

LANDSCAPE GENETICS AND THE EFFECTS OF CLIMATE
CHANGE ON THE POPULATION VIABILITY OF DECLINING
AVIFAUNA IN FRAGMENTED EUCALYPT WOODLANDS
OF THE WEST AUSTRALIAN WHEATBELT

A Thesis submitted for the degree of
Doctor of Philosophy in Molecular Ecology

Antonia Sara Angel BSc (Hons)

School of Veterinary and Life Science Murdoch University
Commonwealth Scientific Research Organisation (CSIRO)
Perth Western Australia

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DECLARATION

I declare that this thesis does not contain any materials previously submitted for a degree at any tertiary education institution. To the best of my knowledge it does not contain any material previously published or written by another person except where due reference has been made in the text.

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Sara Angel

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ABSTRACT

The Rufous Treecreeper (*Climacteris rufa*), Yellow-plumed honeyeater (*Lichenostomus ornatus*) and the Western Yellow Robin (*Eopsaltria griseogularis*) are focal species and were investigated to assess the impacts of climate change and severe habitat fragmentation on the genetics and viability of remaining populations. This study was located within the west Australian wheatbelt where 93% of the native vegetation, including 97% of the York gum, wandoo and salmon gum woodlands have been cleared for agriculture (Saunders, *et al.*, 1989) and where climate modelling predicts hotter and dryer weather conditions (CSIRO, 2005, IOCI, 2002). The Dryandra woodlands contains the largest native vegetation remnants in the central wheatbelt with a combined area of 28 066 ha and provides habitat for a diverse assemblage of flora and fauna many of which are in Decline, Threatened or Specially Protected (NWC, 1991).

The effects of habitat loss and fragmentation on the gene flow and population structure on the Rufous Treecreeper, was assessed within the Dryandra woodlands and across a range of fragmented habitat spanning approximately 100 km. Microsatellite and mitochondrial DNA data was applied to a spatial genetic and phylogeographic analysis. AMOVA shows genetic variation to be higher within populations (78%) than among populations (22%) and populations did not conform to Hardy Weinberg Equilibrium. This infers gene flow exceeds genetic drift across the region and the presence of migration between remnant habitats. Isolation by Distance was not found within Dryandra or across the region and infers the effective dispersal distance of the Rufous Treecreeper exceeds the geographical distance of sampling sites. However a Mantel's Test found a correlation ($r=0.316$, $p=0.004$) with a distance of 28kms, within the

Dryandra woodlands. A Spatial Autocorrelation of microsatellite DNA found a genetic structure of up to approximately 25kms ($V=0.55$) and beyond the Dryandra woodlands, shows genetic discontinuities where dispersal is more likely to occur. Landscape interpolation of genetic distance shows high genetic differentiation within the Dryandra woodlands and decreasing in an easterly direction where habitat size decreases and the distance between habitat increases. The Maximum Difference Delaunay Triangulation shows population boundaries of 12 populations within the woodlands including 3 central populations that are 1.3 km apart. A Bayesian Computation of microsatellites found a Continent-Island pattern of population structure across a distance of 85 km. Ritland's Kinship Coefficient found dispersal patterns amongst populations within the Dryandra woodlands and a genetic neighbourhood size of about 1.7 km. Loiselle's Kinship Coefficient found a unidirectional pattern of migration from the woodlands to smaller, isolated habitats with a maximum dispersal distance of 48 km. A Landscape Interpolation of male and female Rufous Treecreepers show a female bias in dispersal from Dryandra, with higher genetic divergence patterns in isolated remnants where habitat and nesting hollows are limiting.

Rufous Treecreeper mitochondrial DNA (partial cytochrome b gene) data was applied to the Mantel's Test and found no correlation in Dryandra or the surrounding area but did show a positive correlation at a distance of 500kms and infers at least 2 different bioregions within this distance for this species. Results from the Interpolation and Principal Component Analysis show genetic variation decreasing with increasing distance from Dryandra in an easterly and southerly direction. The highest divergence patterns were found in Dryandra, North Yilliminning, Wickepin and Commondine Reserve. Genetic patterns with high similarity were found in Dongolocking and

Highbury sites south- east of Dryandra and are most likely remnant populations that once belonged to a larger, continuous population or gene pool. A geographical distribution of shared mitochondrial haplotypes found a historical range prior to land clearing of approximately 85kms. A genealogy study based on coalescence found the earliest ancestral haplotypes belonged to Dryandra, North Yilliminning and Wickepin populations and should be prioritised for long term conservation purposes. Also, novel sequences of partial cytochrome b gene for the Yellow-plumed Honeyeater and Control Region for the Western Yellow Robin was resolved for further research.

The ecological niche and distribution of the Rufous Treecreeper was assessed using a distance based Redundancy Analysis (db-RDA) and a Habitat Suitability study. The db-RA found slope and aspect explained 29.16% ($p= 0.04$) of the genetic variation (ϕ) of mitochondrial DNA, which infers a relationship between landscape features and historical divergence patterns. Since old growth *Eucalyptus wandoo* trees are a critical habitat requirement for nesting hollows (Rose, 1993) a georeferenced (GIS) habitat suitability map was constructed from a vegetation survey (Coates, 1995) to show the distribution of *E.wandoo* and Rufous Treecreepers within Dryandra. Also using demographic information of the Rufous Treecreeper from a previous study (Luck, 2001) and RAMAS GIS (Akçakaya, 2002), it was estimated that the Dryandra contained enough suitable habitat for a maximum of 158 populations or 1 106 individuals.

The impact of climate change on the Dryandra woodlands and the Rufous Treecreeper was measured by annual rainfall measurements (BOM, 2011), satellite imagery of tree foliage cover of each sampling site and mist net capture recapture data. This study found a declining trend in rainfall patterns and in 2010, the annual rainfall (277.4mm)

fell below the minimum climatic range (350mm) of *E.wandoo* forests. Based on climate modelling (CSIRO, 2005) the predicted reduction rainfall will eventually will negatively impact these forests by inducing a permanent state of drought. A critical threshold of 7.73% foliage cover was found, where foliage cover does not appear to recover foliage cover beyond 11.53% after a reduction to 7.73% in 2003. This indicates a critical threshold of percentage tree canopy cover for the *E. wandoo* in Dryandra. A linear regression found a significant relationship ($p = 0.036$) between previous year's rainfall and percentage foliage cover. This delayed response to rainfall is explained by the defence mechanisms of *E.wandoo* that provide this species with drought tolerance (Veneklaas & Manning, 2007). A logistic regression (GLM) found foliage cover within the same year to be a significant predictor ($p = 0.039$) of Rufous Treecreeper captures. Therefore declining rainfall patterns and tree canopy cover have a direct impact on the abundance of Rufous Treecreepers.

The apparent survival rate estimate for the Rufous Treecreeper was 0.65 (SE 0.13) and 0.303 (SE 0.08) for the Yellow-plumed Honeyeater. Alternate modelling is required for the Yellow-plumed honeyeaters to account for their varied seasonal dispersal patterns and the Western Yellow Robin data could not be used for this demographic study because of small sample size. During 1997 and 1999 adult survival rates for Rufous Treecreepers within Dryandra was 0.76 (Luck (2001) and show the Rufous Treecreepers within the Dryandra woodlands are continuing to decline. A comparison of the two survival rates shows there is a reduction of 0.11 within an 8 year period (a single generation), which coincided with a 5.16% decrease in mean foliage cover during sampling times. This study concludes that climate change is negatively impacting *E.wandoo* forests and that tree foliage cover is not only a significant predictor in

determining the presence of Rufous Treecreepers within the Dryandra woodlands, but also effects the short term survival and long term viability of this focal species.

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CHAPTER 1

INTRODUCTION

1.1 Context of Study

This investigation into declining woodland passerine birds was conducted within the framework of Caughly's (1994) paradigms of conservation biology and Lambeck's (1997) focal species approach. Caughly's small population paradigm investigates threats to populations once they become small and the declining population paradigm investigates the factors causing populations to decline and what may be done to reverse the decline (Armstrong, 2005). Caughley's paradigms serve as a guide to assess extinction risks internally (within populations) and externally (environmental impacts) and makes recommendations to address identified risks. Although these ideas are not unique to Caughley, he arranges questions involving species decline into a logical order of identification, diagnosing and management of extinction risks, from within populations or impacting them (Armstrong, 2005).

In practice, because biodiversity conservation and management is often limited by a lack of funding, knowledge and time for action; the utility of 'single species' as a basis for defining conservation requirements is limited (Roberge, *et al.*, 2004). Lambeck's 'focal species' approach aims to identify a group of species that defines different landscape attributes that must be present, if a landscape is to meet the needs of its resident flora and fauna (Lambeck, 1997). Passerine birds have been previously utilised as focal species (Brooker, *et al.*, 2001, Maron, 2008, Jones, *et al.*, 2010, Maron, *et al.*, 2011, Doer, *et al.*, 2011). This is because they are sensitive to changes in the flora and vegetation structure (Saunders, 1989), to processes relating to agricultural practises

(Lambeck, 1997) and serves as an indicator of the presence of other species (Leibold & Miller, 2004). Therefore, Lambeck's approach in conservation practices is not only efficient, but also serves as a practical method for utilising focal species as biodiversity indicators and for environmental monitoring as well.

By combining Caughley's population paradigms and Lambeck's focal species approach, an assessment of some extinction risk factors affecting declining woodland avifauna can be identified and assessed. Once factors thought to be causing an adverse effect on the viability of species are identified, then efforts to reverse or limit these processes leading to the decline can be addressed. For example, if woodland passerines experience a reduced and highly fragmented habitat for a long period of time and this is found to be the primary cause of inbreeding caused by small population size, then this information can be used to reconnect specific populations using vegetation corridors. Population genetic analysis can determine which populations or individuals can be re-connected with similar genotypes, or in the case of rare genotypes that may need to be conserved through breeding programs. Finally by working within an ecological framework, the reasons for population decline of these woodland passerines may provide explanations or predict a pattern of population decline in other species (Moyle, 2002), especially those with the same habitat requirements (Caro & O'Doherty, 1998).

According to the fossil record the vast majority of species that ever existed on earth over the last 2 billion years are now extinct (Lande, *et al.*, 2003) and shows that mass extinction events follow a 26 million year cycle (Ridley, 1996), with a period of 10-15 million years for biodiversity to recover (Jablonski, 1995 & Erwin, 2001). 1980).

Extinction estimates were in the range from 17 000 species per year in 1972, to 150 000 species per year calculated in 1992 (Hay, 2008). Leakey & Lewin (1996) argue that

even a lower figure of 30 000 species per year, is an extinction rate which is 120 000 times higher than the background (normal) extinction rate (Hay, 2008). The overall loss of biodiversity ranges from the depletion of the number of species at a particular time and place to homogenising species composition among different localities (Lande, *et al.*, 2003). It is estimated that the current mass extinction event began about 11 000 years ago (Lande, *et al.*, 2003) and has coincided with the expansion of human populations (IUCN, *et al.*, 1980).

The exploitation of natural resources is based on a utilitarian view of life which assigns a 'monetary value' to species and natural resources (Norton, 2003). However a Deep Ecology Philosophical argument against this view is based on the fact that humans are biological and therefore dependent on the functions of natural ecosystems, such as the cycling of energy through water, nutrients, soil and a complex array of plants, animals and environmental conditions which our survival depends on (Lovejoy, 1995).

Therefore if natural ecosystems continue to be irreversibly exploited and polluted, then the potential of life giving resources and well being of future persons will have tragically been traded for the interests and monetary values of present persons (Norton, 2003). Unfortunately the current mass extinction event is leading humans into to an ecological crisis, whereby we are now required to protect and restore ecological systems and improve management of declining stocks of natural resources (Norton, 2003).

In 1987, the United Nations Commission on Environment and Development released the Bruntland report, in which 'sustainable development' was defined as the level of development to which meets the needs of the present generation without compromising the ability of future generations to meet theirs (UN, 1987). Unfortunately a broad

approach to sustainability has interpreted the concept of *needs* as human desires and the limitations of *development* imposed by human productive capacities (Norton, 2003). Also in practice, development is based on the concept of continuous economic growth and profit while the long term, total cost of the exploitation of natural resources has been ignored to the detriment of nature's processes and the basic needs of future generations (Shiva, 1992). Sustainability can be more accurately defined as meeting human needs without compromising the health of natural ecosystems (Callicott, *et al.*, 1997) and sustainable development based on new technologies and moderate economic growth can be achieved without the destruction of nature's processes (Shiva, 1992).

The impact of human activity on the Earth's climate is also a major concern with several lines of evidence suggesting global climate change will itself have a major impact on natural systems (IPCC, 2014 & Gryj, 1998). The atmospheric changes are thought to be result of a combination of natural variability and the Greenhouse Effect (IOCI, 2002), but now there is stronger evidence than ever that human activities are the primary cause (IPCC, 2014). Some climate change risks have already materialised, and are having widespread and consequential impacts (IPPC, 2014). It is predicted that by 2030, most of Australia will be warmer by 0.4 to 2.0 °C and 1 to 6 °C by 2070 (CSIRO, 2001). A 3 °C change in mean annual temperature corresponds to a shift of about 300-400 km in latitude or 500 metres in elevation (Hughes, 2000). This means that many species that are sensitive to climate change will need to move away from their current habitat to maintain their preferred climate (CANA, 2005). Climate change is also predicted to alter the quantity, quality and distribution of suitable habitats in a landscape for many plants, animals and insects (Thomas & Hanski, 2004, Gryj, 1998).

Although some species will be able to adapt to climate change, it is expected overall, to reduce biodiversity in individual ecosystems and result in a re- shuffling of species associations (Brasher & Pittock, 1998, Gryj, 1998). Most species are well adapted to short term climate variability, but not longer term shifts in mean climate and increased frequency or intensity of extreme events (IPCC, 2007). During the Ice Ages (Pleistocene period) the average temperature change was 5°C over 10,000 years, which caused major changes in the distribution and abundance of biota during that time (Gryj, 1998). However, the response of species today is likely to differ from past events because the distribution of natural communities is already highly modified, which limits the ability of some species to disperse (Fortin, *et. al*, 2005). In highly modified landscapes the continuity of habitat is critical for poor dispersers where the distances between native habitat is too great to traverse (Saunders, 1989 & Pulin, 2002), but it is less important for species with better dispersal capabilities (Opdam & Wiens, 2002).

Climate change is expected to have a pervasive impact, especially in forest areas that experience a decrease in rainfall and a greater number of wildfires (NBS, 2009) and is likely to have a direct effect on birds, with higher temperatures affecting their life cycles as they respond to changes in seasons and increasing loss of habitat (Saunders, *et al*, 2013 & Baker, 2000). Modelling the distribution of species under realistic climate change scenarios (see Brereton, *et al.*, 1995), suggests that many species would be adversely affected unless populations were able to move across the landscape (Fortin, *et. al*, 2004). Ultimately through natural selection species may be able to adapt to environmental change, but if environmental changes are greater than what a species can cope with, then a species has a high probability of extinction (Frankham, 2002). A direct cause of species extinctions is habitat loss and habitat fragmentation which occurs

at the same time and results in overall reduced habitat area (MacDonald, *et al* 2002, Villard, *et al.*, 1998, Tilman *et al.*, 1994, Burgman, *et al.*, 1998).

Habitat fragmentation occurs when a large continuous area of habitat is reduced and divided into two or more fragments leading to a decrease in habitat and an increase in isolation of patches (Pullin, 2002). Habitat fragmentation also alters the condition of the remaining habitat through edge effects, altered micro-climate, hydrology, increased incidence of environmental catastrophes, incursion of predators and competitors and change of passive emigration from the habitat (Hobbs, 2002, Pullin, 2002). These factors cause the dispersal of species between patches to become weaker, until their habitat falls below a functional fragmentation threshold (Opdam & Weins, 2002). The degree to which habitat loss and fragmentation is biologically relevant will vary among species depending on how each perceives and interacts with the landscape matrix (Cushman, *et al*, 2012).

Through habitat fragmentation, plant and animal species are distributed across the landscape discontinuously (Lindenmayer & Burgman, 2005). How species respond to fragmentation of their primary habitat may depend on the relative suitability and spatial configuration of other elements in the landscape (Opdam & Weins, 2002). For example, some species show great variance in habitat requirements and are therefore limited by habitat availability (Hobbs, 2002), while others are inhibited by movement across the landscape by the vast distances created between remnant habitat patches (Saunders, 1989). As a consequence, landscape stepping stones or vegetation corridors have been used to counter the effects of isolation (Saunders, 1989, Beier & Noss, 1998, Haas, 1995). Findings of another study (CSIRO, 2009), showed that revegetation

corridors had significantly increased bird diversity (57%) and increased species numbers (22%) in the area. However, connectivity of the landscape does not always enhance species survival (Fahrig & Merriam, 1994 & Pullin, 2002, Hobbs, 2002). While vegetation corridors can reduce the isolation of habitat patches by increasing the probability of colonisation, they can also facilitate the movement of predators or pathogens (Thrall, *et al.*, 2000), or cause the failure of individuals to reach another reserve with suitable habitat (Pullin, 2002).

