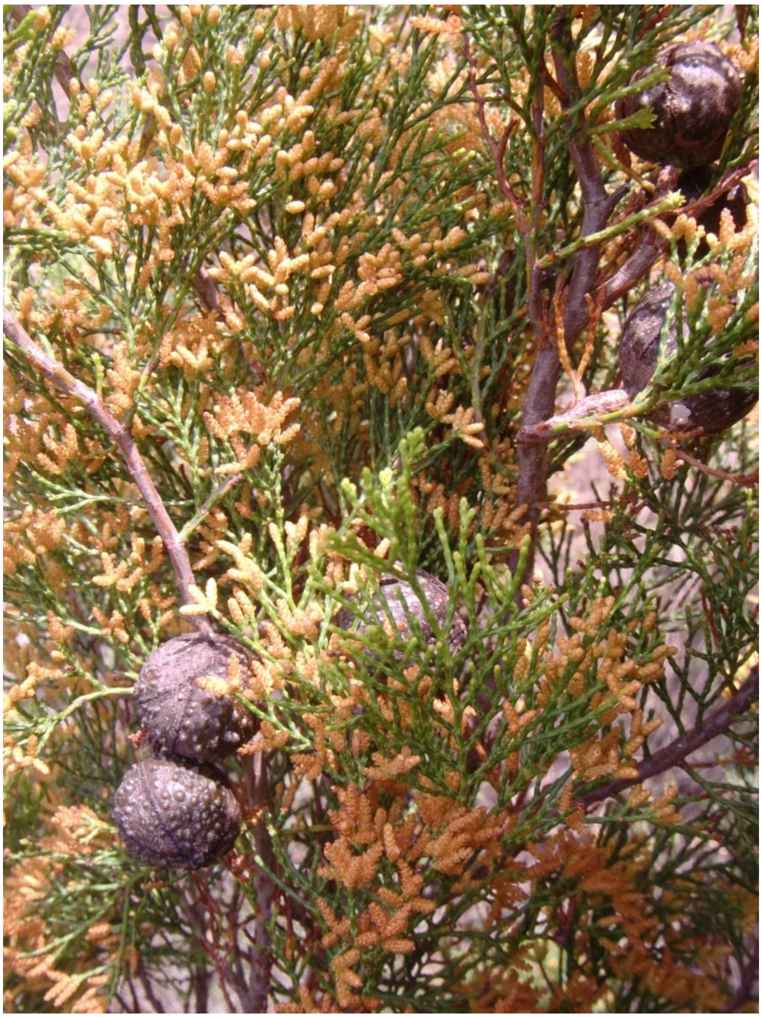
4.8 Supplementary materials

Table S4.1: Characteristics of sampling sites for *Callitris verrucosa* used in this study

Site	Type of burn	Season	Month	Date	Years to last fire	Burn intervals
1	Prescribed burn	Autumn	April	2006	4	
	Bushfire	Summer	December	1977		29
	Bushfire	Spring	November	1966		11
	Bushfire	Spring	November	1959		7
2	Bushfire	Summer	January	1999	11	
	Bushfire	Summer	December	1977		22
	Bushfire	Spring	November	1966		11
3	Bushfire	Summer	December	1977	34	
4	Bushfire	Summer	January	1999	13	
5	Bushfire	Summer	December	1977	35	
6	Bushfire	Spring	November	2006	6	

CHAPTER FIVE

Fire season influences seed dispersal by wind



Seed and flowers on *Callitris verrucosa* at Hincks Wilderness Protection Area, SA
(Photo B. Dunker)

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5.1 Abstract

Fire management, in the form of prescribed burning often results in a shift in fire season from hot and dry summer conditions to cooler, moister conditions in spring or autumn. The effects of this change on plant species which rely on efficient seed dispersal by wind after or during fire are unknown. Variations in wind conditions during the different seasons and changes in the amount of standing vegetation after the burn, resulting from variations in fire intensity, may alter dispersal conditions following fire. Any reduction in dispersal potential arising from a shift in fire season could be of concern for these species if it reduces gene flow between populations and their ability to colonise new sites.

We studied wind-based seed dispersal in different seasons for a serotinous obligate-seeder, *Callitris verrucosa*, growing in a semi-arid environment in South Australia. We measured primary (wind-borne) and secondary (on ground) seed dispersal during different seasons, spring, summer and autumn, using both empirical observations and modelling based on the distribution of wind speeds.
We found that at comparable horizontal wind speeds the seeds travelled further in summer compared to spring and autumn, probably because of more turbulent wind conditions during summer. Prescribed burning carried out during cooler seasons, shifts the seasonal timing of seed release in an obligate seeder. This shift decreases dispersal distances of seeds, which is likely to reduce: gene flow; recolonisation rates, and the ability to colonise new sites.

Policy implications: Wind-mediated seed dispersal distances of a serotinous obligate seeder in semi-arid environments can be negatively impacted by management related changes in fire season. This could reduce re-establishment and population persistence, particularly in a fragmented landscape, and hence lead to the reduction of overall biodiversity.

Keywords: *Callitris verrucosa*, fire intensity, gene flow, mallee, prescribed burning, turbulence, wind speed

5.2 Introduction

Understanding how anthropogenic changes to fire regimes affect persistence of species populations is central to the protection and maintenance of biodiversity in fire-prone ecosystems (Penman et al., 2011, Bennett et al., 2010, Allen, 2008, Johnson and Miyanishi, 1995, Keith et al., 2002). Some effects of changed fire regimes on certain plant population processes, such as recovery from disturbance or seedling establishment, are relatively well understood (Sheuyange et al., 2005, Bradstock et al., 1996, Howe, 1994, Knox and Clarke, 2006). In contrast, understanding the impacts of changed fire regimes on dispersal of plant propagules remains poor yet may exert a strong influence on fire recovery potential. This paper explores how the dispersal distances of seeds from a serotinous tree might be affected by the time of year when the seed-releasing fires occur.

One component of how humans influence fire regimes is through modifications of ignition patterns (Bradstock, 2010, Bowman et al., 2009, Bowman et al., 2012, Pyne, 2010). In various temperate ecosystems around the world, most wildfires occur during the drier and hotter summer months. These fires achieve high temperatures and often burn large areas (Pausas, 2004, Pausas et al., 2009, Meyn et al., 2007, Keeley and Zedler, 2009). However, most managed, controlled burning is carried out during cooler and wetter times with lower winds when fires are easier to control (Bushfire Cooperative Research Centre, 2008, Van Wilgen et al., 2010). As a result these fires generally burn with lower intensity (Gill et al., 2001, Van Wilgen et al., 2010).