According to Wegner (1994), the spatial structure of landscapes can be separated into three separate components of composition (characteristics of patches in a landscape), configuration (spatial arrangement of patches) and connectivity (Burgman, *et al.*, 1998). Connectivity in a landscape depends on the relative isolation of habitat elements from one another and the extent to which the matrix represents a barrier to movement of species (Hobbs, 2002). The degree of connectivity depends on the permeability of the landscape and the ability of the species to move through landscape elements (Hobbs, 2002; Villard, 1998). Also, different species perceive the landscape differently and landscape connectivity will depend on the mobility and habitat specificity of the species involved (Hobbs, 2002). For example Bentley, *et al.*, (1997), found the abundance of some bird species living in narrow riparian remnants was attributable to habitat configuration and for other bird species, the type of vegetation cover proved to have a greater effect (Fahrig, 1997). In another study Lamberson, *et al.*, (1994) found if habitat networks contained large key patches, spotted owls need 30% less habitat area compared to networks with only small patches (Opdam & Weins (2002). Also, very little is known about how the variation in landscape mosaics affects the detection of landscape genetic relationships (Cushman, 2013). In one study, Cushman, *et al.*,

(2012), found that habitat extensiveness and fragmentation were stronger predictors of genetic differentiation than habitat area alone (Cushman, et al., 2013). Therefore, it is important to know the threshold level of habitat loss below which spatial configuration of the landscape becomes a critical factor for species (Opdam & Weins (2002).

A major consequence of habitat loss and fragmentation is that it can reduce population size and change the spatial distribution of remaining subpopulations by confining them to remnant patches (Lindenmayer & Peakall, 2000). Populations that become fragmented into smaller units are at a greater risk of extinction than larger ones (Pullin, 2002). This risk is due to random environmental stochasticity (effect of environmental fluctuations upon a population's demographic parameters), demographic stochasticity (random variation in birth and death rates) and genetic factors such as genetic drift (unpredictable change in gene frequency) loss of heterozygosity (genetic variation), and inbreeding depression (MacDonald, et al., 2002). Tilman et al., (1994), describes a model that predicts a deterministic time lag by which more species become extinct as habitat destruction increases. As these extinctions occur generations after initial habitat fragmentation, they are represented as a future debt caused by current habitat destruction (Tilman et al., 1994). In central Brazil, dry forests were found to have a 35 year or more time lag between deforestation and the effect on the genetic structure of Pfrimer's Parakeet (*Pyrrhura pfrimeri*) (Miller, et al., 2013).

When planning a conservation strategy for species, a landscape perspective improves the probability of species survival because the processes that operate on a large spatial scale, inevitably influence the occurrence and persistence of species at a local scale (Pullin, 2002 and With, 2004). Also, the scale of landscape chosen for any study will

ultimately depend on the questions being asked and the processes or species under investigation (Hobbs, 2002). Both scale and landscape features can be assessed by using remote sensing and Geographical Information Systems (GIS), which displays spatially explicit information of landscape features and allows the user to quantify and analyse the patterns of elements in the landscape (Hobbs, 2002). For example, the west Australian wheatbelt is currently dominated by a mosaic of arable fields, pastures and salt pans, with thousands of small remnants of native vegetation scattered across the landscape (Saunders, *et al.*, 1993). As this habitat has passed the fragmentation threshold, conservation efforts are generally focused on managing and preserving only remnants of native vegetation (Hobbs & Saunders, 1993). The fragmentation threshold is the critical proportion of remaining habitat at which habitat continuity is broken (Opdam, *et al.*, 2002).

The south west of Western Australia (SWWA) is one of 34 global biodiversity hotspots as it is rich in endemic species with over 4000 plant and 100 vertebrate species and simultaneously impacted by vast stretches of agricultural land known as the wheatbelt (WWF, 2014, Bradshaw, 2012). Since European settlement, over 93% of the native vegetation in the central wheatbelt has been cleared for agriculture, including 97% of the York gum, wandoo and salmon gum woodlands (Saunders, 1989). Prior to land clearing, closed forest and woodlands with a crown cover of $\geq 20\%$ and open woodlands with a crown cover of $\leq 20\%$ were the second most common habitat type and regarded as good indicators of agricultural soil (Bradshaw, 2012). For this reason they were more extensively cleared than any other type of vegetation (Saunders 1989, Yates, *et al.*, 2000). In addition to this catastrophic loss of biodiversity, intensive farming has also led to longer term environmental problems such as rising water tables,

increased soil salinity, soil erosion, nutrient leaching and has changed the structure and floristic composition within the majority of the remaining remnants (Close, *et al*, 2004, Hobbs, 2002, Recher, *et al*, 1998, Hatton *et al*, 1993 and Saunders, *et al.*, 1992). The ecological balance has also been upset by the unnatural exclusion of low intensity fire regimes causing the eutrophication of the top soil which favours arbivores and other competitors such as introduced weeds (Jurskis, 2005). Intensive agriculture has also resulted in local and regional extinctions of native flora and fauna (Yates, *et al.*, 2000). Rosenzweig (1965) predicted that only 51% of the original avifauna will continue to persist (Abbott, 1999), with 38% of all the land birds in the area, declining in range and abundance (Saunders, 1989).

Over the last 30 years, the SWWA has also experienced extreme and unpredictable climatic shifts, with a 10-20% decrease in winter rainfall and a gradual and substantial increase in temperature over the last 50 years (IOCI, 2002). This reduction in annual rainfall is associated with changes in large scale atmospheric circulation called El Niño-Southern Oscillation Events (ENSO), are driven by the greenhouse effect and human activity on a global scale (IPCC, 2014, Risbey, *et al*, 2009, CSIRO, 2005), Climate modelling evidence shows there will be a doubling in the occurrences of El Niño events in the future in response to global warming (Cai, *et al*, 2014).

If these conditions persevere, it will reduce habitat quality and food availability, such that where species once persisted, leads to an environment that can no longer sustain them (Thomas & Hanski, 2004). The impact on native vegetation and remnant ecosystems varies, but for many species that have a restricted range or are already confined to small areas, these species are destined towards the possibility of extinction

(AWA, 2002). Species at risk include those with long generations, poor mobility, narrow ranges, specific host relationships, isolate and specialised species and those with large home ranges (DEC, 2005). The predicted loss of existing habitat in the central wheatbelt area of Western Australia is in the range from 40-50% (CANA, 2005). The estimation of future loss of native flora and fauna has catastrophic consequences for biodiversity within this unique area. Government initiatives combined with the mobilisation of a legitimate workforce is critical for the conservation of species and rehabilitation of ecosystems under a hotter and dryer climate scenario. The Ecological Society of Australia encourages scientific research into identifying species and habitats most under threat from projected climate change (Chambers, *et al.*, 2005).

The declining annual rainfall of the SWWA is associated with changes in large scale atmospheric circulation (global warming), driven by the greenhouse effect and human activity on a global scale (CSIRO, 2005). Data shows from 1960 to 1990 there was a decrease in rainfall by 16% and it's predicted that by 2030 there will be a decrease of up to 20% and by 2070, up to a 60% decrease in annual rainfall (CSIRO, 2005). As shown from future climate models, the dryer and warmer weather scenarios for the SWWA will have enormous implications for the remaining native vegetation and for the animals that depend on it for their survival (Saunders, 2005).

Over the last 4 decades *Eucalyptus wandoo* (Blakely) has been suffering crown decline and is hypothesised to be the result of environmental stress (Dalmaris, 2012). Wandoo crown decline is characterised by a thinning of the crown that begins at the branch ends and progresses towards the trunk (Close, *et al.*, 2004). It had not been noticed on a large scale until the mid 1980's and appeared to coincide with a dramatic decrease in

average annual rainfall (Veneklaas & Manning, 2007). Several studies suggest the cause may be climate related, but no empirical evidence to support this has been collected (Zdunic, *et al.*, 2012, Hooper, 2009). The climatic range of *E.wandoo* is between 1000-350 mm annual rainfall (Zdunic, *et al.*, 2012, Yates, *et al.*, 2000) and zones between the isohyets of 400-450 mm and 600-650 mm display the most severe crown decline, with better health at higher rainfall (Mercer, 2003). Wandoo woodlands are very long lived and have developed defence mechanisms to cope with attack by insects and fungi and drought strategies which control over-transpiration, leaf fall, branch dieback and replacement of its primary crown by epicormic growth (Batini, 2004 & Veneklaas & Manning, 2007). However, when a combination of negative impacts are sustained over long periods of time these defence mechanisms may be compromised and fail (Batini, 2004). Old growth eucalypt forests are biologically and evolutionary unique (Bradshaw, 2012) and therefore are a high priority for conservation.

This study investigates the impact climate change has on the habitat, viability and genetic structure of woodland avifauna. In the wheatbelt of Western Australia, species that once lived in a continuous habitat now reside in small patches of remnant habitat that are scattered through a vast and highly modified agricultural landscape (Saunders, 1989). There are two main reasons for making an assessment of the genetic population structure and the population viability of focal species. As focal species are sensitive to changes in their habitat structure and because they have specific habitat requirements, they are strong indicators of the functioning of woodland ecosystems. Therefore their presence, absence or decline is indicative of the environmental health of the remaining woodland remnants. Also by comparing the population genetic structure in continuous

and fragmented woodland systems, the effects of geographical distances between habitat on species distribution and dispersal patterns can be assessed.

Landscape Genetics can resolve the genetic structure of continuous and fragmented populations. It aims to explain observed spatial genetic patterns by the detection of genetic discontinuities and the correlation of these discontinuities with landscape or environmental features (Manel, *et al.*, 2003). This approach attempts to establish a relationship between the variation in the physical environment and observing the effect it has on the population dynamics of species (Schmelzer, 2000, Guillot, *et al.*, 2009). Detecting and understanding restrictions to gene flow can improve the management of species by identifying habitats for either conserving genetic variation or required for population connectivity (Safner, *et al.*, 2011).

Distribution, Climatic Envelope and Ecological Niche Modelling (ENM) are methods that reconstruct species ecological requirements (including abiotic preferences) and predict their geographical distributions (Peterson, 2006). Biogeographic variables such as altitude and salt stress provoke the physiological and behavioural adaptations the geneticists seek to explain, while their presence is presumed to leave a hidden signature in patterns of nucleotide variation (Purugganan & Gibson, 2003). Recent integration of ENM's and phylogeographic studies, have increased the understanding of the processes structuring genetic variation across landscapes (Alvarado & Knowles, 2014). This includes the use of ENMs to identify the potential location of past populations and to test whether niche divergence accompanies species divergence (Alvarado & Knowles, 2014).

Assessing the effects of environmental conditions and management strategies on declining species can be carried out by using a Population Viability Analysis (PVA) (Van Horne, 2002). PVA's are used to integrate various risks that a species faces and estimates a probability of time to extinction (Wade, 2002). The analysis is primarily based on environmental and demographic stochasticity and sometimes includes potential catastrophes (Lande, et al., 2003). However, some of the difficulties in using this method is that the models depend on a complex range of ecological parameters, for which sometimes their values are sometimes largely uncertain or unknown (Beissinger, 2002). Also, the factors that contribute most to extinction risk may differ among species (Lande, et al., 2003) therefore, the aim is to determine which parameters can be reliably and precisely estimated and then to build the PVA models around those parameters (White, et al., 2002).

Landholders and wildlife managers require information regarding specific risks to native species prior to reaching a critical threshold and thereby preventing further declines that drive species towards inevitable extinction (Beissinger, 2002). If habitat fragmentation has adversely affected the dispersal patterns of species, or if habitat quality is having a negative impact on the viability of species, then vegetation corridors can be built to facilitate the movement of wildlife between reserves and remnants. In a survey of changes in forest avifauna in the south west of Western Australia Abbott (1999) predicted that with increasing temperatures, birds restricted to the eastern sectors of the forest, would have to move as competition for water by plant species would ultimately result in more open forests. Chambers (*et al.*, 2005), also suggests that because of the effects of climate change, birds in this area will have to move into higher rainfall areas.

1.2 Study Aims

This study investigates three focal woodland birds living in the highly fragmented west Australian wheatbelt. Landscape genetics, habitat modelling and a viability analysis using demographic data was conducted to investigate and assess suitable habitat, population genetic structure and survival rates of declining species. The long term impact of climate change on the quality of habitat and was also investigated. To promote the recovery of these species, negative impacts were identified and recommendations were made to facilitate the future management of these and many other species that share the same habitat.

- Observe the impact of habitat loss and fragmentation on gene flow and population structure of declining populations of avifauna.
- Determine current species migration patterns and re-construct a genealogy to determine the dispersal range prior to land clearing.
- Observe the interaction between rainfall patterns, quality of habitat and woodland avifauna.
- Investigate the longer term impacts of climate change and species viability.
- Make recommendations for the management and recovery of woodland avifauna.

1.3 Conservation and Landscape Genetics

Conservation of genetic diversity is one of the main issues in conservation biology (Burgman & Lindenmayer, 1998, Caballero & Toro, 2002, Frankham, *et al.*, 2002)).

The extinction of a population in the wild produces an overall loss of genetic diversity within a species because a significant proportion of the total genetic variation has been diminished (Caballero & Toro, 2002). Wild species are most commonly found in populations that are subdivided into smaller units because the geographic distances between populations exceeds their dispersal range or because of ecological and behavioural factors, such as habitat specificity and social interactions (Hendric, 2000).

The spatial subdivision of a population arises from barriers to dispersal, which act to inhibit genetic exchange among all the parts of a population and by doing so, creates a population structure (Donovan & Welden, 2002). The genetic connectivity between populations depends on the level of gene flow that has occurred among the subpopulations (Hendric, 2000). Generally if there is little or no gene flow between populations, then each sub-population evolves independently of the other and if there is too much gene flow between populations, then the whole population can lose its structure by the process of genetic mixing (Donovan & Welden, 2002). The most important evolutionary consequence of gene flow is that it tends to homogenise a population's structure and acts against genetic drift (Richards, 2000).

If a population experiences a prolonged period of isolation and reduced genetic variation, it can lead to inbreeding depression which is correlated to a decrease in viability, survivorship and fecundity (Whelan, *et al.*, 2000). Inbreeding tends to reduce population growth rates and contributes to extinctions, but it is not generally accepted

that inbreeding alone translates into elevated extinctions because demographic and environmental stochasticity including catastrophic events, are thought to have a larger impact on small populations (Brooks *et al.*, 2002). The recovery of such populations can be achieved through founder or re-colonisation events, which can generate further increases of genetic differentiation (Lindenmayer & Peakhall, 2000). However, in unstable environments and over the long term, the cumulative effects of repeated extinction and re-colonisation events, ultimately leads to a serious loss of genetic diversity (Lindenmayer & Peakhall, 2000, Goodnight, 2004, Frankham, 2002 & Thrall, *et al.*, 1994).

Natural selection is defined as an evolutionary force that selects differential genotypes that preserve the most favourable and best adapted variants or traits and eliminates less favourable ones (Lincoln, *et al.*, 1998). According to Moritz (1994), populations that have evolved in isolation have a distinct potential to develop into genetically different populations, population groups or are uniquely adapted to existing environmental conditions (Crandall *et al.*, 2000). However, the ability of species for adaptation is affected by a reduced population size, fragmentation and changes in the environment (Frankham, *et al.*, 2002) and part of preserving evolutionary processes responsible for adaptation, is also conserving genetic diversity (Avisé, 1994, Crandall, *et al.*, 2000).

Adaptive genetic diversity is maintained through the evolutionary forces of random mutation and natural selection, effectively allowing species to respond to changing environmental conditions and providing them with a significantly better chance of survival (Sherwin, *et al.*, 2000, Kohn, 2006, & Frankham *et al.*, 2002). The effects of natural selection can be demonstrated in fragmented populations found at the edge of

their geographic range, living in atypical environments. Although these often small, isolated and somewhat unstable populations have a higher risk of extinction, they also possess a high degree of adaptive genetic diversity and are therefore considered to be a high priority for conservation (MacDonald, 2002, Sherwin, *et al.*, 2000).

The difficulty in making an assessment of genetic diversity of fragmented populations is that historical processes, such as previous population expansions or contractions which may have a greater influence on the pattern of genetic diversity than contemporary processes (Walsh, 2006, Lindenmayer & Peakhall, 2000). However, with the ever increasing availability of genetic markers and analysis techniques, the partitioning of historical from recent phenomena has been possible and has motivated an increasing number of studies including dispersal patterns of species (Berry, *et al.*, 2004, Hansson *et al.*, 2002, Eldridge, *et al.*, 2001, Sumner, *et al.*, 2001, Waser, & Strobeck, 1998 & Rannala & Mountain, 1997), shared ancestry (Luikart & England, 1999) and coalescence within a metapopulation (Pannell, 2003).