For serotinous plants, high temperatures generated by fire stimulate the release of seeds from their woody fruits (Lamont et al., 1993, Nathan et al., 1999). The subsequent germination of the released seeds on the ash bed produces the next generation of recruits to the population. One impact of changes in fire season, and hence in fire intensity, on serotinous plants has already been well studied. Cooler burns are less likely to cause canopy scorch, reducing the amount of seed released

(Knox and Clarke, 2006) and the subsequent success of seedling recruitment (Bond et al., 1984). If fire kills the established standing plants, populations could then decline because their persistence as seeders relies entirely on seed release and seedling regeneration (Bradstock et al., 1996, Knox and Clarke, 2006). There are also differences in the success rate of seedling establishment linked to the weather conditions that seeds experience after their release. In general, wet conditions following seed release result in higher germination rates for seeds and more successful seedling establishment (Thomas et al., 2010, Bond and van Wilgen, 1996, Enright and Lamont, 1989, Bond et al., 1984, Hodgkinson, 1991, Heelemann et al., 2008).

Another less explored impact of a changed fire season could be the dispersal distance of fire-released seeds, a parameter of substantial importance for population persistence (Keith, 2012). Seed dispersal contributes to gene flow between existing populations and consequently influences the persistence of plant populations over time (Nathan and Muller-Landau, 2000). For serotinous plant species with aerial seed dispersal, this might depend on the prevailing wind conditions during and after a fire, which could vary among seasons for two reasons. First, local turbulences can be caused by solar heating of the ground surface; hence it is likely that the wind after fire will be less turbulent on cooler days in spring and autumn than during hotter days in summer (Carl von Ossietzky Universität, 2007, Sullivan et al., 2012, Kuparinen et al., 2009). Updrafts associated with turbulence may be more important for determining seed dispersal distance than horizontal wind speed (TackenbergPoschlod and Bonn, 2003, Tackenberg, 2003). A study in boreal forests showed that there was a positive correlation between air temperature and wind-driven dispersal distances (Kuparinen et al., 2009). Thus, for serotinous species with wind-driven dispersal, cooler season burns may cause seeds to travel shorter distances. Second, less intense fires usually leave more intact and taller vegetation that will act as both, a wind break, reducing wind speed, and an obstruction to prevent dispersal for wind-borne seeds (Schurr et al., 2005, Soons et al., 2004).

Together these factors might reduce dispersal of seeds that are released by cool season management fires, relative to those released by natural summer wild-fires.

Mallee woodlands are fire-prone ecosystems (Noble and Bradstock, 1989) found widely across temperate semi-arid environments in southern Australia, but in many regions they are highly fragmented by cereal and sheep farming (Driscoll and Henderson, 2008). Fire management has an important role in the conservation of the remaining fragments of mallee (Management Plan - Mallee Parks of the Central Eyre Peninsula, 2007).

In this study we asked whether the season of seed-releasing fires in mallee could affect seed dispersal dynamics in *Callitris verrucosa*, a serotinous obligate gymnosperm. In this species adult plants are killed by high intensity fires. Therefore its population persistence relies on efficient and extensive seed dispersal from the adult plants after fire (Bradstock et al., 2006). To determine how seed dispersal of *C. verrucosa* is affected by fire season, we measured primary seed dispersal in the wind conditions experienced during spring, summer and autumn, and we assessed the effect of vegetation cover on the secondary dispersal of seeds along the ground. We used both empirical observations and modelling to study the effect of the seasons on the seed dispersal of *C. verrucosa*.

5.3 Material and methods

5.3.1 Study region

The study was conducted in Hincks Wilderness Protection Area (33°45' S, 136°03' E) a 67,000 ha reserve on the Eyre Peninsula, South Australia. The region is semi-arid, with an average annual rainfall of 340 mm. The main topographic features are white sand dunes interspersed by hard, reddish-brown swales. The dominant vegetation type in the reserve is mallee woodland, characterised by multi-stemmed eucalypts. Common trees are *Eucalyptus costata, E. socialis* and

Callitris verrucosa. Common understory species include the shrub *Melaleuca uncinata* (Smith, 2012, Joe Tilley, Ranger Hincks Conservation Park, on published data 31/01/2014), and the hummock grass *Triodia irritans* (Driscoll and Henderson, 2008).

5.3.2 Study species

Callitris verrucosa (A.Cunn. Ex Endl.) F.Muell., a native mallee-pine, from the family Cupressaceae, is a canopy dominant of mallee vegetation and a serotinous obligate seeder (Bond and van Wilgen, 1996). It grows as a small stunted tree often with several trunks, usually up to 3 m, and rarely up to 6 m high. The plant is monoecious, with female cones about 3 cm in diameter and densely warted. Cones contain 5 to 35 seeds, depending on the age of the tree (Bradstock and Cohn, 2002). Each seed has two wings attached with the actual seed located in the middle. The two wings generally make up more than 70% of the total width. Seed including wing width ranges from 2 to 6 mm, and mass from 2 to 30 mg. Cones are solitary or clustered on short lateral branches and increase in number with tree age (Bradstock and Cohn, 2002). Cones mature in 18 to 20 months, then remain closed on the trees for many years until opened by fire (Earle, 2011). Trees produce their first fruits at an age of 10 to 15 years and can live for 250 years (Bradstock and Cohn, 2002, Zimmer et al., 2010).

Adult and juvenile plants are normally killed by fire if their leaf canopy is completely scorched. Fire initiates the release of seeds, as cones open after exposure to high temperatures (pers. obs.) Seeds remain inside open cones until released and dispersed by wind. The time frame of seed release and dispersal depends on weather conditions and can take place over several days or weeks after a fire. Seed release and dispersal are promoted when there are strong turbulent winds and little or no rain (pers. obs.). Essential conditions for seed germination and success of post fire seedling establishment include sufficient rainfall coinciding with cool ambient temperatures in the winter following seed dispersal (Bradstock and Cohn, 2002, Adams, 1999).

In Hincks Wilderness Protection Area, *C. verrucosa* occurs in clusters of various sized individuals as well as scattered and isolated individual trees. Within the reserve, *C. verrucosa* only grows in areas with white sandy soils often associated with elevated dune topography (pers. obs.), as reported for this species at other locations (Harden, 1990, Zimmer et al., 2010).

5.3.3 Empirical measures of seed dispersal

We measured seed dispersal using daylight visual tracking during three different seasons: spring; summer, and autumn. We chose six release locations, three locations in each of two sites about 6 km apart that had both recently undergone a planned burn, one in spring 2009 and the other in autumn 2011. Within those two sites the three release locations were positioned 800 m apart from each other along a transect that was placed parallel to and 100 m from the burn edge within the site.

Seed release experiments were conducted in October (spring) 2011, January/February (summer) 2012 and April (autumn) 2012. At these times, the sites had a post-fire age of 0.5 to 2.5 years and limited regrowth of vegetation had occurred. Considering the factors that can cause alterations in wind conditions over time our main concern was changes in vegetation cover. Based on personal observation in the field, which showed little changes in vegetation cover for the period 0.5 to 2.5 years post fire, we rejected this concern (compare Figure 5.1) and therefore we assumed that the test conditions were similar to those at the time when *C. verrucosa* normally releases its seeds (i.e. within weeks of a fire).