The immediate evolutionary potential of a population is determined by heritability (a phenotypic variability that is genetically based) (Frankham, *et al.*, 2012, Lincoln, *et al.*, 1998). Phylogenetic inferences are based on the inheritance of ancestral characteristics and are defined by changes in these characteristics over evolutionary time (Swofford, *et al.*, 1996). The stable inheritance of these characteristics (quantitative traits) is mediated by the genome and their analysis involves the direct comparisons of slowly evolving nucleotide sequences such as those found in the gene coding region of mitochondrial DNA (Avice, 1994). Phylogeography is finding the distribution of

genealogical lineages by relating DNA sequence trees to geographic origins of mitochondrial haplotypes (Frankham *et al.*, 2002).

The potential geographic range of any species is limited by the suitability of environmental conditions; including interactions with other species and the distribution of species is influenced by historical factors (including vicariance events) and dispersal patterns (Avice, 1994). Historical demographics of populations have been profoundly influential to phylogeographic patterns over micro evolutionary time scales because of their inevitable impact on the structure of gene genealogies (Avice, 2000). The branching process theory and coalescent theory address the connections between demography and pedigree based genealogies (Avice, 2000). The branching process models produce evolutionary trees constructed from contemporary individuals that have only a subset of lineages that existed in the past which left no descendants to the present day and therefore cannot be represented on the genealogy (Harvey & Steers, 1999). However, the coalescent model provides a description of genealogical relationships by simulating evolutionary processes by going backwards along the lineages that gave rise to that sample at the most recent common ancestor (Marjoram & Tavaré, 2006). It predicts that ancestral haplotypes will be the most frequent sequences sampled within a population and allows networks to incorporate the often non-bifurcating genealogical information associated with population divergences (Clement, *et al.*, 2000).

A more recent method for making inferences of past history of populations and species is Approximate Bayesian Computation (ABC). It is a likelihood-free approach for Bayesian inferences based on a rejection algorithm method that applies a tolerance of dissimilarity between summary statistics from observed and simulated data (Nakagome,

et al., 2012). The approach is carried out in three successive steps. It uses repeated simulation data under plausible evolutionary scenarios and generates simulated data sets (Cornuet *et al.*, 2013). At each step, if the data that are produced match the observed data, the parameter value that is being generated is accepted (Majoram & Tave, 2006). The set of accepted parameter values are then used to approximate the posterior distribution through a local linear regression procedure (Cornuet *et al.*, 2013). The posterior distribution or the most appropriate algorithm, is determined by factors such as complexity of the model and size of data set being considered (Majoram & Tave, 2006).

One of the main challenges of conservation plans is to be able to identify the spatial scales at which species are able to disperse and then restore the ecological processes that promote species viability (Luque *et al.*, 2012). Since the degree to which a population is connected across a broad landscape increases with increasing ability to disperse (Cushman & Landcuth, 2012), by identifying barriers to dispersal as such as distance between habitat patches, then the dispersal range of species in the landscape can be determined (Peterson, 2006). Individual species that are not able to disperse across small gaps in the distribution of their natural habitat result in highly fragmented populations that are at possible risk of inbreeding depression through lack of gene flow (Cushman & Landcuth, 2012, Veit, *et al.*, 2005, Johnson, *et al.*, 2003, Couvet, 2002). One of the ways the genetic structure of these fragmented populations can be elucidated is with Landscape genetics. It aims to detect the genetic discontinuities across a habitat area and correlates these discontinuities with geographical and environmental features by using different spatial modelling techniques (Manel *et al.*, 2003).

Spatial models that describe the expansion of populations include (a) the Island Population Model, (b) the Continent Island Model, (c) the Stepping Stone Model and (d) IBD (Isolation By Distance) Model (Rockwell & Barrowclough, 1987, Frankham, *et al.*, 2002 & Hedrick, 2000). The assumption of Wright's Island Model, is that each population receives and gives migrants to each other and is composed of the same number of individuals (Whitlock & McCauley, 1999). This model describes gene flow among equal sized demes, which are independent of the distance between each other and where immigrant individuals come from a common gene pool, consisting of a mixture of all genotypes (Rockwell & Barrowclough, 1987). The main disadvantage of this model is the inability to describe localised dispersal between demes (Rousset, 2004).

The Continent-Island Model assumes unidirectional gene flow. This Model assumes Island populations receive migrants from a large source, with reciprocal gene flow having a negligible effect on the allele frequency in the source population (Hedrick, 2000). The Stepping Stone Model describes neighbouring or surrounding populations that exchange migrants are assumed to be colonial, of equal size and that gene flow is mostly directed towards adjacent colonies (Rockwell & Barrowclough, 1987). In both the Continent Island and the Stepping Stone Models, the consequences of gene flow such as inbreeding and fitness, will largely depend on dispersal rates and population size (Frankham *et al.*, 2002).

The equilibrium theory of Island Biogeography by MacArthur and Wilson (1967) demonstrates the number of species inhabiting an island is related to island area, the distance from the mainland and determined by immigration and extinction (Lincoln, *et*

al., 1998). This theory was initially utilised for studying the effects of habitat fragmentation, but the differences between true islands and habitat fragments became apparent and in time it was superseded by the Metapopulation Theory (Attiwill and Wilson, 2003). The Island –Mainland metapopulation model is based on mainland or conservation reserve that provides a source of colonists to nearby populations of varying size and isolation (Attiwill and Wilson, 2003). The second metapopulation model is the Levin’s Model (1969) and built on by the IF (Incidence Function) approach (Hanski, 1997). This combined approach, models populations that inhabit a collection of variable habitat patches and depending on the suitability, size and isolation of habitat, each population has its own likelihood of extinction and recolonisation (Hanski, 1997). The metapopulation theory has been applied in Australia to the conservation of species such as the Leadbeater’s Possum (*Gymnobelideus leadbeateri*) and the Euro (*Macropus robustus*) (Attiwill and Wilson, 2003). However because of the detailed and sometimes difficult to measure ecological information required for this modelling, accounts for the relatively few species that have been modelled using this method (Attiwill and Wilson, 2003).

While geographically restricted gene flow creates a genetic structure, genetic drift is occurring locally via sub-populations that exchange genes at a rate which is dependent on distance (Hardy & Vekemans, 1999). As individual progeny of wild species typically do not disperse long distances away from a maternal parent, this is how the process of the build up of genetic isolation by distance within populations starts (Epperson, 2003). If the dispersal of species is localised in space, it is expected that genetic similarity will be greater between individuals from closer subpopulations (Rousset, 2004). When the rates of migration among populations depend on the

distances separating them, spatial patterns display symmetry both within and among spatial dimensions (Epperson, 2003). This leads to a positive correlation between genetic and geographic distances and gave rise to the Isolation by Distance (IBD) Model proposed by Wright (1943) (Palsson, 2004, Rousset, 2004).

Standard spatial patterns of isolation by distance are best studied by using pairwise measures of genetic similarity or correlations among populations (Epperson, 2003). The values of correlations at short distances as well as the form of the decrease with distance depend on the number of spatial dimensions as well as the rates and distances of dispersal (Epperson, 2003). This relationship of genetic relatedness between pairs of individuals and their geographic distances makes it possible to estimate a neighbourhood size ($4D\pi\sigma^2$) (Rousset, 2004, Manel, et al., 2003, Sumner, et al., 2001). However Wright (1943), showed that limited dispersal produces locally inbred demes and that unless there is random mating within neighbourhoods, there is no basis for using neighbourhood size as a spatial unit (Epperson, 2003). Wright used the inbreeding coefficient F_{IS} within blocks or demes to partially examine the genetic results of isolation by distance (Epperson, 2003). Although the different theories of population expansion and their proposed population models may not precisely fit real populations, they do however, give a close approximation to many situations and enable us to evaluate the effect of limited gene flow (Hedrick, 2000). Exactly which model to choose largely depends on the geographical connectedness of populations (Rockwell & Barrowclough, 1987).

Spatial patterns of genetic diversity can reveal how species and their genes are distributed across their native range and is largely dependent on the amount of

migration and natural selection (Epperson, 2003). This is crucial to conservation genetics because spatial distributions inform expectations about habitat fragmentation, and is directly related to the containment of genetic diversity within versus among fragments, including the levels of inbreeding within fragments (Epperson, 2003). The analysis of the spatial patterns of genetic divergence has traditionally been performed by Wright's F statistics and related techniques such as Φ -statistics (Φ) and G_{ST} estimates, but are criticised because they cannot give detailed descriptions of the 'spatial patterns' of genetic divergence (Diniz-Filho, *et al.*, 2000). Also, these statistics measure the relative proportion of the total variation that exists among populations rather than within populations (Epperson, 2003). Explicit spatial methods such as autocorrelation analysis and matrix comparison techniques such as the Mantel Test (1967) have been used to overcome this difficulty and describe in more detail the spatial patterns in genetic data (Diniz-Filho, *et al.*, 2000).

Mantel's Tests can measure the association between genetic distance and an environmental variable such as forest cover, temperature (Manel, *et al.*, 2003) or geographical distance (Geffen, *et al.*, 2004). It is a regression in which the variables are themselves distance or dissimilarity matrices that summarise pair-wise similarities among sample locations (Urban, 2003). Plotting pairwise geographic versus genetic distances can determine whether there is genetic Isolation by Distance or geographical barriers to gene flow (Guillot, *et al.*, 2009). Mantel correlograms test whether or not synchrony changes with distance, relative to the overall data set (Koenig, 1999). A partial Mantel test can be used to compare three or more variables (Manel, *et al.*, 2003) and is the most common method of performing partial regression analysis for genetic distances, but the validity of the approach has been questioned (Pilot, *et al.*, 2006).

Rousset (2002) found that the Partial Mantel Test was prone to bias, but had no concern of such when using the simple Mantel's Test.

Within any spatial analysis, it is recommended to include methods that do not make any assumptions of population boundaries or genetic structure beforehand (Manel, *et al.*, 2003). Spatial Autocorrelation is a multivariate method that identifies correlations among the genotypes of mapped individuals and identifies distance classes of genetic structure without prior knowledge of the scale (Loiselle, *et al.*, 1995). It tests whether the observed genotype of an individual at one location is dependent on the genotype of an individual at another location and is independent of any prior assumptions about the underlying population structure (Manel, *et al.*, 2003, Barbujani, 1987). Moran's I Coefficient (Moran, 1950), summarises the strength of allele frequency between distance classes of sample populations as a function of their geographical distance (Arnaud, *et al.*, 2001).

Another autocorrelation statistic A_y , is the average genetic distance between pairs of individuals that fall within a particular distance class (Miller, 2005). A Two Dimensional Local Spatial Autocorrelation Analysis (2D LSA) based on the method of Smouse & Peakhall (1999), allows the investigation of local patterns and defines a local subset as an individual and its n nearest neighbours, based on the n pairwise comparisons (Double, *et al.*, 2005). The 2D LSA method only differs from standard autocorrelation analysis by the way individuals are selected for a particular distance class (Peakall & Smouse, 2006). The main advantage of using Spatial Autocorrelation based on individuals rather than on populations, is that it is useful in investigating within population dispersal patterns, when there are no clear population boundaries

(Temple, et al., 2006). Although there are different methods of measuring Spatial Autocorrelation, it cannot identify the specific location of a genetic discontinuity or genetic boundary (Manel, *et al.*, 2003). Populations on each side of a genetic barrier can have much lower genetic correlations than expected based on the short distances between them (Epperson, 2003).

Some techniques to detect genetic boundaries within a landscape are the Maximum Difference Monmonier Algorithm and the Bayesian Clustering approaches (Manel, et al., 2003). The statistical wombling approach is a barrier detecting method used to identify the slope of a surface (gene frequencies in populations viewed as a surface) that is steep in the zone separating populations on opposite sides of a barrier (Epperson, 2003). The barrier detection methods try to detect areas of abrupt genetic discontinuities, whereas the clustering methods look for homogenous spatial domains (Guillot, et al., 2009). All these methods are considered direct methods of edge or boundary detection because rather than looking for clusters of individuals, they focus on boundaries between dissimilar individuals (Safner, et al., 2011). One of the limitations of the Womble approach is that it cannot be used in situations where there is gametic disequilibrium such as in small population sizes, bottlenecks, inbreeding, admixture and departures from Hardy Weinberg equilibrium (Manel, et al., 2003). The Maximum Difference Monmonier Algorithm is a geographical regionalisation procedure can be used to detect the location of barriers to gene flow by identifying large genetic differences along connectivity networks (Miller, 2005). These connectivity networks are formulated by a Delaunay Triangulation procedure, which connects adjacent geographical positions of the samples on a map and results in a network that connects all the samples (Mantel, et al., 2003).

Another approach to identify spatial genetic patterns is through the use of Principal Component Analysis (PCA) (Manel, 2003 & Guillot, 2009). PCA is a method to visually represent the relative similarity among individuals and distinction among sampling locations (Menozzi, *et al.*, 1978). It's an ordination method used to correlate and analyse the patterns of variation, by standardising or simplifying the raw data first (Gardiner, 1997). It explains the covariance structure of data by means of a small number of components (Hubert, *et al.*, 2003). Each component has an associated eigenvalue which is a measure of the proportion of the total variability in the data (Gardiner, 1997). When analysing genetic data, the PCA does not classify all individuals into discrete populations, or linear combinations of populations; rather it outputs each individual's coordinates along axis of variation (Patterson, *et al.*, 2006). Since PCA was first introduced to the field by Cavalli-Sforza in 1978, it has become a standard tool in population genetics (Patterson, *et al.*, 2006). PCA can cluster allele frequencies of spatially referenced populations (Piertney, *et al.*, 1998) and can generate synthesis maps, which can be used to visualise clines (Manel, 2003) or evolutionary history (Menozzi, *et al.*, 1978).

Principal Coordinate Analysis (PCoA) is a method that uses a matrix of Euclidean Distances between individual data points and finds a coordinate space, where the distances are exactly preserved (Higgins, 1992). The calculation involves converting the distance matrix into a centred matrix, which is then decomposed into its eigenvalues and eigenvectors (Ramachandran, *et al.*, 2005). Each eigenvector is then divided by its square root of its corresponding eigenvalue, to yield principal coordinate scores for each population in the distance matrix (Ramachandran, *et al.*, 2005). PCoA has been used to

find meaningful patterns in an aligned set of DNA sequences (Higgins, 1992) and allele frequencies (Ramachandran, *et al.*, 2005).

Graphical representations of genetic patterns across a landscape can also be constructed by using a connectivity network of sampling areas and assigning calculated inter-individual genetic distances to landscape coordinates (Miller, 2005). An interpolation procedure is then used to infer genetic distances at locations on a uniformly spaced grid overlaid on the entire sampled landscape (Miller, 2005). There are different applications of this type of interpolation procedure. For example, Menozzi, *et al.*, (1978), used the Principal Components of population allele frequencies, as variables in the interpolation procedure. This method plots geographical distances with genetic similarities so they can be visualised at the same time, but it cannot provide a statistical analysis of the pattern of change of genetic frequencies (Manni, *et al.*, 2004). However by using genetic similarities via Principal Components, a pattern of evolutionary history of populations can be inferred (Menozzi, *et al.*, 1978).

1.4 Microsatellite DNA Analysis

All genetic diversity originates from mutation and the highest levels of genetic diversity is found in non-coding DNA such as microsatellite DNA (Frankham, 2002). Microsatellites are motifs of Short Tandem Repeats (STR) of DNA sequences between 2 and 5 nucleotides long and are inherited in a co-dominant, Mendelian fashion (Loew, 2002, Frankham, 2002). On each side of the repeat unit are flanking regions (Primmer & Ellengren, 1998) of unordered DNA (30-50 bases long), where locus specific primers are designed (Primmer & Ellengren, 1998).

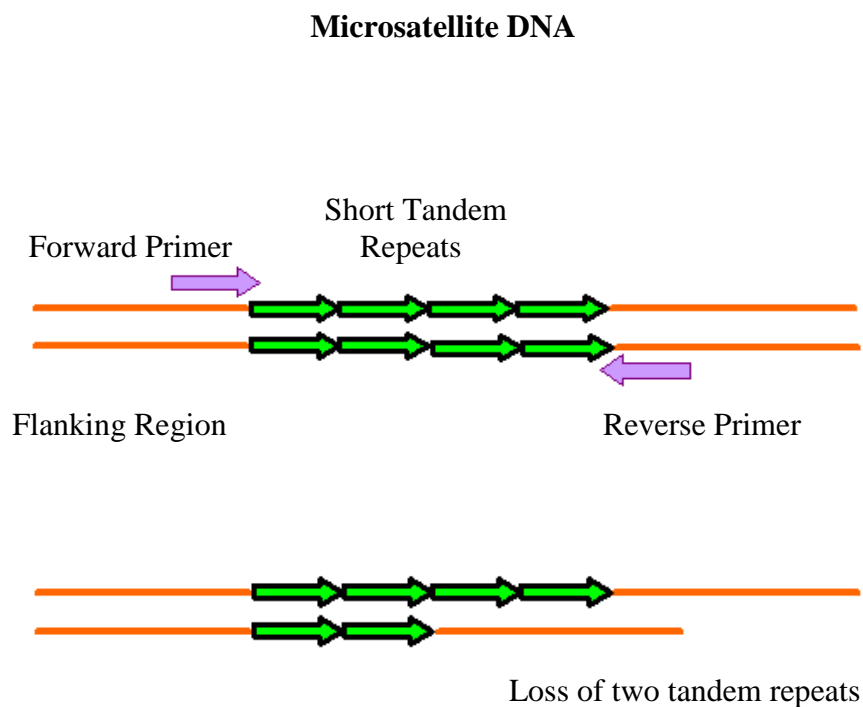


Figure 1.4 Simplified diagrams of microsatellite DNA and how fragments of different length are created.