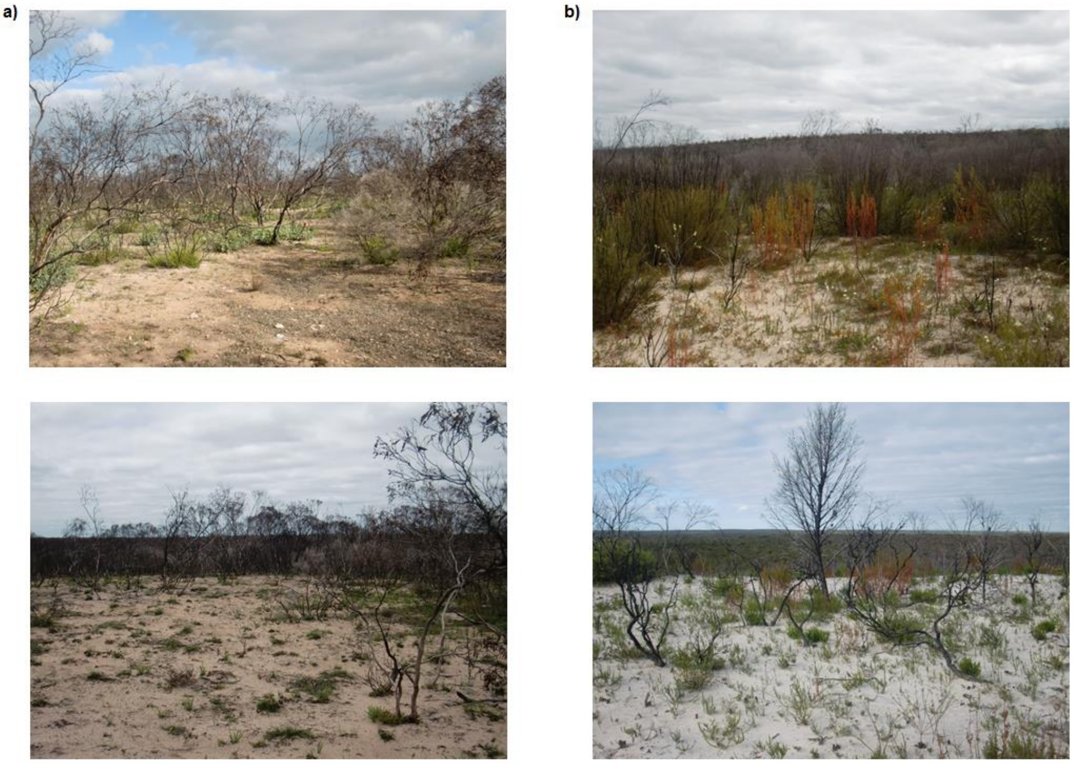

Figure 5.1: a) Two different field sites in an area burned in 2011, b) two different field sites in an area burned 2009, both taken in 2012 at Hincks Wilderness Protection Area.

Seeds were collected from other populations of *C. verrucosa* within the reserve. At each release location and in each season, 20 randomly chosen seeds were released from each of three different heights (1 m, 2 m and 3 m) giving a total of 360 seeds released per season. The seeds were given a dot of fluorescent coloured dye to make relocation easier. We tested one hundred of the seeds with dye (8.3±4.3 mg; 3.0±.05 m/s) and found no significant difference in the falling time (t_{198} =0.943; P =0.346) compared to 100 seeds without dye (9.8±4.9 mg; 3.1±.07 m/s). In the field trials, the 20 seeds were released at each height individually during daylight by placing them on a small platform on a mast, and allowing them to be carried away by the wind. The different heights represented the natural height of cones carried by young trees soon after they reach maturity (1 m), the height of cones at the top of the canopy of fully grown mature trees (3 m), and a common height (2 m) at which many cones were found on plants in the reserve (Table 5.1). The released seeds were followed by eye and the linear distance from the release point to where the seed first landed was measured to the nearest cm using a tape measure. We also measured wind speed (Model Kestrel 3000 Pocket Weather Meter, Nielsen-Kellerman, USA) at the release platform, at the moment of take-off for each seed. Seed release experiments across all locations and sites were conducted over 1 to 2 days within a season, with release of the 20 seeds for each height taking 60 to 240 min depending on the wind conditions. Seeds were only released within a horizontal wind speed range of 8 to 25 km/h. At lower wind speeds seeds would not take-off and at higher wind speeds seeds could not be relocated. While this limited our ability to detect long-distance seed dispersal that might be associated with higher wind speeds, we could still address our main question about dispersal differences among seasons. Using a similar range of horizontal wind speeds in each season allowed us to identify any seasonal effect associated with seasonal differences in turbulence.

Table 5.1: Distribution of seed cones and plant heights of *Callitris verrucosa* observed in the study area (N = 98 trees)

Percentile	Lowest cone (m)	Highest cone (m)	Plant height (m)
25th	0.26	0.57	0.50
50th	0.35	0.88	0.87
75th	0.50	1.39	1.33
Max.	1.03	3.40	3.49

To understand the effect of standing vegetation on the on-ground seed dispersal, we established groups of 10 seeds on the ground within 10 m of each of the six previous release locations. The seeds were treated with fluorescent coloured dye. After four days a UV light was used to search for the seeds at night, and to record the distance moved since release. This was repeated at each location in spring, summer, and autumn. To quantify the potential obstruction to movement from standing vegetation, we estimated the percentage of vegetation cover up to 1 m height in a 5 m radius circular area around each release point.

5.3.4 Modelling seed dispersal

Seed dispersal of *C. verrucosa* was modelled using the program PAPPUS (Tackenberg, 2003), a trajectory model that simulates seed dispersal by wind. The modelling study complemented our empirical observations independently because PAPPUS uses wind speeds measured over a longer period of time and therefore incorporates more of the variation in wind speeds that can occur within and among seasons. It produces predictions of seed dispersal distance based on high-precision wind profiles, measured in the field at a frequency of 10 Hz. PAPPUS has performed well in predicting seed dispersal of other species over longer distances (Tackenberg, 2003).

Wind measurements were taken on a sand dune crest close to one release location in the site that was burned during autumn 2011. Measurements continued for two weeks in each season (spring, summer and autumn) including the time during which seed release experiments were conducted. An ultrasonic anemometer (Model WindMaster (Part 1590-PK-020), Gill Instruments Ltd, Lymington, UK) measured horizontal wind speed, horizontal wind direction, and vertical wind speed every 0.1 s, producing a dynamic, three dimensional wind speed vector. The measured values were used later to simulate the course of the wind vector in PAPPUS. The anemometer measured wind at a height of 2 m, and PAPPUS then generated wind profiles for 1m and 3m by extrapolation. Generated values for all three heights were used in the model and included in the analysis.

PAPPUS derives predictions for seed dispersal distances taking into account wind profile, landscape slope (hilly, sloping upwards/downwards, even), surface roughness (influenced by vegetation height and density), and terminal falling velocity of the seeds. We used the pre-set landscape feature “rolling hills” in PAPPUS that we considered was comparable to the sand dunes found in the study area. To determine the terminal falling velocity, we dropped 100 randomly chosen seeds from 10 m height under calm conditions inside a building. Falling duration was timed with an electronic watch (compare Hammill et al. (1998)). The overall average seed falling rate (3.0 ±0.6 m/sec) was then used as the falling velocity in PAPPUS (Tackenberg, 2003).

PAPPUS simulated 1000 random seed releases and related dispersal distances based on the windprofiles generated over the two weeks anemometer recording period. This was repeated for each season and at each release height (1, 2 and 3 m).

5.4 Results

5.4.1 Wind conditions each season

The mean horizontal wind speed at the time when seeds were released (Table 5.2) increased significantly with height ($p < 0.001$) but there was no significant difference among seasons ($p = 0.808$; Kruskal-Wallis 1-way ANOVA). However the means of the continuous anemometer readings of the horizontal wind speed at 2 m height over the 2-week period were significantly different among seasons ($p = 0.017$; Kruskal-Wallis 1-way ANOVA) with the highest wind speeds in summer (Table 5.2). A similar, and marginally significant trend was observed for anemometer readings of mean vertical wind speeds (spring 0.22 m/s ±0.01; summer 0.30 m/s ±0.03, autumn 0.21 m/s ±0.03) ($p = 0.08$; Kruskal-Wallis 1-way ANOVA) (Figure. 5.1a). We measured the range of vertical wind speeds within 60 second periods, at five times in the day, applied at three hourly intervals from 0600 – 1800 h. We used the difference between the lowest and the highest values among the 1800 measurements of vertical wind speed in three 60 second periods, immediately after each designated time on three consecutive days (Figure 5.1b). We interpreted higher variability to indicate greater turbulence. Turbulence increased as the day progressed and was consistently as high or higher, at each time of day, in summer than in the other two seasons (Figure 5.1b). An overall frequency distribution of vertical wind speeds showed that extreme values were more frequent in summer although the mean values were not significantly different among seasons (p= 0.78; Kruskal-Wallis 1-way ANOVA) (Figure. 5.1c).

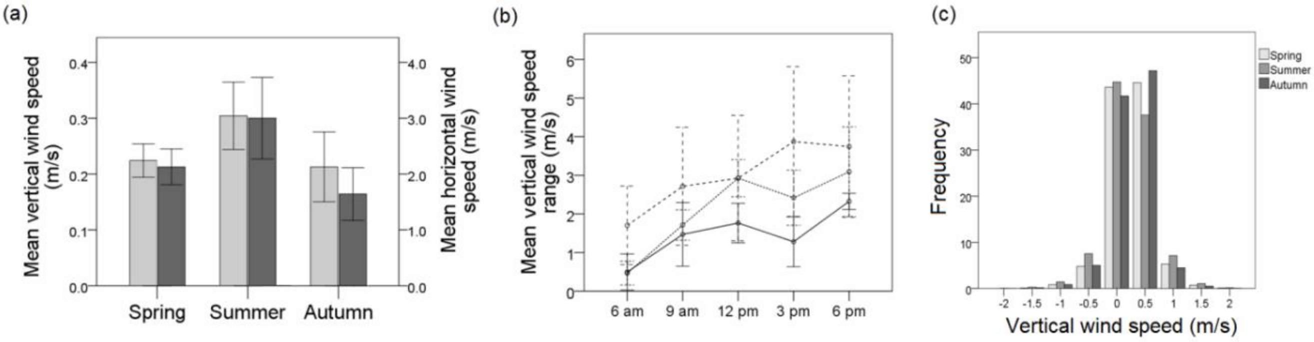

Figure 5.1: Wind profiles for the different seasons (a) mean vertical and horizontal wind speeds for spring summer and autumn. The dark grey bars present horizontal wind speed and light grey bars the vertical wind speed. (b) Range of vertical wind speeds over the period of a day based on wind data measured over three days. Error bars display the standard error. (Spring = dotted line; Summer = dashed line; Autumn = solid line) (c) Frequencies of vertical wind speeds measured over the period of one week. On the x-axis the positive values are upwards movement and negative values represent downwards movement of vertical winds.

Table 5.2: Mean horizontal wind speed (m/s) (SE) at the moment of each seed release, at different release heights and seasons, and mean horizontal wind speed (m/s) (SE) measured at 2 m height with the anemometer over a timeframe of 7 days

Season	1m (S)	2m (S)	2m (P)	3m (S)
Spring	1.7±0.76	2.1±0.89	2.1±0.15	2.8±0.94
Summer	2.0±1.03	2.1±1.03	3.0±0.36	3.4±1.37
Autumn	1.9±0.94	2.4±0.95	1.6±0.23	2.9±1.21

(S)eed tracking by eye
(P)APPUS

5.4.2 Empirical measures of seed dispersal

Most released seeds landed close to the release point. In spring and autumn 90% of the seeds were wind dispersed less than 3 m and in summer less than 4 m. The maximum observed dispersal distances were from seeds released at 3 m height (7.5 m in spring; 10.6 m in summer; 6.6 m in autumn) (Figure 5.2).

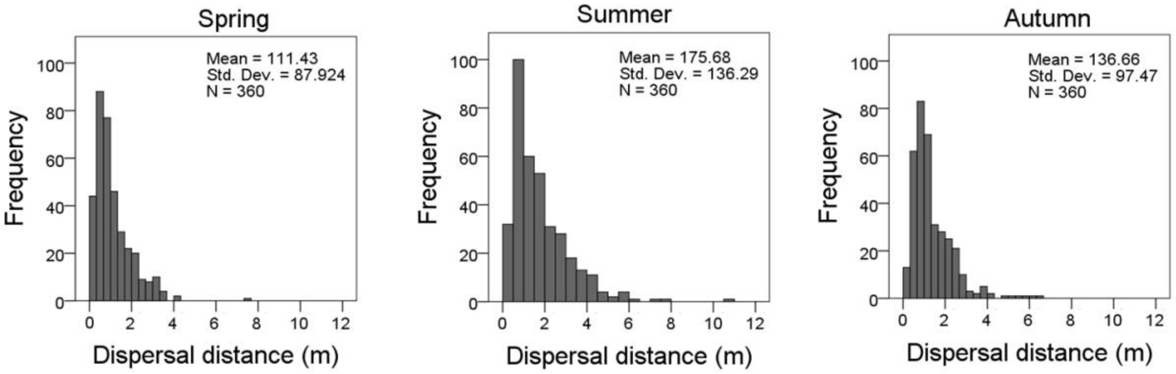

Figure 5.2: Distribution of accumulated seed dispersal distances for seeds released from 1m, 2m and 3 m heights in spring, summer and autumn.