Microsatellites contain an abundant source of DNA mutations (Zhang *et al.*, 2003). They can be discriminated by a difference in repeat unit, which is generated by replication slippage mutation and causes an allelic length variation at a particular locus (Ellegren, 2000 & Eisen, 1999). The rate of mutation may be positively correlated with the number of repeat units (Primmer & Ellengren, 1998) and studies show that in some species the rate has a bias towards gender and age effects (Ellengren, 2000, Primmer, *et al.*, 1998, Ibaruchi, *et al.*, 2004). The high allelic variability and the high average mutation rate (approximately 10^{-3} to 10^{-4} nucleotides/generation) is not only species specific but within a species, it can be variable between individuals at different loci, or among individuals of a population at the same locus (Ellegren, 2000, Loew, 2002) For this reason, microsatellites are very useful for estimating gene flow among sub populations (Schlotterer, 1998).

Neutral genetic markers (in the absence of selection) infer gene flow and therefore are preferred as indicators of population structure (Hardy & Vekemans, 1999). As microsatellite DNA is non-coding, it is generally assumed to be a neutral marker and widely used for routinely used in genetic studies (Zhang, 2003). However, this assumption of neutrality is not always valid for non-model species, which make up the vast majority of studies of natural populations (Nielsen, *et al.*, 2006). Although microsatellites are unlikely to be the target of natural selection, their linkage to a genomic region that is under selection (functional gene), is expected to cause a deviation from neutral expectations (Schlotter, 2002). For example, evidence for selection at microsatellite loci can be found in studies of genetic hitch-hiking and local selective sweeps (Schlotterer, 2002 & Nielsen, 2006). Also microsatellites that amplify across species will often show high conservation across large evolutionary distances

(Zhang et al., 2003, Primmer et al., 1998). Therefore, neutrality testing and carefully assessing microsatellite data prior to further analysis, is highly recommended.

It is important to distinguish between the various models of microsatellite mutation because they have different assumptions (based on the underlying theory) and therefore, will make different predictions about population variability (Jarne & Lagoda, 1996). The simplest, most commonly used models describing microsatellite mutations are the Infinite Allele Model (IAM) model, Stepwise Mutation Model (SSM) and the Two Phase Model (TPM) model. The SMM describes the mutation as the addition or deletion of a single tandem repeat by one unit and infers that alleles of the same size are more closely related, but not necessarily Identical by Descent because of convergence, parallelism or reversion events (Neff, *et al.*, 1999, Estoup *et al.*, 1995). Because of this, the resolution of genetic markers evolving under the SMM may strongly decrease with evolutionary divergence between populations (Estoup, *et al.*, 1995). The TPM model is analogous to the SMM model, but with the mutation of one repeat unit (one phase) in addition to infrequent, large jumps in repeat number (Di Rienzo, *et al.*, 1994). The IAM model involves the mutation of any number of tandem repeats resulting in an allele state, not previously encountered in the population and therefore unique alleles are Identical by Descent (Estoup & Cornuet, 1999 & Neff *et al.*, 1999). As the mutational process of microsatellites is far more complex than previously thought, these commonly used models represent an oversimplification of the true mutation process (Ellegren, 2000). For example, multi-step mutations may often give rise to novel alleles and could explain the apparent fit of microsatellite data to the IAM (Neff, *et al.*, 1999). Secondly, homoplasy describes alleles that are Identical in State (IIS), without being Identical by Descent and therefore, disregarding homoplasy leads to underestimating the actual

divergence between populations, which may be problematic for the SMM and TPM (Jarne & Largoda, 1996).

There is a potential for null alleles or allele dropout in microsatellite data, by a failure to detect an allele that actually exists in the template (Van Oosterhout, *et al.*, 2006). Null alleles are common in microsatellite analysis and it is important to partition their effects from other biological causes of deviation from Hardy-Weinberg Equilibrium (Van Oosterhout, *et al.*, 2006). In the presence of null alleles at a particular locus the observed heterozygosity would be largely underestimated, resulting in an excess of homozygotes over and above the effects of inbreeding and reduced gene flow (Chybicki & Burczyk, 2009, Campagne, *et al.*, 2012). Also, null alleles can affect genetic differentiation measures causing overestimation of F_{ST} and large error rates in parentage assignment, mating system, behaviour and dispersal parameters (Chybicki & Burczyk, 2009). Null alleles can also arise through sequence polymorphisms which occur within the repeat region, the flanking region or in the primer binding region of microsatellite DNA (Butler, 2005).

The most common method used to measure genetic variation within and among populations, is by calculating the allele frequencies of individuals, found in each population (Rockwell & Barrowclough, 1987, Frankham, *et al.*, 2002, Rousset, 2004). Generally very similar allele frequencies in different sub populations reflects a high rate of migration or recently fragmented populations and conversely, different allele frequencies in subpopulations results from populations with severely restricted gene flow (Frankham, *et al.*, 2002). Wright's Fixation Index describes the genetic variation in a total population (F_{ST}), sub-populations (F_{IS}) and of individuals (F_{IT}) (Hedrick, 2000).

F_{ST} is a measure of the genetic differentiation over subpopulations and ranges from 0, indicating no differentiation (a panmictic population) and increases to 1 in value, indicating the fixation of different alleles in sub populations (Gibbs, *et al.*, 2000 & Frankham, *et al.*, 2002). Positive F_{IS} values indicate a deficiency of heterozygotes and negative values indicate an excess of heterozygotes (Hedrick, 2000). The two main sources of heterozygote deficiency are inbreeding within populations, or by the mixing of different gene pools (Wahlund Effect) (Frankham, *et al.*, 2002). The interpretation of F_{ST} depends on the assumptions of non-selection of the DNA marker, that populations are at Hardy Weinberg Equilibrium (HWE) and a microsatellite mutation model (Selkoe & Toonen, 2004).

The Hardy Weinberg Law describes the frequencies of genotypes in large populations that do not change through the process of inheritance; rather they remain constant from generation to generation (Solomon, *et al.*, 1993). This can be seen in situations of gametic equilibrium that exist under the conditions of a large randomly mating population, with emigration, immigration, mutation and natural selection having a small effect (Solomon, *et al.*, 1993). However, deviations from Hardy Weinberg Equilibrium exist and involve the non-random gametic association of alleles at different loci in a population (Mueller, 2004). This occurs because of selection and non random mating (Rousset, *et al.*, 1995), or when the spatial scale chosen for sampling sites is larger than the true scale of the population (more than 1 population is sampled), which causes an excess of homozygotes (Wahlund Effect) (Selkoe & Toonen, 2006).

The Analysis of Molecular Variance (AMOVA) was initially introduced as an extension of the analysis of gene frequencies (Michalakis & Excoffier, 1996). When a population

sample is small, the allele frequency estimates alone, may not reflect the actual allele frequency in the whole population (Hedrick, 2000). AMOVA is a method which partitions the variance of gene frequencies into hierarchical group memberships, with the resulting genetic structure of populations analysed as variant percentages of individuals, within populations or between populations (Excoffier, et al., 2005). The different variance components are combined to produce a global F-statistic as variance component ratios (Excoffier, et al., 2005). The variance components of AMOVA can be used to calculate phi-statistics (Φ), which summarize the degree of differentiation among populations, relative to the total variance (Excoffier, et al, 1992).

The bottleneck effect is when populations that undergo large reductions in effective population size and experience a loss of genetic variation, through genetic drift (Lincoln, *et al.*, 1998). This increases the probability of inbreeding depression due to the fixation of deleterious alleles, which ultimately reduces adaptive potential and increases the probability of population extinction (Garza & Williamson, 2001, England, et al., 2003, Luikhart & Cornuet, 1998). The most common cause of bottleneck populations are habitat loss, founder events, disease outbreaks, and environmental catastrophes, which makes them more vulnerable to stochastic changes in population size or composition (England, et al., 2003). It is often difficult to determine whether a population has recently experienced a bottleneck because of levels of genetic variation and historical population sizes and are seldom known (Luikart & Cornuet, 1998). When conducting a genetic analysis, it is important to be able to detect bottlenecked populations because they violate the assumption of equilibrium, which is integral to most methods of estimating gene flow (Davies et al., 1999). Knowledge of recently bottlenecked populations which may not have had time to adapt to problems associated

with having a small population size, enables adaptive management strategies to be implemented (such as translocations) and thereby avoiding an increased risk of extinction (Luikart, et al., 1998).

The Assignment Test uses allele frequency of genotypes to assign individuals to populations with the highest likelihood (Cegelski, *et al.*, 2003). Assignment Tests assume the population is in HWE and that the marker used to generate the data is not under natural selection (Waser & Strobeck, 1998). Provided there are no events that cause departures from HWE (such as small population size, bottlenecks and inbreeding), Bayesian Clustering approaches can identify a population structure by assigning individuals to populations by a likelihood method, thereby resolving hybrid zones, migrants and admixed individuals (Pritchard, *et al.*, 2000, Griebeler, 2006) and origins of recently founded populations (Elderidge, 2001) clines (Jones, *et al.*, 2005) and sex-biased dispersal (Hansson, *et al.*, 2003).

Marker based inferences of relatedness (quantitative inheritance) in natural populations are used to study sibship structure, kin selection, inbreeding depression and isolation by distance in continuous populations (Hardy, 2003). Estimating the relatedness between individuals from a wild population, allows the study of social behaviours such as kinship or dispersal and can provide wildlife managers with vital information to minimise the potential of inbreeding of threatened species in captive breeding programs (Boulin, et al., 1996). For this reason, relatedness can be used as a more appropriate measure of genetic distance for assessing small populations (Reynolds, et al., 1983). Some advantages of using a relatedness measure, is that it is independent of assumptions of a mutation process (Rousset, 2002), and does not assume Hardy-

Weinberg genotypic proportions or Wright's Inbreeding Coefficient (Vekemans & Hardy, 2004).

There are two main methods to estimate relatedness (Oliehoek, et al., 2006). The first categorises individuals into a limited number of discrete classes of relatives such as full-sib, half sib, parent-offspring or unrelated relationships and the second estimate of relatedness, is based on a continuous scale (Oliehoek, et al., 2006). The first relatedness estimator is not based on a continuous scale and can lead to incongruous assignments because of the relationship between individuals. For example, if A-B are full-sibs and B-C are full sibs then the relationship between A and C may be something other than full sibs (Fernandez & Toro, 2006). In other words, the exclusion of three homozygotes for different alleles being full sibs cannot be done in a pair wise comparison basis (Fernandez & Toro, 2006). However, a second type of relatedness measure uses a continuous scale and has a biological range of -1 of unrelatedness, to +1 of being highly related (Kelunen & Ritland, 2005).

Relationship estimates involves the identity of homologous alleles either between two individuals (relatedness), or within individuals (inbreeding) (Ritland, 1996). The Kinship Coefficient (r) between two individuals represents the probability of two alleles (one randomly sampled from each individual), that are Identical by Descent (IBD) (Ritland, 1996). The Inbreeding Coefficient represents the probability of both alleles at a diploid locus within a single individual that share Identity in State (IIS) (Rousset, 2002). By making specific assumptions about the origins of alleles IIS, estimation of the probability that genes differ in individuals within subpopulations are IBD by using a measure of coancestry (Loiselle, et al., 1995). Molecular coancestry is the probability

that two alleles randomly taken at the same locus (one from each individual), are equal or average across loci (Fernandez & Toro, 2006). The concern here is not so much with the variation of gene frequency as in Wright's F_{st} , but rather the pattern of inheritance (Ritland, 1996),

Genetic relatedness based on Kinship or Coancestry Coefficients are designed to measure the divergence between populations caused by genetic drift and are most suited for short term evolution studies (Reynolds, *et al.*, 1983). In Ritland's (1996) Method of Moments Estimator (MME) of kinship, estimates are found for each allele at each locus and then combined into a single estimate using optimised weights. By utilising the information provided by alleles of differing frequency, this method reduces the small sample bias and is ideal for individual level estimates (Ritland, 1996). Vekemans & Hardy (2004), conducted a study of genetic relatedness and spatial genetic structure in plants and found that Ritland's estimator proved to be the most powerful when using highly polymorphic markers, but gives a downward bias when rare alleles occurred (Vekemans & Hardy, 2004).

The relationship between Relatedness (r) and geographical distance can give an indication of the spatial scale at which population differentiation occurs (Sweigart, *et al.*, 1999). Although Autocorrelation Coefficients such as Moran's I is used to find correlations among the genotypes of mapped individuals, it does not provide a surrogate measure of genealogical relationships (Temple, *et al.*, 2006). However, Loiselle, *et al.*, (1995) Kinship Coefficient is relative to the product of Moran's I Spatial Autocorrelation Statistic (Vekemans & Hardy, 2004), but is used to resolve the spatial scale of inheritance in natural populations, based on Identity by Descent (Loiselle *et al.*,

1995 and Hardy, 2003). This Identity by Descent estimate of coancestry declines gradually for pairs of individuals with increasing geographic distance (Loiselle, et al., 1995). Vekemans and Hardy (2004) found Loiselle's (1995) Statistic performed well in determining the spatial genetic structure of plants, despite low polymorphisms and for individuals that were selfing with a high F_1 (first filial generation).

The Molecular Coancestry Coefficient according to Caballero & Toro (2002) is defined as similar to the Genealogical Coancestry Coefficient of Malecot (1948) (Fernandez & Toro, 2006). Molecular Coancestry is used to assess the genetic diversity between individuals in subdivided populations and is also related to the genetic distance of individuals and the geographical distances separating them (Gutierrez & Goyache 2004, Hardy & Vekemans, 1999). The Molecular Coancestry Coefficients such as Kinsub and Kindist, measure alleles as identical or not and have values of 0, $\frac{1}{4}$, $\frac{1}{2}$ and 1, between individuals and can be averaged within populations (Gutierrez & Goyache 2004). Generally, kinship based diversity measures conserve the founder population allele frequencies, whereas genetic distance measures will conserve populations in which allele frequencies are the most different (Eding & Meuwissen, 2001).

Genetic Distances are measures which summarise the differences of genotypic data in an overall measure of differentiation between a pair of populations, or of individuals within a set of populations (Kalinowski, 2002). However, different types of evolutionary forces can cause genetic distances to vary greatly among gene regions or loci (Nei & Tateno, 1975). At some loci, gene substitution proceeds quickly by the aid of directional selection, and at other loci the similarity of genes is maintained by balancing selection (Nei & Tateno, 1975). The purpose of genetic distance data is not

to demonstrate that two populations are different, but to reveal how different they are (Kalinowski, 2002).

The various methods and modifications for measuring genetic distances have at the basis of each measure, a specific evolutionary and statistical model. As inferences from each model produce different genetic distances (Kalinowski, 2002), it is important to understand the assumption of each chosen model prior to conducting the analysis.

Rousset's Genetic Differentiation between individuals, models continuous populations where spatial clustering of individuals is difficult to define (Rousset, 2000). It can specifically estimate neighbourhoods and dispersal distances in continuous populations (Rousset, 2000). This distance measure is based on Malecot's Lattice Model (1951), where individuals are equivalent (probability of identity) with members of another deme of a continuous population (Rousset, 2000). The Tomiuk & Loeschcke Genetic Identity measure is based on the Infinite Allele Model (IAM) of microsatellite mutation, with a constant mutation rate which is related to evolutionary time (Tomiuk & Loeschcke, 2003). Roger's Genetic Distance is based on the assumption of no selection and no mutation and therefore does not consider features of evolutionary processes (Nei & Tatenko, 1975). However, it is dependent on gene frequencies and can cause problems with geometric distances (Reynolds, *et al.*, Cockerman, 1983).