We analysed the influence of season, seed release height and horizontal wind speed on the observed air-borne seed dispersal distance with a generalized linear mixed model (GLMM). The model was fitted using the glmer function of the lme4 software package (Bates et al., 2011) and the predictSE.mer function of the AICcmodavg software package (Mazerolle, 2011) in R (R Development Core Team, 2011). Site (separating the two different burnt sites) was fitted as a random effect to account for potential impacts of pseudo-replication from measuring dispersal distances of multiple seeds within the same site. The statistical significance of the relationships was assessed using the Wald test in R. There were significant effects for each main factor and all of their two-way and three-way interactions (Table 5.3). Dispersal distance increased with wind speed at each release height, the increase was consistently greater in summer than in the other two seasons, and the increase with wind speed was lowest in spring for seeds released at 1 m, while it was lowest in autumn for seeds released at 3m (Figure 5.3).

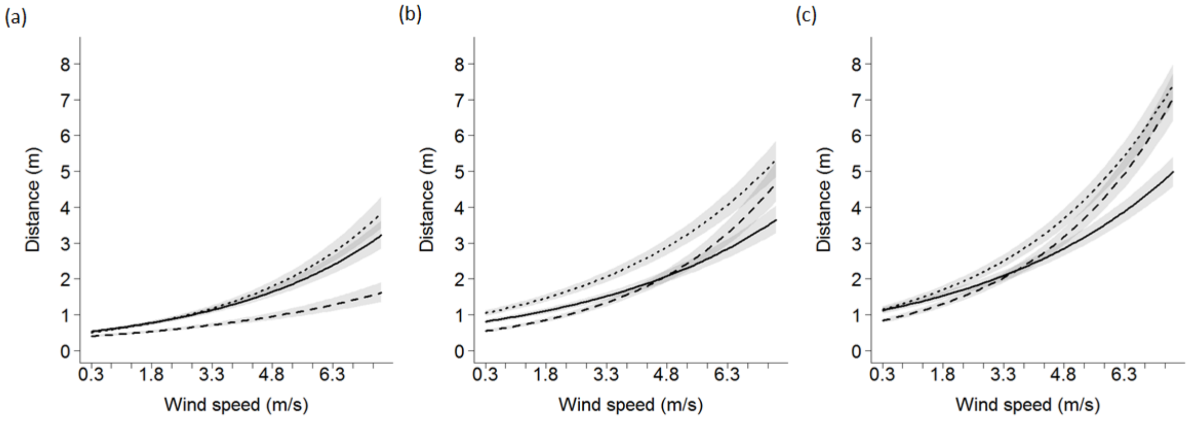

Figure 5.3: Dispersal distance of seeds released over a range of wind speeds from heights of (a) 1 m; (b) 2 m; and (c) 3 m. Grey area = 95% confidence limits. (Spring = dashed line; Summer = dotted line; Autumn = solid line).

Since seeds dispersed further with higher wind speed, we adjusted dispersal distances for wind speed at the time of release (distance dispersed / wind speed) and repeated the analysis with factors season and height of release. There was still a highly significant interaction effect between season and height on the adjusted dispersal distance (Table 5.3). Adjusted dispersal distance increased with release height in each season except in summer which had equally long dispersal (and the longest mean values in the data set) at both 2 m and 3 m release heights (Figure 5.4).

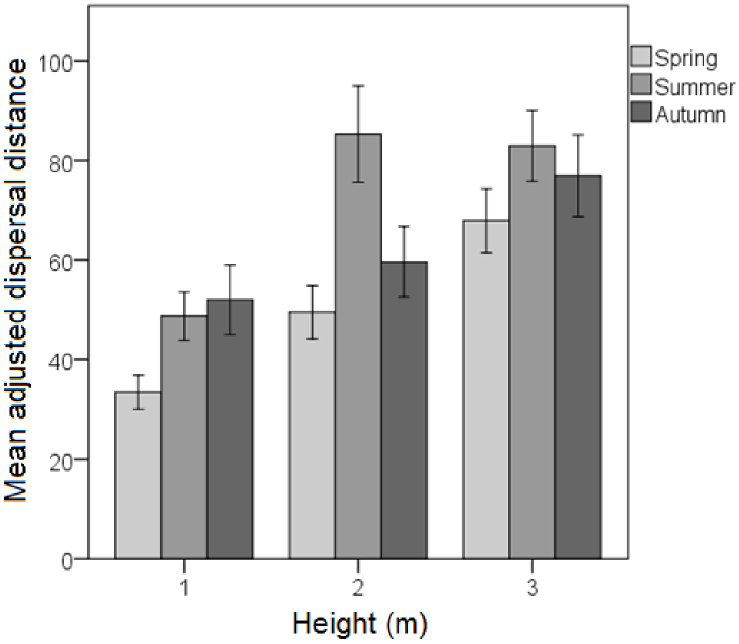

Figure 5.4: Mean adjusted dispersal distance for wind speed (distance (m)/ wind speed (m/s)) in each season and at each release height. Error bars display the standard error

Table 5.3 Results of Wald tests for influence on (a) absolute seed dispersal distance of season, release height and wind speed; and (b) seed dispersal distance relative to wind speed of season, and release height

	a		b	
Factor	z-value	P-value	z-value	P-value
Season	59.1	<0.001	520.5	<0.001
Height	702.5	<0.001	615.3	<0.001
Wind speed	27.1	<0.001	-	-
Season : Height	136.9	<0.001	615.3	<0.001
Wind speed : Height	16.5	<0.001	-	-
Wind speed : Seasons	29.8	<0.001	-	-
Season : Height : Wind	85.9	<0.001	-	-

5.4.3 On-ground dispersal

Out of the 180 seeds released we considered dispersal distances of the 161 (89%) that could be relocated. Dispersal distances after four days on the ground were short. Most seeds (90%) were found within 0.5 m from their release point, and the furthest dispersal was 1.4 m (Figure. 5.5a). GLMM analyses, as above, showed a highly significant interaction between season and vegetation cover (Table 5.4) with seeds travelling similar short distances in all three seasons when cover was high, but seeds travelling much further in summer than in the other two seasons when cover was low (Figure 5.5b).

Table 5.4: Results of Wald test for influence on seed dispersal distances on the ground of percent vegetation cover at <1m height and season

Factor	z-value	P-value
Vegetation cover (%)	-2.5	0.01
Season	181.0	<0.001
Season : Vegetation cover (%)	109.6	<0.001

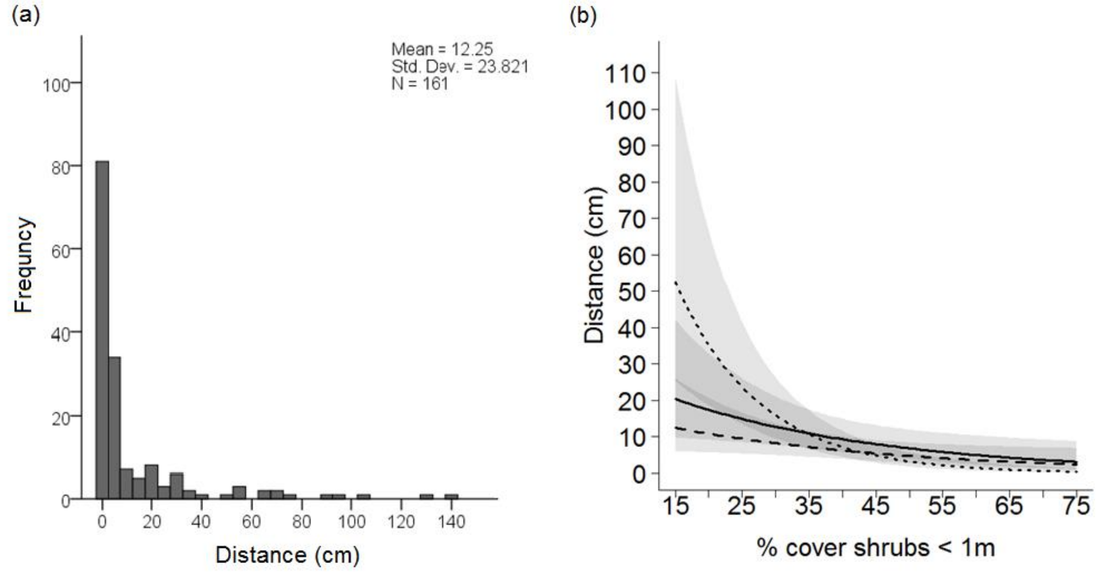

Figure 5.5: (a) Distribution of on-ground seed dispersal distances for *Callitris verrucosa* combining data from all three seasons. (b) Relationship between on ground dispersal distance and percent vegetation cover up to 1 m height for each season. Grey area = 95% confidence limits. (Spring = dashed line; Summer = dotted line; Autumn = solid line).

5.4.4 Wind-borne dispersal: Comparison of empirical study with PAPPUS model

The PAPPUS models generated higher frequencies of seeds dispersed in distance categories over 5 m, and had some seeds dispersed further in each season than the empirical observation (Table 5.5). This was probably because empirical observations were avoided when wind speeds were high. Nevertheless, there were three consistent outcomes from the two methods. More seeds dispersed distances further than 5 m in summer than in other seasons, maximum distances were greater in summer than in other seasons, and no seeds were either observed or predicted to disperse further than 25 m from the parent tree in any season.

Table 5.5: Proportion of seeds in the dispersal distance spectra for daylight visual tracking and for the trajectory Model PAPPUS accumulated from all distributions for 1m, 2m and 3 m heights

Method	Season	0-5m	>5-10m	>10-15m	>15-20m	>20-25m
Daylight visual tracking						
	Spring	0.997222	0.002778	0	0	0
	Summer	0.972222	0.025	0.002778	0	0
	Autumn	0.986111	0.013889	0	0	0
Trajectory Modell PAPPUS						
	Spring	0.791136	0.202971	0.005893	0	0
	Summer	0.587536	0.289979	0.114243	0.008157	8.57E-05
	Autumn	0.871943	0.107993	0.019443	0.000621	0

5.5 Discussion

Wind-borne dispersal distance of seeds was influenced by an interaction between season, height and wind speed. Seeds released from greater heights or in stronger winds tended to disperse further in all seasons. However, there was an additional impact of each season. The dispersal distances for the same wind speeds and same release heights were higher in summer. Tackenberg (2003) reported that for variations in seed dispersal distance, horizontal wind speeds were less important than thermal updrafts and turbulent air conditions. Our results can be explained if hotter temperatures in summer create more turbulences and updrafts, allowing seeds to be dispersed further in that season (Kuparinen et al., 2009). Since seed release of *C. verrucosa* only occurs after fire, this finding suggests seeds will disperse further after fires in summer than after fires in other seasons.

Earlier studies that have shown that seeds disperse further during stronger winds, included autocorrelated turbulent fluctuations within the vertical wind velocity (Soons et al., 2004, TackenbergPoschlod and Kahmen, 2003, Bullock and Clarke, 2000, Nathan et al., 2001). However, even in low wind velocity, seeds dispersed further in turbulent air (Soons et al., 2004, Tackenberg, 2003). In summer, solar heating of the ground creates instability in the atmosphere and causes thermal updrafts favouring seed uplift and long distance dispersal (Sullivan et al., 2012, Kuparinen et al., 2009, Nathan, 2005, Wright et al., 2008). In our measured wind profiles the range of vertical wind speeds over the duration of a day was highest during summer. The low reflecting power but higher radiation power of black ash associated with the post-fire environment exacerbates these effects, increasing the occurrence of "willie-willies" or "dust devils", local convective circulations, and similar turbulences (Oke et al., 2007). In addition to the thermal effects the dry thunderstorms in summer, with gusty and erratic winds, potentially enhance seed dispersal (Heidorn, 2005, Sullivan et al., 2012). Our data are consistent with these generalisations, suggesting that the wind dynamics found during summer are more favourable for promoting seed dispersal compared to spring and autumn. Natural wildfires are more common in summer. Seeds that are released by those fires are likely to be dispersed further by wind than seeds released by controlled burns in autumn or spring.

Our study also suggested that the secondary dispersal of seeds on the ground will be reduced after non-summer fires. We infer that the higher wind speeds we recorded in summer at 1 m heights, were also present at ground level (boundary layer effects) and that this explained the higher dispersal distance of seeds along the ground during summer in conditions where ground vegetation cover was low. We also found that seeds deposited in sites with higher vegetation density had lower secondary dispersal, probably because the vegetation acted as a wind break. Height and density of the surrounding vegetation greatly influence wind velocity on the ground and hence seed dispersal potential and distances (Schurr et al., 2005, Soons et al., 2004). Prescribed burns in autumn or spring generally have lower intensity (Bradstock and Auld, 1995) and will leave more understorey vegetation and more obstructions to seed movement than summer wildfires. Secondary wind dispersal, after a seed has initially landed on the ground, can markedly alter the seed dispersal shadow, and may have an important influence on the long distance movement of seeds and spatial patterning of plant populations (Schurr et al., 2005). Thus both primary wind-borne dispersal following seed release from the tree, and secondary dispersal along the ground can be synergistically affected by an anthropogenic shift in fire season from summer to either autumn or spring, resulting in an overall reduction in the seed dispersal capability of *C. verrucosa*.