Nei's (1972) Standard Genetic Dissimilarity Coefficient is based on the IAM and assumes that an ancestral population has split into various subpopulations, diverging via genetic drift and mutation (Reif, *et al.*, 2005). Nei's (1978) Genetic Distance is an extension of Nei's (1972) Coefficient and is a modification of the original Cavalli-Sforza Chord Distance (1967) (Kalinowski, 2002). As this chord distance is not based

on any specific genetic model or Euclidean Distance, it is not recommended for detecting phylogenetic relationships (Rief, *et al*, 2005). However, Nei's *et al.*, (1983) Dissimilarity Coefficient is more appropriate for recovering true evolutionary trees (Rief, *et al*, 2005). In relation to microsatellites, Takezaki & Nei (1996) proposed Genetic Distance values of $a = 0.1$ to represent trees for different species, and $a = 0.004$ for trees constructed for populations within a species.

1.5 Mitochondrial DNA Analysis

Mitochondrial DNA (mtDNA) is a double stranded, closed, circular molecule which is present at a high copy number in each cell (Zischler, 1999). As a rule mtDNA is a haploid genome that is maternally inherited in all animal species (Awise, 2000, Frankham, et al., 2002, Ishibashi, et al., 1997), except for some bivalve families (Rokas, et al., 2003, Ballard, et al., 2004). The genome contains about 37 genes coding for 22 transfer RNA's (tRNA's), 2 ribosomal RNA's (rRNA's) and 13 messenger RNA's (mRNA's) that are involved in electron transport and oxidative phosphorylation of the mitochondria (Roques, *et al.*, 2004, Kvist, 2000).

Vertebrate mitochondria are ~16kb are extremely compact with no introns and few intragenic spacers (Broughton, *et al.*, 2001). In comparison to nuclear coding genes, mitochondrial genes evolve at 5-10 times faster because of an error prone replicating system, which lacks a proof reading capability and thereby increasing the probability of a single DNA base substitution becoming fixed (Zischler, 1999). Among the protein coding regions of the mitochondria, different sites and genes are found to evolve at widely different rates (Awise, 2000, Palumbi, 1996).

Genes that evolve at a constant rate, give rise to a molecular clock which predicts the relative constant mutational rate and enables the estimate times of species divergence, by comparing their gene sequences (Page & Holmes, 1998). The Molecular Clock Hypothesis predicts the relative constant mutational rate of animal mtDNA, to be approximately 2% sequence divergence per million years (Hillis, *et al.*, 1996).

However, not all sequences conform to this hypothesis as there can be an acceleration of molecular evolution over short timescales due to neutral and slightly deleterious

mutations (Penny, 2005). Another way to explain rate variation is with the Generation Time Hypothesis and is based on principal that the mutation rate is set to generational rather than chronological time (Page & Holmes, 1998).

The neutral rate of evolution estimates mutations that arise through a single substitution of one nucleotide for another without a change of amino acids (Solomon, 1993). These synonymous, or neutral mutations are neither beneficial nor injurious and therefore evolve more rapidly than non-synonymous or replacement substitutions because of the functional constraints acting on proteins (Veuille, 2000). Knowledge of mtDNA neutrality is essential for analyses involving phylogenetic relationships between closely related taxa (Moritz et al. 1987), genetic distance estimates (Avice, 2000), historical bottlenecks (Weber, et al., 2004), or to analyse hybrid zones (Ritchie, et al., 2001). According to Nei (1987) nucleotide diversity is calculated as π (π); the average number of nucleotide differences per site between two sequences and the Jukes and Cantor correction uses the average of the values for all comparisons (Rosas, et al., 2003). For low levels of polymorphism, both methods give similar estimates (Rosas, et al., 2003).

Although the coding regions of the mitochondria are highly conserved, the gene order within the mitochondria has been shown to vary among taxa (Desjardins & Morais, 1990, Roques, *et al.*, 2004). A rearrangement of the gene order is found in vertebrates, with birds possessing a unique arrangement involving ND6 and glutamic acid, which is not found in any other vertebrates (Roques, *et al.*, 2004).

Gene Re- arrangement in Mitochondrial DNA

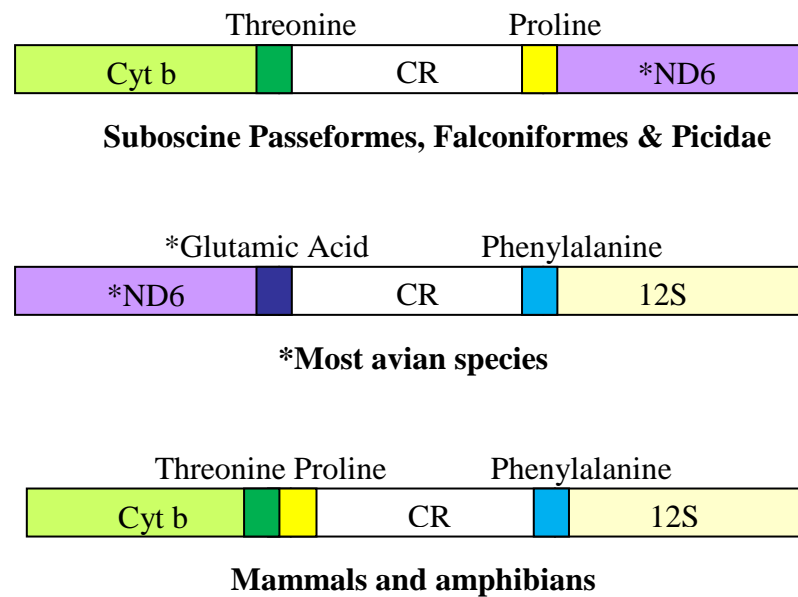


Figure 1.5a Simplified gene map of the gene re-arrangements of the mitochondria, diagram reproduced from Mindell *et al.*, (1998).

This novel gene order is thought to have resulted from the transposition of a segment of the tRNA glutamic acid and ND6 genes or alternatively, the tRNA proline, tRNA threonine and cytochrome b genes (Desjardins & Morais, 1990). Bird mitochondria also have a displaced O_L (origin of light strand replication), similarly to other taxa prone to gene order changes such as reptiles (Mindell *et al.*, 1998). This provides evidence of parallel evolution of mitochondria, with independent origins because of the re-arrangements found in relatively divergent taxa (Mindell *et al.*, 1998).). When sequencing the Control Region of mitochondrial DNA, it is useful to consider the different possibilities of gene arrangement for primer selection in flanking regions, especially in birds.

The CR is the only large non-coding region of the mitochondria, ranging in size from about 1000 to 1760bp (in bird species) and is responsible for transcription and replication of the mitochondrial genome (Kvist, 2000, Ritchie & Lambert, 2000). It is one of the most rapidly evolving parts of the mitochondrial genome with a rate of 10-20 times faster than average rates for most other mtDNA coding sequences and thus, it is one of the most commonly used markers for phylogenies of closely related species with high resolution for population analysis (Desjardins & Morais, 1990 & Ruokonen & Kvist, 2002) and biogeographic studies (Tarr, 1995 & Baker *et al.*, 2001).

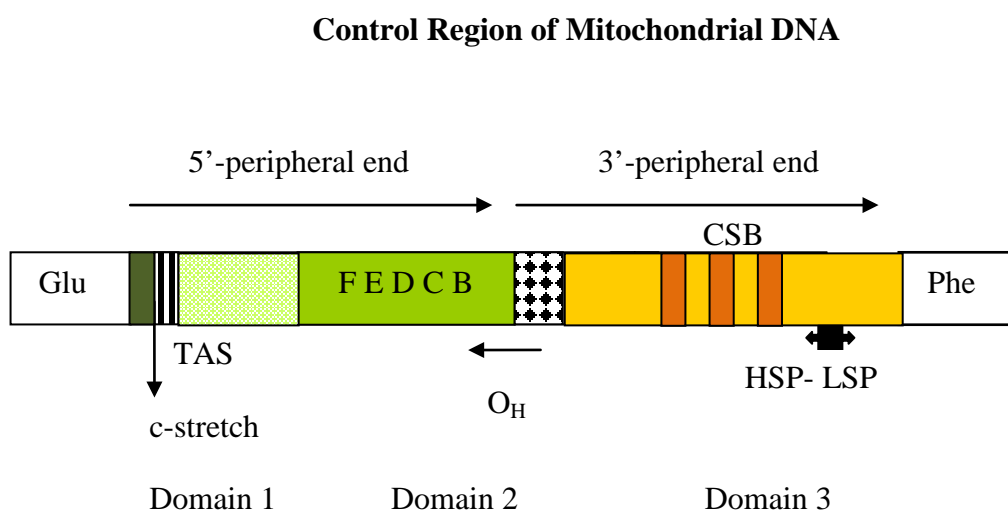


Figure 1.5b Simplified gene map of the Control Region, reproduced from Ruokonen & Kvist, (2002).

Most of the variability within the control region is found in domains 1 and 3 (Ruokonen & Kvist, 2002, Roques, et al., 2004). Repeat DNA sequences (SSR's) have also been found in the Control Region (CR) of many species, including Penguins and range in size from 3bp to 777bp (Ritchie & Lambert, 2000). The highly conserved regions of the CR include the C-stretch with a TAS termination sequence in the first domain at the 5' end, followed by the F-E-D-C-B box in the second domain (Ritchie & Lambert, 2000,

Ruokonen & Kvist, 2002). At the 3' peripheral end, resides the origin of heavy strand replication (O_H), the conserved sequence blocks (CSB's) and the heavy and light strand transcriptional promoter (HSP-LSP) sites (Ritchie & Lambert, 2000).

Cytochrome b (*cytb*) is the most widely used gene for phylogenetic studies because it is easily compared with other studies (universal metric), is the best choice for resolving relatively recent evolutionary history and for the relative ease of analysis (Kvist, 2000, Sherwin, *et al.*, 2000, Christidis, *et al.*, 1996). Although the control region is considered the fastest evolving part of the mitochondria due to the lack of coding constraints (Roques, *et al.*, 2004, Tarr, 1995), other studies show that *cytb* transitions occur at approximately the same rate or faster (Ruokonen & Kvist, 2002). The molecular structure of *cytb* is made up of 8 conserved trans-membrane helices (A-H), where most of the variable positions are located and connected by intra or extra membrane domains (Kvist, 2000). These transmembrane helices can generate sufficient genetic variability so that differential haplotypes can be detected within and between populations in relatively short evolutionary time spans and conserved enough for clarifying deeper phylogenetic relationships (Kvist, 2000).

Prior to the sequencing of the mitochondrial DNA (mtDNA), it is recommended that a pure isolate of mtDNA is prepared to eliminate any interfering effects of mitochondrial pseudo genes (Bensasson, *et al.*, 2001). Nuclear copies of mtDNA (pseudogenes or numpts) are transferred from the mitochondria to the nucleus via transposition events and are considered relics of ancient mtDNA (Bensasson, *et al.*, 2001). Evidence of nuclear copy mtDNA contamination produces DNA sequence ambiguities, PCR ghost bands and unexpected phylogenetic placements (Sorrenson *et al.*, 1998). Pseudo genes

have been found in numerous animal studies including birds (Pereira, *et al.*, 2004) and have been used to infer ancestral states (Bensasson, *et al.*, 2001 & Sorrenson *et al.*, 1998). Prior to amplification it is possible to separate nuclear from mtDNA products by using a cloning procedure, designing new primers targeted to regions where ambiguities reside, using tissue that is rich in mtDNA (muscle) and by physically separating mtDNA from other cellular components by differential centrifugation (Bensasson, *et al.*, 2001, Palumbi, 1996 & Sorrenson *et al.*, 1998).

Prior to the analysis of mitochondrial DNA sequences, tests for the neutrality and recombination events are conducted to ensure the assumption of evolutionary models are not being violated. A neutrality test is based on the neutral hypothesis, whereby it compares the number of synonymous and non-synonymous (replacement) variation of DNA within and between species. Under neutrality, the ratio of replacement to synonymous substitutions between species should be the same as the ratio of replacement to synonymous polymorphisms within species (Rozas, *et al.*, 2003).

Although a rare occurrence in animals, there is undisputable evidence for recombination and rearrangements of the mitochondrial genome (Abbott, *et al.*, 2005, Rozas, *et al.*, 2003). The presence of homologous DNA recombination in mitochondria is thought to function primarily as part of the replication and repair system (Rogers, *et al.*, 2004).

When recombination does occur, it changes the patterns of descent, generating some individuals which contain more than one mtDNA haplotype (heteroplasmic) (Ballard, *et al.*, 2004). Heteroplasmy is detected by the presence of two nucleotides at a single site with a frequency that has been found to remain stable over time in the same individuals (Butler, 2005). It is also thought to be inherited from both parents (paternal leakage) (Kvist, *et al.*, 2003) and seems to occur at higher rates in hybrid individuals (Ballard, *et*

al., 2004). It is important to determine whether a recombination event has altered the mtDNA of an individual because the resulting phylogeny will differ from the true underlying history of a species (Rokas, et al., 2003).

Identifying mitochondrial haplotypes is done by grouping samples possessing the same polymorphic nucleotides (Butler, 2005). Phylogenies estimated from mitochondrial haplotypes have been useful for testing hypothesis about historical biogeography at geographic scales where migration rates are low (Moritz & Hillis, 1996). Observed distributions of pair wise differences are assumed to be influenced by population expansions, genetic drift and mutations (Zischler, 1999). Generally, a unimodal distribution of pairwise differences is associated with a scenario of population expansion, whereas multimodal distributions can be associated with populations that have been of constant size over a longer period of time (Zischler, 1999).

Inferring a Phylogeny is an estimation procedure by which a “best estimate” of an evolutionary history, based on incomplete information contained in the data (Swofford, *et al*, 1996). Traditional methods of phylogeny reconstruction pose a number of difficulties for resolving relationships among genes at the population level as they require large numbers of variable characters, which are uncommon in population level studies (Clement, *et al.*, 2000). However coalescence is based on the Neutral Theory and proposes that current sequences in a population and can be traced back through time, to a point (root of the tree) at which they coalesce to a single individual sequence (Frankham, 2002). The probability of parsimony is calculated by comparisons of DNA pairwise differences, until the probability exceeds 0.95 (Clement, 2000). By applying coalescent theory to estimate the expected distribution, it is possible to distinguish

among different scenarios of population history (Harvey & Steers, 1999). Distance based analysis have an advantage at these levels because the relatively small genetic distances are not super imposed with nucleotide substitution models (Avice, 2000). The theory also predicts that the most ancestral haplotypes of a population will be the most frequent sequences found within populations (Clement, *et al.*, 2000).

Molecular information can also be constructed into gene trees which represent genetic distances of species (Avice, 1994). The nodes of gene trees represent taxonomic units and branches are pathways connecting the nodes (Avice, 1994). The Neighbour-Joining Method (Saitou & Nei, 1987) is related to the traditional cluster analysis method, but it has the advantage of not assuming that all lineages have diverged by equal amounts, rather it is additive (Swafford *et al.*, 1996). The raw data is provided as a distance matrix and the tree is constructed by linking the least distant pair of nodes by a common ancestral node (Swafford *et al.*, 1996). By using this process the gene tree is algorithmically constructed and pruned until a definition of the preferred tree is made into a single statement.

1.6 Ecological Niche, Climate Change and Population Viability

Although Darwin and Wallace founded the concepts of the struggle for existence and genetic variation, it was Ford (1964) who first published the term Ecological Genetics which specifically deals with the adjustments and adaptations of wild populations to their environment (Lowe, et. al., 2004). This apparent genetic variation and its relationship to the environment was supported by many animal case studies including the Banded Snail *Cepaea nemoralis* (Cain & Shepphard, 1950, 1954) and led to Ford's view of ecological fieldwork and laboratory genetics as being two inseparable investigative methods (Lowe, et. al., 2004). Understanding the patterns of gene flow and local adaptation requires a detailed knowledge of how landscape characteristics and ecological niches structure populations (Crandall, et al., 2000, Manel, et al., 2003).

Adaptive genetic variation is so important to species persistence, that the interpretation of patterns of gene diversity should be investigated within the context of ecological variables (Kohn, et al., 2006 & Purugganan, et al., 2003). Adaptation to local environments has important implications in conservation as translocations are likely to be more successful when populations or individuals are moved to similar rather than different environments (Frankham, et al., 2002). When conserving wild species, the aim is to target the gene regions with the greatest significance to population viability such as those under strong and consistent Darwinian selection and whose variation is most important for adaptation (Sherwin, et al., 2000 & Kohn, 2006). Therefore because of the link between genetic and ecological processes, they need to be studied together (Whelan, et al., 2000). Ecological exchangeability allows for individuals to move between populations which occupy the same ecological niche or selective regime (Crandall et al., 2000). Crandall et al., (2000), has defined management units within

species, based on genetic and ecological exchangeability. This method formulates categories of population distinctiveness based on rejection (-) or failure to reject (+) the null hypothesis of ecological and genetic exchangeability for both recent and historical time frames (Frankham, et al., 2002).