A potential limitation of this study is that we primarily examined the proximal portion of the dispersal kernel, the probability density function that describes the pattern of primary seed dispersal distances away from the source. Long distance wind dispersal was not empirically detected because we avoided observations during times of exceptionally high wind speed. Long distance dispersal along the ground was not detected if widely dispersed seeds were beyond our search zone, and because dispersal may have been greater over longer periods than our four days of observation. The PAPPUS modelling method avoided some of the limitations in estimating initial wind-borne dispersal, but extreme wind conditions may have been missed during the sampling period of 2 weeks. However, we suggest that the relationships revealed from the inner portion of the kernel, and specifically the longer dispersal distances in summer, are likely to be maintained in the tail of the distribution. Therefore we argue that our results reflect the potential importance of fire season in generating long-distance dispersal of fire-released seeds, and hence the importance of fire season for recolonisation and rescue effects in populations of *C. verrucosa* and other serotinous obligate seeders. An additional effect we have not considered here is that there may be a minimum temperature threshold for the release of seeds during seed abscission. If fewer seeds are released by the lower temperatures of non-summer burns, then even fewer seeds are likely to be dispersed into the longer distance categories (Soons and Bullock, 2008, Green, 2005).

We conclude that the application of prescribed burning as a fire management tool, when carried out during cooler seasons compared to natural wildfires, could reduce dispersal distances of *C. verrucosa* seeds, and reduce recolonisation rates, subsequently increasing the risk of local extinction. This potential risk will be intensified if fire frequency, and thus adult tree mortality, increases in response to future climate change or to inappropriate fire management, and if fewer viable populations of adult trees are retained as seed sources. To improve understanding of this topic there are several questions that could be addressed in the future. Would we find the same difference among seasons if we observed seed dispersal at higher wind speeds or the conditions experienced within minutes or weeks of a fire? How do threshold temperatures for seed abscission affect seed dispersal? And how is seedling establishment and survival after dispersal influenced by different fire seasons? Each of these questions needs to be asked about *C. verrucosa*. However, the broader general findings might be applicable to other serotinous obligate seeders in fire-prone ecosystems that rely on seed dispersal by wind for population persistence and re-establishment (Hammill et al., 1998a). We suggest that this study could be used as a guideline to study effects of different fire season on other species that are likely to be affected by those changes. Hence, our results will have broader implications for the role of fire management in the conservation of a range of serotinous tree species, and the ecosystems they populate.

5.6 Acknowledgements

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CHAPTER SIX

Summary and conclusions

Recently burned site at Hincks Wilderness Protection Area in South Australia

(Photo B. Dunker)

6.1 Summary of research outcomes

Fire is a prominent feature of mallee vegetation and it is widely understood that changed fire regimes can impact on community composition and the persistence of species (Van Wilgen et al., 2010, Bradstock et al., 2006). Natural fire regimes in semi-arid mallee woodlands are suggested to show frequencies ranging from 40 years to more than 300 years between fires, depending on rainfall, vegetation type and substrate (Gibson, 2013). While long intervals are generally a result of relatively slow fuel build-up from *Triodia* hummocks and eucalyptus litter, and relatively infrequent ignition sources from lightening, short fire intervals are driven by man-made ignition sources, climate change and fuel availability provided by herbaceous understory such as ephemerals and spear grass (Bradstock and Cohn, 2002, Gibson, 2013, Noble, 1986). The timing of fires can vary between hot and dry seasons including late spring and summer (natural fires) or cooler and wetter seasons during early spring and autumn (prescribed burns). The alteration of environmental context (generally clearing for farmland or urban settlements) in which mallee vegetation occurs demands new knowledge to understand interaction between biodiversity and fire regimes (Bradstock and Cohn, 2002).

In the context of improving our current knowledge of how fire regimes affect biodiversity, this research provides information on the present state of genetic diversity within populations of two species, *Triodia irritans* and *Callitris verrucosa*, which are important components of fire-prone mallee vegetation on the Eyre Peninsula in South Australia. I assessed spatial genetic structure and genetic diversity of *T. irritans* and *C. verrucosa*, in sites exposed to different fire regimes. This research has generated novel data on primary seed dispersal for *T. irritans* and *C. verrucosa*, and on the influence of seasonal wind variation on seed dispersal in *C. verrucosa*. The two study species present different functional traits. They were *Triodia irritans*, a hummock grass and potential resprouter (uses obligate seeding as well as re-sprouting) after fire, and *Callitris verrucosa*, a native mallee pine and serotinous obligate seeder. The dispersal of seeds and the distribution of dispersal distances, including the potential for long distance dispersal, were studied because gene flow through seed dispersal is one of the factors influencing: population structure; re-establishment success; genetic diversity, and genetic structure of populations as well as spatial extension rate of species. Knowledge of seed dispersal particularly over long distances, provides information on the recolonisation ability of each species which is an important factor in their regional survival under adverse fire regimes (Driscoll et al., 2010). The knowledge of seed dispersal improves understanding of how disturbance affects populations, their genetic diversity and spatial structures (Banks et al., 2013). My study aimed to improve understanding of seed dispersal distributions and gene flow patterns as well as likely changes in population genetic diversity and structure under different fire regimes. This was achieved by using three different, but complementary approaches for measuring seed dispersal and the assessment of population genetic variation at sites with different fire histories, including various fire frequencies and times since the last fire. This understanding can be used to inform future predictions of how the two study species will cope with different fire regimes, different climate scenarios given the current extent of habitat fragmentation, and hence estimate the potential risks of population decline or extinction. The generated information could also possibly be used to suggest the likely fate of other species with the same or similar functional traits, but verification would be required. The gained understending will help to identify plant species that may be vulnerable to future changes and hence in need of conservation attention and careful monitoring of population size and genetic diversity.

The outcomes from this study can be summarised as follows:

1. Three methods were used for measuring the distribution of seed dispersal, including: a direct tracking method based on seed tracking by eye; a mechanistic model based on wind data

measured in the field, and an indirect genetic approach using population assignment. Each of these established and commonly applied methods produce only a fraction of the complete range of probable dispersal distances. They make different assumptions, involve different biases and produce estimates of different precision. Based on their complementary strengths and weaknesses, application of all three methods (empirical, mechanistic and genetic) in a combined approach produces a more holistic understanding of seed dispersal (Chapter 1).

2. Primary seed dispersal by wind for *T. irritans* covers only short distances of less than 10 m under strong wind conditions, while genetic methods detected rare seed dispersal up to distances of 3 km. Historical gene flow between sites appears to be high. These findings suggest that secondary seed dispersal on the ground by wind or other secondary dispersal vectors and possibly pollen dispersal play an important role in the overall genetic connectivity of *T. irritans* populations (Chapter 1+2).

3. *Triodia irritans* population genetic diversity was similarly high under various fire regimes. There was no evidence for clonality in this species. Microsatellite data showed levels of polyploidy up to hexaploidy. We found little evidence for inbreeding in *T. irritans*, which was generally low. Genetic structure in *T. irritans* is likely to be a result of extensive seed dispersal rather than fire history. Size distributions of plants at different burn age suggest seedling establishment increases with time since fire (highest number of small plants found 13 years after fire) up to a particular age at which establishment of seedlings becomes negligible (observed at 32 years after fire) (Chapter 2).

4. Primary seed dispersal by wind for *C. verrucosa* measured at maximum release height (5 m) is up to 40 m under strong wind conditions while genetic methods detected individuals that

dispersed seed over distances of up to 8 km. Very low genetic diversity (among the lowest recorded for conifers) was observed in *C. verrucosa.* Different theories were suggested that could explain this finding, such as past bottleneck, founder effect or common species characteristic, which would however require verification (Chapter 1+3).