Understanding what constitutes habitat for a species is critical given that habitat loss is considered to be the most important factor that has led to the decline of many extinct, threatened and vulnerable species (Burgman & Lindenmayer, 1998). Habitat selection occurs at the evolutionary level and at the local level (Heglund, 2002). At the local level (or fundamental niche), species environment models are based on the assumption that an individual selects a general location according to certain landscape, topographical feature (Heglund, 2002) or climatic variable (Austin, 2002). Methods of for identifying habitat are based on detecting associations between environmental attributes and the presence or absence of species (Lindenmayer & Burgman, 2005). By combining data from several habitat characteristics that are important to species survival with occurrence or abundance of species, a multiple regression can then be used to rank each environmental factor according to its importance for a species (Akçakaya, *et al.*, 1995). This results in a function that links habitat characteristics to a Habitat Suitability Index (HIS) function (Akçakaya, *et al.*, 1995) and is a means of assessing habitat quality (Van Horne, 2002).

Another approach to habitat modelling is to identify ecological spatial patterns with a Multivariate Analysis method (Manel, *et al.*, 2003). However, some of the problems encountered in the analysis of multivariate ecological data include misbehaving data, complex experimental designs, scale of sampling or spatial and temporal variability

(Anderson, 2002). These factors have the potential to violate the basic assumptions of statistical inference and as a consequence, they can be responsible for erroneous conclusions (Anderson, 2002). However, a Distance Based Redundancy Analysis (db-RDA) can improve on this method as it does not rely on assumptions of multivariate normality (Legendre & Anderson, 1999). Rather, it's based on a matrix of distances (including non euclidean) or dissimilarities, computes all the principal coordinates and then uses redundancy analysis, to analyse the relationship between the principal coordinates and the explanatory variables (Legendre & Gallagher, 2001). Variables thought to be related to the most important determinants of species composition as well as those which are easy and inexpensive to measure, can be surprisingly informative and should be tested (Palmer, 2007).

To generate realistic information requires knowledge of how species respond to their environment and of environmental processes (Austin, 2002). No consensus yet exists on the best choice of spatial modelling as all methods are fraught with difficulties (Austin, 2002). Since vegetation is dynamic and changes over time in response to seasons and disturbances, a single time of observation may not reflect its true condition (Wallace, *et al.*, 2006). However, time series land satellite imagery can provide information on changes in vegetation and can be used to provide vital information for conservation and natural resource management questions (Wallace, *et al.*, 2006). Time series land satellite imagery can detect tree declines within a range of dates and locations and can be used to demonstrate the dynamics of crown cover by changes of a spectral image index called the Projected Foliage Cover (PFC) (Zdunic, *et al.*, 2012). This is the percentage of the field site occupied by the vertical projection of foliage and is the product of both tree crown cover and crown density estimates (Bland, *et al.*,

2006). The percentage foliage cover can be collected over time periods and can be incorporated with other species or environmental variables to allow for the dynamics of change in woodlands to be observed.

Quantitative studies as well as recent modelling studies are consistent in showing that species composition varies continuously along environmental gradients and that each species shows individualistic distribution patterns in relation to environmental variables such as climate and local variables such as topographic position (Austin, 2002). To model the effect of multiple variables simultaneously, rather than using additive models with limited range or regression models that could predict values below zero or above 1, the probabilities are better carried out on a transformed scale using logistic regression analysis (Daalgard, 2002). Logistic Regression analysis belongs to the class of Generalised Linear Models (GLM) models which are basically characterised by their response distribution and link function which transfers the mean value to a scale in which the relation to background variables is described as linear/additive (Daalgard, 2002). Any GLM model can be used with the same algorithm which allows the user the freedom to define their own models by defining suitable link functions.

Population Viability Analysis utilises qualitative methods that link habitat variables using functions created subjectively with ecological knowledge and then using explicit statistical models to link the occurrence of a species with habitat parameters (Lindenmayer & Burgman, 2005). As the quantitative assessment of risk for a species or population is based on detailed population models (Lande, et al., 2003), the accuracy of future trajectories of population size is dependent on the careful construction and parameterisation of these models (Beissinger, 2002). Also because of the difficulties of

understanding the causes of population change and modelling stochastic events, PVA's are not likely to predict accurately the patterns of population increase, stasis or decrease (Van Horne, 2002). A sensitivity analysis of population models is critical in deciding which aspects of life history or ecology require additional studies and to improve model accuracy or to assist in decisions of which management actions are likely to be the most effective in improving mean population growth rate and reducing extinction risk (Lande, et al., 2003).

Population demography is an approximate expression of a host of factors that influence individual fitness and population viability, such as habitat quality, habitat distribution, food quality, weather conditions and successful breeding (Marcot, 2006). Often ecologists count animals, assume a census and derive parameters of interest such as abundance and survival, directly from these counts (White, *et al.*, 2002). As many animals are not easily observed in the wild because of their size, preferred habitat, behaviour or other aspects of their ecology, population estimates can be made using captured animals that are marked and released (Williams, *et al.*, 2002). The advantage of the capture recapture method is that the accuracy does not depend on an assessment of the amount of habitat, but the disadvantage is that the accuracy does depend on capturing a large proportion of the population (Southwood & Henderson, 2000). A basic prerequisite for this method is finding an appropriate technique for marking animals and releasing them unharmed and unaffected back into the wild so that they can be recognised on recapture (Southwood & Henderson, 2000).

Various assumptions underlie all methods of capture recapture analysis such as parsimony, which is the use of the smallest possible number of parameters to model a

situation (Southwood & Henderson, 2000). The maximum likelihood procedure is used to estimate parameters that maximise the probability of the observed data and requires knowledge of the underlying distribution of a random sample (Williams, *et al.*, 2002). The goodness of fit procedure uses sample data to investigate the mathematical structure of a distribution rather than specific values for its parameters (Williams, *et al.*, 2002). As open populations change under some combination of birth, death, immigration and emigration, it is necessary to sample such populations at least three times (Southwood & Henderson, 2000).

The purpose of this investigation was to explore the interaction of declining avifauna and the quality of habitat under changing environmental conditions. Based on a multi-disciplinary approach to the analysis, a combination of landscape genetics, climate and habitat modelling and a demographic study using species capture- recapture data was employed. The study area is situated within a global Biodiversity Hotspot and is of high priority for conservation (WWF, 2014). If the region continues to experience higher temperatures and declining rainfall patterns as predicted (CSIRO, 2005) it is highly likely this will increase the stress already imposed on the system and in effect drive many more declining species towards extinction. Therefore it is particularly relevant and urgent to find critical elements that species are most sensitive to, in order to facilitate species survival.

1.7 Study Species

The Rufous Treecreeper (*Climacteris rufa*), is a small songbird that forages over trunks and main branches of both standing and fallen trees and eat ants as a main source of food (Schodde and Mason, 1999). In Western Australia, the Rufous Treecreeper occurs in the primeval forests of the southwest region in jarrah, karri, yarri, bullich, wandoo forest and associated heath, but not in dieback (*Phytophthora cinnamomi*) affected jarrah (Abbot, 1999). In agricultural lands, the critical patch size for the Rufous Treecreeper given for a 10% chance of occurrence is 10 ha (Brooker, *et al.*, 2001). Treecreepers hold their territory permanently (Schodde & Mason, 1999), breed in spring; laying 2-3 eggs in tree hollows (Saunders & Ingram, 1995), have an estimated life expectancy of 8 years and a maximum movement ability of 12 km (BTO, 2014 and ABBBS, 2014).



Figure 1.7a Rufous Treecreeper

Rose (1993), estimated that the Rufous Treecreeper requires a minimum size nesting hollow of 20 cm and trees with hollows of this size are between 60 and 150 years old, having a structural complexity of old growth woodland (Luck, 2002). This species is an uncommon resident in the remaining woodlands throughout the Wheatbelt, where it is declining in range and abundance (Saunders & Ingram, 1995) and has become extinct in the Swan Coastal plain (Abbot, 1999).

The Yellow-plumed honeyeater (*Lichenostomus ornatus*), is an arboreal songbird that have tongues evolved especially for feeding on nectar and pollen (Schodde & Mason, 1999). The yellow plumed honeyeater is an uncommon resident in the south western woodlands and mallee and a rare vagrant in the extensively cleared districts of the central wheatbelt (Saunders & Ingram, 1995). According to Seventy (1997), as a consequence of extensive clearing this species has penetrated the jarrah and wandoo forest, preferring areas where it is less tall and more open (Abbott, 1999). This species feeds on nectar, flowers as well as insects and breeds in spring, nesting 2-3 eggs in a low tree (Saunders & Ingram, 1995). It is also parasitized by the Pallid Cuckoo and Shining Bronze Cuckoo (Storr, 1991). The Yellow-plumed Honeyeater has an estimated life expectancy of just over 9 years and a maximum movement ability of 555 km (ABBBS, 2014).



Figure 1.7b Yellow-plumed honeyeater

The Western Yellow Robin (*Eopsaltria griseogularis*, Race: *griseogularis*) lives in the primeval forests of the southwest region including jarrah (but not in dieback affected jarrah), wandoo, powder bark and associated heath (Abbott, 1999). Ford (1963), observed geographical variation in the coloration of the rump (yellow to green), of robins belonging to the east and western parts of the southwest forest regions (Abbott, 1999). This species is rare or extinct on the swan coastal plain (Storr, 1991) and an uncommon resident in woodland, mallee and shrub land throughout the wheatbelt (Saunders & Ingram, 1995). In the West Australian wheatbelt, Lambeck (1997) found the western yellow robins occur in remnant vegetation larger than 20 ha and less than 2 km from other patches of native vegetation (Abbott, 1999) and have been known to move locally (up to 7.5 km), provided the presence of well vegetated road verges, used as corridors between habitats (Saunders & Ingram, 1995). The Western Yellow Robin has an estimated life expectancy of just over 8 years and a maximum movement ability of 6 km (ABBBS, 2014).



Figure 1.7c Western Yellow Robin

The following investigations were conducted on each target species. Initially, it was intended to use all three species for complete analysis but for various reasons this was not possible. For example, only 15 individual Western Yellow Robins were captured in the Dryandra woodlands with 1 re-capture and therefore no survival estimate could be estimated for this species. Also after some experimentation, no published microsatellite primers proved to be successful in amplifying DNA from the Yellow-plumed Honeyeaters. Despite these difficulties, as much data as possible was collected. The Rufous Treecreeper was the most complete analysis.

Table 1.7 Types of analysis conducted on each target species.

Analysis	Rufous Treecreeper	Yellow-plumed Honeyeater	Western Yellow Robin
Microsatellite DNA	Dryandra and Regional sites examined	Unsuccessful	Unsuccessful
Mitochondrial DNA Cytochrome b	Dryandra and Regional sites examined	Sequence only. No population analysis	Not completed
Mitochondrial DNA Control Region	Unsuccessful	Unsuccessful	Completed
Linear Regression of Tree Foliage Cover, Rainfall and Bird Captures	Completed	Completed	Not enough samples
Survival Estimates	Completed	Completed	Not enough samples

For information on Diversity of species captured at each sampling site, please refer to Appendix 11, Species Catch List for Dryandra (2003-2010). Also, for number of individuals caught at each sampling site in the capture-recapture study please refer to Results (Tables 3.5.5 a & 3.3.5b).

1.8 Study Area

This investigation was conducted in the wheatbelt region of Western Australia which is Australia's only global Biodiversity Hotspot (WWF, 2014) and where 97% of woodlands have been cleared for agriculture (Saunders, 1989). This has impacted the size and quality of available habitat, created vast distances between habitat patches and caused geographical isolation between populations (Hobbs, 2002, Lindenmayer & Peakall, 2000). Although the avifauna in this investigation are known to exist across five different types of remnant woodland communities in the south west (Barrett, *et al.*, 2003), this study was conducted within the Wandoo Vegetation zone only. With the predicted rapid decrease in rainfall in this area (CSIRO, 2005) and given the stress already imposed on the habitat system, species that have a restricted range or are already confined to small areas are destined towards the possibility of extinction (AWA, 2002).

Feather samples for DNA analysis were collected from 20 different sites spanning approximately 100kms (Fig. 1.8a). Please refer to Appendix 12 for Location Coordinates for all sampling sites. This included 12 sites of the Dryandra Woodlands and 8 regional sites. Sampling sites were spatially orientated in order to compare relatively close populations to more distant populations and to investigate gene flow within and between geographically separated populations. Dispersal distances within a continuous habitat and across fragmented habitat were also investigated. This information aims to provide details of species requirements to facilitate vegetation corridor planning that supports the movement of species throughout the region while conserving genetic diversity.

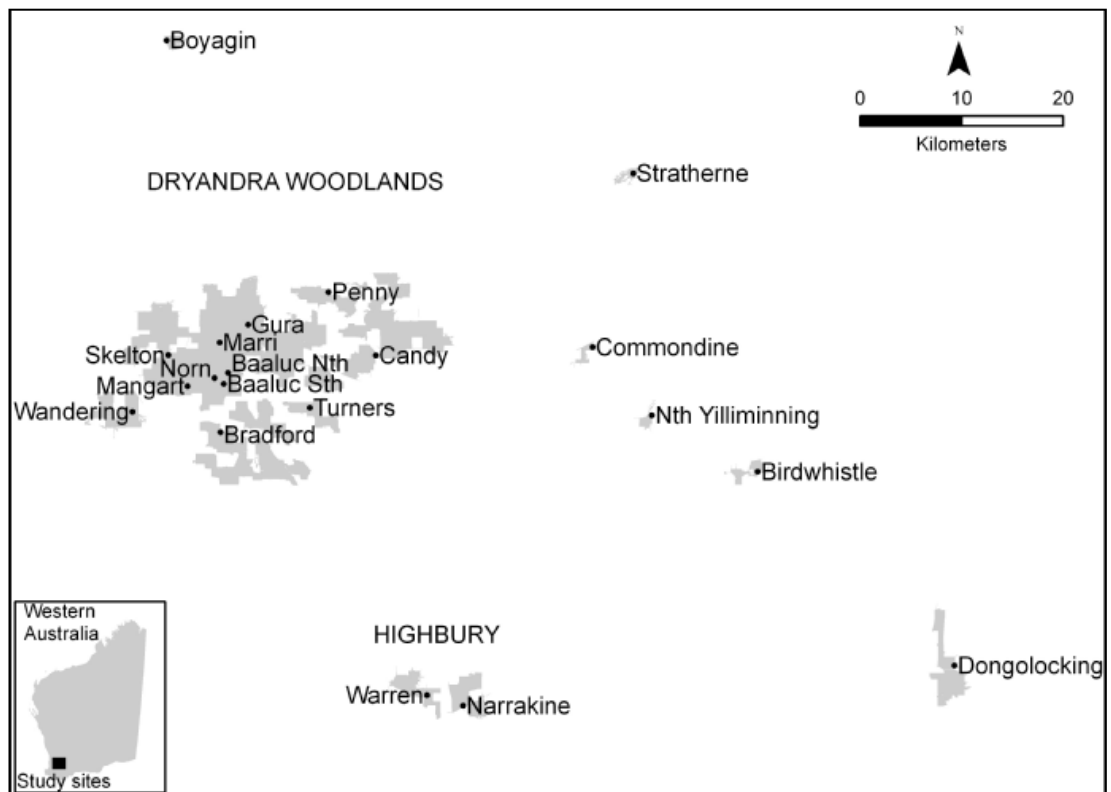


Fig. 1.8a Map of the Dryandra Woodlands and other genetic analysis study sites in grey.

The Dryandra woodlands is a collection of 10 blocks (including Highbury), with a combined area of 28, 066 ha and contains some of the largest native vegetation remnants in the central western wheatbelt (CALM, *et al.*, 1995). Previous logging of *Eucalyptus wandoo* (*E. Wandoo*, Blakely) and Powder bark wandoo (*E. Accendens*, Fitzgerald) for saw logs, fencing posts and firewood eventually forced the closure of a saw mill at Dryandra in 1977, as areas of Dryandra could no longer sustain Wandoo production (CALM, *et al.*, 1995). Despite these activities, the Dryandra woodland contains 816 identified native plant species (CALM, *et al.*, 1995) within 12 different vegetation communities (Coates, 1993) and provides habitat for a diverse assemblage of fauna that includes 24 mammal species (5 Threatened) and 66 species of birds (2 species are Threatened and 2 are Specially Protected Fauna) (NWC, 1991).