5. Fire season was found to influence dispersal distance of wind dispersed seeds in *C. verrucosa*. Natural fire seasons which are related to hotter weather conditions seem to promote longer seed dispersal distances because they reduce vegetation cover, which obstructs seed movement, and increase the frequency and strength of updrafts (Chapter 4).

6.2 Limitations of current study

i) Limited site replication

The number of site replicates was limited due to time and financial considerations. All sites were located in the same park and there were limited options to replicate sites with comparable fire history. This reduced the significance of the results. Conclusions based on the results have to be taken with care. The sampling of certain characteristics of the study species was incomplete because the importance of these characteristics was not recognized at the beginning of the study again causing a reduction in the informative value of the study.

ii) Logistic constraints that prevented experimentation under high wind conditions.

The empirical method was limited to moderate winds (max. 25 km/h) as visual relocation of seeds dispersed at higher wind speeds was difficult. This has caused a bias towards shorter seed dispersal distances making it difficult to compare empirical results with estimates from the mechanistic model which sampled a wider range of wind speeds. A solution for this would have been to verify the accuracy of the mechanistic model (PAPPUS) using the wind data from the exact same time as the empirical seed release was carries out (compare Tackenberg (2003).

iii) Constraints on markers (e.g. high ploidy).

The initial plan of using microsatellites for both study species was compromised because of a failure to develop an adequate number of polymorphic microsatellite loci required to study population genetics for *C. verrucosa*. As a result, the less informative method of using Amplified Fragment Length Polymorphism was used. Microsatellites developed for *T. irritans* are limited in their information content as a result of high polyploidy levels in *T. irritans* (Dufresne et al., 2014). However, the fact that I observed a similar pattern in the genetic structure for each species at the shared sampling sites suggests that the findings are still reliable. This suggestion is based on the belief that using two different genetic markers and methodologies for the two species and finding a similar pattern amongst their sites would be an unlikely coincidence.

6.3 Implications for future fire management

The two species included in this study displayed not only different seed dispersal patterns and levels of gene flow but also very different levels of population genetic diversity. While *T. irritans* displayed shorter primary seed dispersal as well as gene flow distances than *C. verrucosa*, *T. irritans* population had higher genetic diversity. In contrast, *C. verrucosa* was found to have very low population genetic diversity within Hincks Wilderness Protection Area. Both species showed no significant differences in genetic diversity and spatial genetic structure amongst sites displaying various fire regimes.

The results showed that, for *C. verrucosa*, the height at which seeds are released plays an important role in how far seeds disperse. Seeds released from greater heights travelled much further;

maximum dispersal for 3 m release height was 20 m while a release height of 5 m produced a maximum dispersal distance of about 40 m. This observation suggests that long fire intervals, which would allow this species to reach its maximum height, could be beneficial for maintaining gene flow over greater distances. There may be trade-offs in implementing long fire intervals, depending on the density of *Callitris*. Bradstock and Cohn (2002) found that in long unburned sites (less than 100 years), *Callitris* could overtop mallee eucalypts resulting in elimination (or reduction) of the latter. This may cause a reduction of overall biodiversity in those sites, as *Callitris* dominated mallee was found to have fewer bird species compared to other mallee vegetative communities (Bradstock and Cohn, 2002). Additionally, old *Callitris* may supress fire events, which can reinforce the process of longer fire intervals, leading to further reduction of mallee eucalypts. Nevertheless, I believe that it may be appropriate to create and/ or sustain a mosaic of different burn ages that includes some long unburned patches. The persistence of long unburned patches could be increased by creating fire breaks around them. This management approach to ensure conservation of species has been suggested by other studies (Bradstock and Cohn, 2002, Smith, 2012, Gibson, 2013). Additionally, fires during summer appear to be more advantageous for a wind dispersed serotinous obligate seeder, such as *C. verrucosa* to experience (natural fire season) because wind conditions at that time of year promote greater seed dispersal distances. Whether this information should be considered for management purposes is debatable, considering the high risks involved with summer fires, compared to spring and autumn, and the differences in distances measured for the different seasons.

There was no evidence of a decrease in *T. irritans* population genetic diversity up to approximately 30 years after fire. However, whether fire frequencies could be extended to much more than 30 years without causing a loss of genetic diversity in populations of *T. irritans*, has not been answered. Based on the findings I would suggest that past fire mosaics may be suitable to maintain connectivity of populations within Hincks Wilderness Protection Area because past gene flow in both species appeared to be high within sites and sufficient between sites to result in little genetic variation among the sites.

6.4 Future research suggestions

Of potential concern for *Triodia irritans* are fire regimes with long fire intervals as they are presumed to reduce population densities due to die back of old individuals, increased competition and reduction of re-establishment success. Long fire intervals could further reduce gene flow, not only through decreasing population densities but also due to higher surrounding vegetation which could inhibit dispersal of seed and possibly pollen. A loss of population genetic diversity under low fire frequencies could reduce the resilience of *Triodia irritans* to future climate changes as genetic diversity provides potential for adaptation to change (Barrett and Schluter, 2008). An earlier study by Bradstock (1989) found reduced numbers or absence of *Triodia* seedlings in populations that were more than 20 years old. Our study only included one site that had not been burned within the last 30 years. We therefore suggest that a future study might look at genetic diversity and genetic structure in older populations of *T. irritans* (incorporating a bigger gradient of times since the last fire, e.g. 20, 40, 60, 80 years) to assess the need for fire occurrence at a particular population age in order to avoid loss of its genetic diversity.

Genetic diversity in *Callitris verrucosa* was found to be particularly low. It is not known whether this is a result of: i) a bottleneck associated with past fire regimes within the region; ii) founder effects that persist since initial population establishment; or rather iii) a common and widespread characteristic of this species. Studies that investigate population genetic diversity in *C. verrucosa* populations across the Eyre Peninsula as well as more broadly across other regions would help to resolve these alternative explanations. Additionally, information on palaeo fire regimes across the

Eyre Peninsula would be required to detect evidence of high fire frequency in the past, which could have caused a population bottleneck.

Pollen dispersal is another major factor contributing to gene flow within and particularly between populations, as wind can certainly carry pollen further than seeds. In the results on population genetic structure of the study species, I was unable to differentiate between the effects of seed and pollen dispersal on the overall gene flow. However, there are methods that allow this differentiation like combining markers of different inheritance. Nuclear and paternally inherited markers occur in both pollen and seed and therefore measure overall gene flow, while for maternally inherited markers genes only dispersed in seeds (Ennos, 1994, Ouborg et al., 1999). Another more recent method by Moran and Clark (2011) is a hierarchical Bayesian approach integrating genetic and ecological data. A future study using such methods would help to shed more light into gene flow patterns of the study species.

6.5 References

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