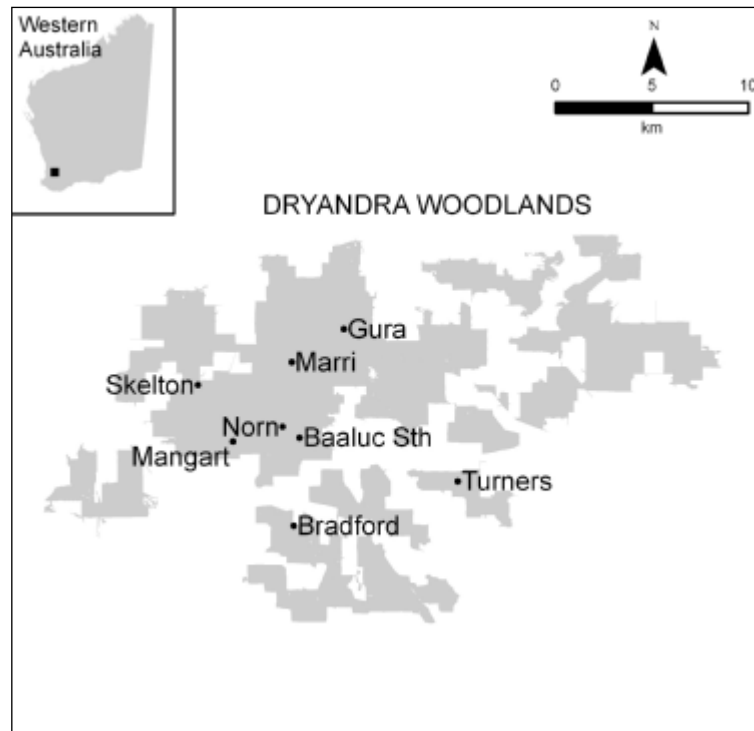


Fig. 1.8b Map of the Dryandra Woodlands showing 8 data collection points for the analysis of foliage cover, capture-recapture rates and rainfall.

Sites for avifauna sampling were selected and based on observed vegetation preferences such as mature wandoo forest and tracking through the bush listening for bird calls. Access to potential sites was also a consideration as some road tracks were unsafe or where fenced farm land made it impossible to pass through. Also close distances to main roads where the noise and dust created by passing traffic may have disturbed the birds, was avoided. Avifaunal demographic data was collected by a mist net capture recapture study and percentage tree foliage cover calculated from land satellite imagery was collected from 8 sites within the Dryandra woodlands (Figure 1.8b) and obtained from the Department of the Environment and Conservation.

Site 1 Norn Road

Location: E 492310.38 N 6369954.84

This site consists of sparse, mature wandoo woodland with patchy regrowth of young wandoo (*Eucalyptus wandoo*). The under storey consists of *Gastrolobium* shrubs 0.5-1m high and the occasional group of Grass Tress (*Xanthorrea* sp.). Adjacent to this site is a large area of shrub mallee (*E. drummondii*). Amongst the fallen, deadwood Wandoo branches, the groundcover consists of a variety of grasses and flowering annuals such as *Conostylis setigera*, *Stackhousia monogynai* and everlasting daisies of the Asteraceae family.



Figure 1.8(c) Norn Road.

Site 2 Mangart Road

Location : E 489648.32 N 6369157.94

This site resides within a low altitude water course area. It contains sparse, mature wandoo trees with mid-dense jam wattle (*Accacia acuminata*) understorey.

Groundcover consists of annual grasses with patches of *Gastrolobium* species, interspersed with *Hybertia* species. The jam wattle branches are covered in lichens and there is an abundance of dead wood and branches on the ground.



Figure 1.8(d) Mangart Road.

Site 3 Skelton Block

Location: E 487756.74 N 6372207.43

Compared to all other sites, this site is the most open. It consists of sparse mature wandoo woodland with a *Gastrolobium* sp. understorey. This site lies within 200 meters of a field crop and is part of a flood plain that leads to a water drainage area. A large patch of rock sheoak (*Allocasurina huegeliana*) is situated nearby on an elevated embankment.



Figure 1.8(e) Skelton Block.

Site 4 Bradford Block

Location: E 492901.91 N 6364631.06

This site shows evidence of previous logging and is predominantly mature wandoo but has a mixed, patchy understory of sheoak (*Allocasurina fraseriana*) on down ward slope areas and parrot bush (*Dryandra sessilis*) on the gravelly mounds. There is also a natural patch of brown mallet (*Eucalyptus astringens*) at the edge of the plot on a rising slope.



Figure 1.8(f) Bradford Block.

Site 5 Gura Road

Location: E 495605.46 N 6375194.82

Gura Road contains a mature and open wandoo woodland with a sparse understorey of jam wattle and mid-dense *Gastrolobium* ground cover. This site is adjacent to a brown mallet (*Eucalyptus astringens*) plantation; which, when in flower many Yellow-plumed honeyeaters could be seen feeding in the canopy.



Figure 1.8(g) Gura Road.

Site 6 Marri Road

Location: E 492799.34 N 6373431.8

This site lies central to the largest remnant in the Dryandra complex and is relatively even in elevation. It is mature wandoo woodland with sparsely distributed marri (*E. calophylla*) and an occasional jam wattle. There is an abundance of fallen deadwood timber on the ground and the understorey is mainly *Gastrolobium* sp. in small patches 0.5m high to continuous and dense thickets of 1.2m high in adjacent areas.



Figure 1.8(h) Marri Road.

Site 7 Baaluc Road (south)

Location: E 493213.14 N 6369379.66

This site is characterised by a downward sloping valley with mature stands of wandoo. There is evidence of previous logging activities in the area. There is a very sparse distribution of Marri on one side of the site and parrot bush (*Dryandra sessilis*) at the back, on the top of a breakaway.



Figure 1.8(i) Baaluc Road (south).

Site 8 Turners Block (Borranning)

Location: E 501718.4 N 6367029.21

This site resides on a small remnant that is completely isolated from the main Dryandra block and has a slight sloping aspect. It shows evidence of previous logging activity and contains mature wandoo, with patches of rock sheoak (*Allocasurina huegeliana*), powder bark wandoo (*E. astringens*), Marri, Shrub Mallee (*E. drummondii*) and mixed Kwongan heath. Although site is mapped as Turners Block, analysis results refer to this site as Boranning.



Figure 1.8(j) Turners Block (Borranning)

CHAPTER 2

METHODS

2.1 Sample and Data Collection

Demographic data and feather samples for DNA analysis were collected during 5 field trips during 2003, 2004 and 2007. Within the Dryandra woodlands each of the 8 (1 hectare) sampling sites, was visited twice a day from 6-11am and 4-7pm. Each day a different site was visited (morning/afternoon) until all 8 sites (map Figure 1.8b) were visited and the sampling round completed. All other 12 regional sites (map Figure 1.8a) were visited on a single day to collect genetic samples. The target species were captured using 12 mist nets (9x12m, 3x9m). Each individual bird had an identification band carefully attached to its leg and a sample of feathers was collected. Band numbers, bird measurements and weights was recorded on specimen sheets and sent to the ABBBS (Australian Bat and Bird Banding Scheme).

The non-invasive genetic sampling technique involved taking about 20 feathers from the breast area of each individual and placing them into a sealed envelope. Each envelope is labelled with each individual's identification band number, location and date of capture. The feather samples were stored in dry, cool conditions (Taberlet, et al., 1999) until the DNA was extracted. Data from a total of 162 individual Rufous Treecreepers was collected, 133 from Dryandra (see Table 3.5.5a) and 33 from Regional sites. There were 216 Yellow-plumed Honeyeaters sampled from Dryandra (see Table 3.5.5b) and 38 from regional sites. There were 15 individual Western Yellow Robins from Dryandra and 13 from regional sites. DNA from all three species was amplified but was only partially successful, due to financial, time and technical

constraints. The genetic data generated from the Rufous Treecreeper samples were the most successful and include microsatellite DNA (68 individuals) and part of the cytochrome b gene (mtDNA) sequences (25 individuals). However, the Control Region of the Western Yellow Robin was obtained as well as part of *cytb* gene from the Yellow-plumed Honeyeater. These sequences have not been previously published.

Land satellite imagery information (spectral index images) was used to create Projected Foliage Indices (% foliage cover) of the *E.wandoo* trees and was collected from 8 x 1 ha georeferenced sampling sites within the Dryandra woodlands (see Figure 3.5.3). This satellite imagery information of foliage cover in Dryandra has been collected for over 20 years and was obtained from the Department of Environment and Conservation (Behn, 2011). Annual rainfall measurements from the Wandering Weather Station, situated 20 km from the Dryandra woodlands was collected from the Australian Government Bureau of Meteorology (BOM, 2011) and used as an function of climate change (see Figure 3.5.4). .

Bird capture-recapture data was used to explore the impact declining rainfall patterns on the habitat quality and population viability of these populations. Rufous Treecreeper and Yellow-plumed Honeyeater data was used to calculate survival estimates, but the rare occurrence of the Western Yellow Robin, whose capture total was only 15 individuals with only 1 recapture, could not be used to calculate a survival estimate.

2.2 Genotyping and DNA Sequencing

Two different types of DNA markers were used for the genetic analysis. Non-coding, microsatellite DNA was used to detect recent patterns of gene flow and population spatial structure. Mitochondrial DNA was used to identify the spatial distribution of genetic differentiation across the region and to infer a historical range of expansion. Investigating these evolutionary processes is vital for conserving genetic diversity over the long term.

Extraction and quantification of DNA

The QIAamp[®] DNA Micro Kit available by QIAGEN was used to extract DNA from feather samples according to instructions. A fluorometer (Hoefer Quant 200) was used to measure the concentration of extracted DNA from each individual. The DNA was stored at -20 °C (working samples) and -70 °C (stored samples).

2.2.1 Microsatellite DNA

Microsatellite DNA from Rufous Treecreeper (RTC) individuals were amplified using 8 locus specific primers, originally designed for the Brown Treecreeper, *Climacteris picumnus*. The microsatellites were detected using a DNA 3730 capillary sequencer and their fragment lengths calculated (scored) using GeneMapper[®] software by Applied Biosystems (ABI).

Primer Development

Microsatellite amplification experiments were conducted on the Rufous Treecreeper (RTC), Yellow-plumed Honeyeater and Western Yellow Robins, with microsatellite primers specifically designed for Australian passerines and primers that amplified

across-species (see Appendix 1). However, locus specific primers designed for the Brown Treecreeper by Doer, 2004 (unpublished), were used for the final amplification of microsatellite DNA in the RTC. DNA sequencing of these microsatellites (via a cloning procedure) was conducted to test if the same locus was being amplified in both species and to generate sequence information for designing new, more specific primers for the RTC. Amplification of PCR products using primers (with a universal fluorescent tag, attached to an M13(-21) tail, as described by (Schuelke, 2000). This method proved to be unsuccessful, therefore, the BTC primers were synthesised with a standard labelled fluorescent tag. These were NED (black), PET (red), 6FAM (blue) and VIC (green).

PCR reagents and reaction conditions

A 100uL PCR (Polymerase Chain Reaction) mix was prepared with 50ng DNA, 5p mole of each primer, 10 mM (of each) dNTP, a X1 PCR buffer and 1U HotStarTaq[®] DNA polymerase. A gradient of magnesium chloride concentration (20-25mM) and annealing temperatures of 45°C to 60°C was used to optimise PCR reaction conditions.

Separation of products on an agarose gel

A 1% DNA agarose gel with 1% TAE buffer was prepared and 10uL of PCR product was loaded into each well to separate products. A 1000 bp DNA ladder with 100bp increments was used to scale the PCR products. The DNA agarose gel was run at 80V for 40 minutes (see Appendix 2 for photos).

PCR Clean-up

With the remaining 90uL PCR products, a sodium acetate method was used to remove excess reagents that may interfere with the subsequent cloning processes.

Cloning procedure

This procedure was undertaken using the Promega pGEM[®]-T Easy Vector Systems kit with JM109 competent cells. Ligation and Transformation of vector was carried out as to Manufacture's protocol. Eight tubes of transformation vectors, (each containing a particular microsatellite DNA) were inoculated onto LB/ampicillin/IPTG/X-Gal plates, using 3 different dilutions of 1uL, 20uL and 100uL per plate. These plates were incubated over night at 37°C and the next day transformant cells were selected by their white colour or antibiotic resistance. Cells that did not successfully ligate with Rufous Treecreeper DNA, were blue in colour.

Culture of clonal cells

A single white colony (transformant) that was isolated and regular in size and shape was selected, cultured in 5mL of LB broth and incubated at 37°C overnight. This selection was repeated for 6 individual colonies, for each different microsatellite DNA. After overnight incubation, each broth was checked for turbidity (bacterial growth) and any broths that had little bacterial growth were further incubated for another 7 hours.

Extraction of plasmid DNA

As the Rufous Treecreeper microsatellite DNA is incorporated into the bacterial plasmid DNA by the previous ligation/transformation procedure, an extraction of plasmid DNA from the bacteria is necessary. This procedure was carried out using a

QIAGEN plasmid extraction kit (QIAprep[®] Spin Miniprep Kit) and following the recommended protocol.

Sequencing of plasmid DNA

The sequencing was carried out on an ABI 3730 48 capillary machine. The protocol is based on recommendations made from the Big Dye Terminator v3.1 Cycle Sequencing protocol from ABI. A PCR sequencing reaction was performed using 10ng DNA extracted from plasmid DNA kit, 2uL Dye terminator mix, 1.6 pmoles of reverse primer and made up to 5uL volume with de ionised water.

Sequence Comparisons of Microsatellite DNA

The Rufous Treecreeper sequences produced were aligned using CLUSTALW (V1.83). Sequences of the Brown Treecreeper were found in Genbank (Accession Numbers AY894981-AY894988).

Modification of New Primers

Modified primers were then designed using the RTC cloned sequences and the Brown Treecreeper microsatellite sequences found in GENBANK (NCBI). Flanking the repeat motifs, sequence information was screened for improvement of the G:C ratios (sequences of 45-50% GC content in 18-22bp primer). Only some of original the primer sets were modified in order to improve primer binding to template DNA (see Appendix 3).

Final PCR Reaction

The modified microsatellite primers were optimised using QIAGEN HotStarTaq[®]. A 50uL PCR reaction was prepared according to instructions. Loci 3, 4, 6 and 8 were amplified using 1.5mM MgCl₂ and a 57°C annealing temperature. Locus 7 was amplified with 2.5mM MgCl₂ and a 45°C annealing temperature, locus 1 with 2.0 mM MgCl₂ at 58°C and Loci 5 and 2 primers were amplified using 2.0 mM MgCl₂ and a 59°C annealing temperature.

Separation of microsatellites on an agarose gel

A 1% DNA agarose gel with 1% TAE buffer was prepared and 10uL of PCR product was loaded into each well with a 1000 bp DNA ladder. The agarose gel was run at 90V for 30 minutes. This step is used as a final check for microsatellite products, before continuing with the fragment length analysis.

Fragment Length Analysis

The PCR product from each individual was prepared into a 96 well plate and sealed with a septum. Each well contains 2uL PCR product, 15uL Formamide and 0.1uL LIZ size standard dye. Fragment Analysis of each microsatellite allele, for each individual was conducted on a 3730 Capillary DNA Sequencer and Genemapper[®] software was used to call allele sizes.

2.2.2 Mitochondrial DNA

Mitochondrial primers were synthesised for amplification of the genomic DNA that included the control region (CR) and cytochrome b gene. Whole DNA extract was used to amplify these regions from the Rufous Treecreeper, the Yellow-plumed Honeyeater and the Western Yellow Robin.

2.2.2.1 Amplification and Sequencing of Control Region

The amplification of the control region of the mitochondria from the Western Yellow Robin was carried out with a selection of primers from Tarr, *et al* (1995) and Sorenson, *et al* (1999). See Appendix 1 for primer sequences.

PCR reagents and conditions

A 25uL PCR (Polymerase Chain Reaction) mix was prepared with 20-50ng DNA, 5p mole of each primer L16743 and H1248, 10 mM (of each) dNTP, a X1 PCR buffer and 1U HotStarTaq[®] DNA polymerase. A gradient of magnesium chloride concentration and annealing temperatures of 45°C to 60°C was used to optimise PCR reaction conditions. An optimum DNA concentration of 50ng/uL, annealing temperature of 59°C and 1.5mM MgCl₂ was found.

Separation of products on an agarose gel

A 1% DNA agarose gel with 1% TAE buffer was prepared and 10uL of PCR product was loaded into each well to separate products. A 1500 bp DNA ladder with 100bp increments was used to scale the PCR products. The DNA agarose gel was run at 90V for 30 minutes (see Appendix 2).

Purification of DNA

Purification of PCR product was conducted using a QIAGEN MinElute PCR Purification Kit. The basic protocol was followed except for when discarding flow-through, the flow through was passed through the column a second time, and then discarded. The reason for repeating this step was to maximise the quantity of DNA trapped inside the column rather than discarding it.

Quantification of Purified PCR Product

A fluorometer (Hoefer Quant 200), was used to measure the concentration of purified PCR product from each individual. A concentration of at least 20ng/uL was sufficient for the subsequent sequencing reaction. Dilutions of 10ng/uL were used for the sequencing reaction.

Preparation of DNA Sequencing Reaction Mix (1:4)

The reagents used for the sequencing reaction were 2uL Dye terminator mix, 1uL Reverse primer (3.5pmol), 2uL purified PCR product (10ng/uL DNA). The sequencing reaction was prepared on ice, in small Eppendorf tubes and when complete, it is stored in a freezer, covered in foil paper to protect the sequencing reaction mix from light.

PCR Sequencing Reaction Conditions

1. Hold on 96°C for 10 seconds. Wait until temperature is reached, then place samples in the PCR machine.
2. 25 cycles of 96°C for 10 secs. 59°C (*optimum annealing temperature*) for 5 seconds and 60°C for 4 minutes.
3. Hold on 14°C.

Ethanol Precipitation of Sequencing Reaction

1. To a 0.5mL tube, add 12.5uL 100% ethanol, 0.5uL of 3M sodium acetate (pH 5.2) and 0.5uL 125mM EDTA (disodium salt).
2. Add 5uL of the sequencing reaction into the 0.5mL tube containing the ethanol, sodium acetate and EDTA.
3. Mix by pipetting up and down and leave on ice for 20 minutes.
4. Microfuge at maximum speed for 30 minutes at room temperature.
5. Discard the supernatant, removing as much of the liquid as possible.
6. Rinse pellet by adding 125uL of 80% ethanol.
7. Microfuge on maximum speed for 5 minutes at room temperature.
8. Discard the supernatant, removing as much of the liquid as possible.
9. Dry the sample in the speed vac for 15 minutes. Check there is no trace of moisture left.

2.2.2.2 Amplification and Sequencing of Cytochrome b

Fifty mitochondrial cytochrome b gene sequences from within the same family group of species were selected from GENBANK (<http://www.ncbi.nlm.nih.gov>). The DNA sequence alignment editor BioEDIT software (Hall, 1997) was used to find highly conserved gene regions and allows a multiple sequence alignment, showing consensus sequences within coding regions of a transcribed gene. Specific primer sets were then designed from these highly conserved genes, which also flanked the Control Region. The intention of designing new primers was to increase specific binding and to have knowledge of the exact location primers initiate DNA replication (see Appendix 1 for primer sequences). With a set of primers which have a known location within a gene region, prediction of the expected length of the DNA product can then be calculated. Also, consideration also had to be given to particular gene orientation of the mitochondria, as at least 2 different orientations were possible (see Figure 1.5a).

PCR reagents and conditions

A 25uL PCR (Polymerase Chain Reaction) mix was prepared with 20-50ng DNA, 5p mole of each primer L436/12S and cytb/H739 (RTC), primer R496/R858 (YPH), 10 mM (of each) dNTP, a X1 PCR buffer and 1U HotStarTaq[®] DNA polymerase. With each primer tested, a gradient of magnesium chloride concentration and annealing temperatures of 45°C to 60°C was used to optimise PCR reaction conditions. An optimum DNA concentration of 50ng/uL, For RTC, a DNA concentration of 50ng/uL, annealing temperature of 55°C and 2.0mM MgCl₂ was found. For YPH, a DNA concentration of 20ng/uL, annealing temperature of 59°C and 2.0mM MgCl₂ was found.

Separation of products on an agarose gel

A 1% DNA agarose gel with 1% TAE buffer was prepared and 10uL of PCR product was loaded into each well to separate products. A 1500 bp DNA ladder with 100bp increments was used to scale the PCR products. The DNA agarose gel was run at 90V for 30 minutes. See Appendix 2 for gel photo.

Purification of DNA

Purification of PCR product was conducted using a QIAGEN MinElute PCR Purification Kit. The basic protocol was followed except for when discarding flow-through, the flow through was passed through the column a second time, and then discarded. The reason for repeating this step was to maximise the quantity of DNA trapped inside the column rather than discarding it.

Quantification of Purified PCR Product

A fluorometer (Hoefer Quant 200) was used to measure the concentration of purified PCR product from each individual. A concentration of at least 20ng/uL was sufficient for the subsequent sequencing reaction. Dilutions of 10ng/uL were used for the sequencing reaction.

Preparation of DNA Sequencing Reaction Mix (1:4)

The reagents used for the sequencing reaction were 2uL Dye terminator mix, 1uL Reverse primer (3.5pmol), 2uL purified PCR product (10ng/uL DNA). The sequencing reaction was prepared on ice in small Eppendorf tubes and when complete, stored in a freezer and covered in foil paper to protect the sequencing reaction mix from light.

PCR Sequencing Reaction Conditions

4. Hold on 96°C for 10 seconds. Wait until temp. is reached, then place samples in the PCR machine.
5. 25 cycles of 96°C for 10 secs., 55°C for RTC, 59°C for YPH (*optimum annealing temperature*) for 5 seconds and 60°C for 4 minutes.
6. Hold on 14°C.

Ethanol Precipitation of Sequencing Reaction

10. To a 0.5mL tube, add 12.5uL 100% ethanol, 0.5uL of 3M sodium acetate (pH 5.2) and 0.5uL 125mM EDTA (disodium salt).
11. Add 5uL of the sequencing reaction into the 0.5mL tube containing the ethanol, sodium acetate and EDTA.
12. Mix by pipetting up and down and leave on ice for 20 minutes.
13. Microfuge at maximum speed for 30 minutes at room temperature.
14. Discard the supernatant, removing as much of the liquid as possible.
15. Rinse pellet by adding 125uL of 80% ethanol.
16. Microfuge on maximum speed for 5 minutes at room temperature.
17. Discard the supernatant, removing as much of the liquid as possible.
18. Dry the sample in the speedvac for 15 minutes. Check there is no trace of moisture left.

2.3 Spatial Analysis of Microsatellite and Mitochondrial DNA

After the amplification of microsatellites for all RTC individuals was completed and scored, this data was then analysed using a selection of statistically rigorous software programs. Initially, a test for the neutrality of microsatellites was conducted to make sure the microsatellites were not under selection. Then tests under Hardy Weinberg Equilibrium were conducted to assess whether populations are in equilibrium with gene flow and genetic drift and to ensure the basic assumptions of genetic analysis methods were not being violated. Genotyping failures, null alleles and inbreeding coefficients were estimated. The spatial genetic structure was investigated by testing various relatedness and genetic distance measures, between individuals and different populations within a range of geographical distances. Genetic distances between populations were interpolated with geographic locations to understand how populations are distributed across the landscape as well as the population structure within short geographical distances. Kinship Coefficients and Genetic Distances were used to investigate dispersal patterns and neighbourhood structure by constructing Neighbour Joining trees. Also, a large scale (regional) genetic structure was found using a phylogenetic approach. Although many different programs were experimented with, only the results that best described the genetic structure and distribution were used in the final analysis.

Neutrality Test

The Rufous Treecreeper microsatellite data was tested for selection/neutrality using DETSEL (<http://www.univ-montp2.fr/~genetix/detsel.html>) software program.

Null Alleles Genotyping Errors and Inbreeding Coefficient

The detection of null alleles and inbreeding was carried out with a Bayesian approach INEST 2.0 (http://www.ukw.edu.pl/pracownicy/strona/igor_chybiki/), as it has better statistical properties than the maximum likelihood method.

Hardy Weinberg Equilibrium

A permutation test for heterozygosity excess was conducted with INEST 2.0 (http://www.ukw.edu.pl/pracownicy/strona/igor_chybiki/).

F-Statistics

Weir and Cockerham's (1984) methods of calculating Wright's F-statistics for measuring variation of individuals, within populations, between populations (Fit, Fis, Fst) was carried out with Tools For Genetic Population Analysis (TFGPA) v1.3 (Miller, 1999). GENEPOP (<http://genepop.curtin.edu.au/>) was used perform population differentiation.

Test for Bottlenecked Populations

The Bottleneck program written by Piry, Luikart & Cornuet, available from <http://www.ensam.inra.fr/URLB>, was used for detecting recent effective population size reductions from allele frequency data. The Sign Test, Standardized Differences Test and Wilcoxon Test for loci fitting the IAM & SMM (microsatellite Mutation Models) (Cornuet & Luikart , 1996).

Assignment Test

The assignment of individuals to population membership was conducted using the program STRUCTURE Version 2.0 (Pritchard, Stephens & Donnelly, 2000).

Analysis of Molecular Variance (AMOVA)

GenAlEx Version 6.0 was used for AMOVA. This program assesses how much genetic variation is distributed within and between populations. The program was written by Peakall & Smouse, (2006) and available from <http://www.anu.edu.au/BoZo/GenAlEx/>

Spatial Analysis

The following different spatial analysis techniques were carried out with RTC Microsatellite DNA using Alleles in Space (AIS) computer software (Miller, 2005). Maximum Difference Delaunay Triangulation (Miller, 2005), which generates an image of a geographical connectivity network between sites and their corresponding genetic dissimilarities, or genetic boundaries between individual pairs, a Mantel Test for the spatial correlation of genetic and geographic distances, a Spatial Autocorrelation of individuals among specific distance classes and a Landscape Interpolation of RTC microsatellite DNA, to conduct Landscape Interpolation of genetic distances.

Approximate Bayesian Computations (ABC) of Microsatellite DNA

DIYAC was available from <http://www1.montpellier.inra.fr/CBGP/diyabc/> and uses the ABC approach to make inference on past history of populations and was used to find a regional population structure.

Principle Component Analysis of Microsatellite DNA

Initially, Reynolds and Nei's (1972) genetic distances and two measures of co-ancestry were tested with PCA (Principal Component Analysis) and MDS (Multi-Dimensional Scaling) techniques. The Kinship Distance (KinDist) and the Molecular Coancestry Matrix (Kinsub) were generated from Molkin software (Gutierrez & Goyache 2004).

All methods were tested and compared. Eventually, Kinsub was selected as input data for a PCA, with PRIMER 5 v5.2 software. The interpolation of Principal Component scores was carried out with GIS ArcView Nearest Neighbour method.

Rufous Treecreeper Genetic Distance Trees from Microsatellite DNA

Nei's original (1972) and unbiased (1978) Distance and Identity Measures, Wright's (1978), modification of Roger's (1972) distance, were found using Tools For Population Genetic Analysis (TFPGA) V 1.3 (Miller, 1997) software. Reynolds Distance was found using Molkin v2.0 (Gutierrez & Goyache, 2004). Cavalli-Sforza & Edwards and Tomiuk & Loeschcke Genetic Distances and tree topology and Rodger's (1972) Identity were found using POPDIST software (Guldbrandtsen, *et al.*, 2000). Nei's Standard Genetic Distance was also tested using GenAlEx V.6.0 software (Peakall & Smouse, 2006) and Neighbour Joining Trees (NJ) were constructed using MEGA Version 4 (Tamura, *et al.*, 2007). The Rufous Treecreeper microsatellite data set was analysed by each method and the results were compared for statistical significance, including a bootstrapping procedure (when possible).

Rufous Treecreeper Kinship Tree from Microsatellite DNA

Kinship Coefficients were conducted at the individual level (pair-wise genetic distances) using SPAGeDI Version 1.2 (Hardy & Vekemans, 2002). Several methods of kinship measures were tested and compared, including Rousset Distance (2000), Queller & Goodnight (1989), Wang (2002) Li *et al.*, (1993) and Loiselle (1995) and Ritland (1996). Data from Loiselle (1995) and Ritland (1996) was selected to create Neighbour Joining (NJ) Trees with PHYLIP Version 3.66 (Felsenstein, 2004). These NJ

trees were sent to MEGA Version 3.2 (Kumar, Tamura & Nei, 2004) for export into CTree Version 1.00 (Archer & Robertson, 2007).

Preliminary testing of a vast selection of published primers for highly conserved regions of mitochondrial DNA was conducted before a region of relatively high variability was found. These variable sequences (haplotypes) were amplified in and then used to identify the spatial distribution of genetic differentiation across the region and to infer a historical range of expansion. Initially, mitochondrial DNA sequence analysis requires tests of recombination, selection and neutrality to avoid violating the assumptions of evolutionary models.

Mitochondrial Gene Order

DNA sequences generated from initial testing of primers (forward and reverse) from Cytochrome b, ND6, control region and 12S genes of the mitochondria, to find the specific gene order in the Rufous Treecreeper and the Yellow-plumed Honeyeater. Sizing of DNA fragments was determined by gel electrophoresis and sequencing of these fragments confirmed gene order.

Editing and Identification of Target DNA Sequences

Original chromatograms were viewed and ambiguous bases corrected using FinchTV version 1.4.0 was available from Geospiza, Inc.; Seattle, WA, USA, (<http://www.geospiza.com>). BioEDIT (Hall, 1999), was used to find and join the two segments of cytochrome b (*Cyt b*), and Control Region (CR). The control region sequence was blasted at GENBANK (NCBI) for identification and a complete sequence

of cytochrome b gene was sent to the conserved domain search (NCBI) for identification and verification of amplified sequences.

Tests for Neutrality

A test of neutrality was conducted using DnaSP software (Rozas, et al., 2003).

Test for Recombination

DnaSP (Rozas, et al., 2003) was used to calculate the estimate of the recombination parameter, which is based on the variance of the average number of nucleotide differences between pairs of sequences.

Spatial Analysis

The Mantel Test was performed on the Rufous Treecreeper mitochondrial DNA, to find a spatial correlation of genetic and geographic distances and a spatial autocorrelation was used to find a pattern of individuals among specific distance classes. These analysis methods were conducted using Alleles in Space (AIS) computer software (Miller, 2005).

Natural Neighbour Interpolation of Mitochondrial DNA

A matrix of nucleotide diversity phi (π) (Nei 1987, equation 10.5) between each population was calculated using DnaSP software (Rozas, *et al*, 2003). This data was then applied to a PCA, with PRIMER 5 v5.2 software (Clarke, *et al*, 2006). The interpolation of principal component scores was carried out with GIS ArcView Nearest Neighbour method.

A total of 25 individual mitochondrial sequences were organised into 10 different haplotypes by a multiple sequence alignment of Cytochrome b (Appendix 4). The following analyses were the conducted with these 10 mitochondrial haplotypes.

Coalescent Tree of RTC Mitochondrial DNA

A gene tree of RTC cytochrome b sequences was conducted to assess their genealogies within the Dryandra region. This was calculated with TCS software which implements the estimation of gene genealogies described by Templeton, *et al.*, (1992) and conducts a cladogram estimation method also known as statistical parsimony (Clement, *et al.*, 2000). Ten haplotypes were used for this analysis. One individual Rufous Treecreeper sequence from Norseman, approximately 500kms away was used as an outlier to compare the rest of the data with. The sample was obtained from the Birds and Mammals Museum, Victoria, Australia and is registered as B17991. This sequence was accessed through GENBANK (NCBI), located at <http://www.ncbi.nlm.nih.gov/genbank/>.

2.4 Ecological Niche, Climate Change and Population Viability

Based on Ecological Niche Modelling (ENM), abiotic preferences of a species were investigated with a spatial analysis of genetic distances and several landscape elements. A Distance Based Redundancy Analysis (db-RA) was used to investigate if a relationship exists between mitochondrial genetic divergence estimates and landscape features such as aspect, slope and habitat fragmentation, where habitat is either continuous or not. The analysis of Rufous Treecreeper microsatellite and mitochondrial DNA were initially tested, but eventually only mitochondrial DNA sequences were found to be significantly correlated with landscape elements. Phylogeny applied to an ENM method can identify the potential location or species range in the past and identify landscape elements that may be important to species present or future habitat as well (Alvarado-Serrano, *et al.*, 2014).

To identify species requirements and to be able to predict the geographic distributions of species, an analysis of the Rufous Treecreeper (RTC) habitat was conducted. Tree hollows found in old growth *Eucalyptus wandoo* trees, have been identified as being extremely important nesting habitat for the Rufous Treecreeper (Luck, 2001, Rose, 1993). Therefore, old growth *E.wandoo* was used as a function of habitat suitability (Akçakaya *et al.*, 1995, Lindenmayer & Burgman, 2005). Also, information of species territory size (Luck, 2000) was used in combination with a constructed habitat suitability map, to model number of interacting family groups (populations) residing within a limited availability of habitat (Akçakaya, 2002).

Since climate modelling of the SWWA predicts the weather will become drier and hotter (CSIRO, 2005), temporal variation in rainfall patterns was observed and the data

assessed to determine the impact of a continual decrease in rainfall on the habitat quality and avifauna viability within the Dryandra woodlands. Land satellite information of annual percentage tree foliage cover (Behn, 2011) was then used to assess the effects of declining rainfall on the quality of habitat and bird captures. The capture-recapture data collected from mist net sampling within the Dryandra woodlands was used to calculate a survival estimate for each species. A General Linear Model (GLM) was applied to the data to find a relationship between species capture, habitat tree canopy cover and rainfall patterns. To assess the trend of tree canopy cover, a time series regression analysis was conducted and future trajectories of tree canopy cover were made.

Distance Based Redundancy Analysis of RTC Microsatellite DNA

DISTLM v.5 (Anderson, 2004) tested various genetic distance and kinship measures Loiselle (1995), Ritland (1996) Kinship Coefficients, Nei's (1972) Distance and Roussett's Distance (2000) as predictor variables, geographic distances as covariables and slope, aspect (direction of slope) and habitat fragmentation as response variables. Habitat fragmentation was represented as binomial 1 or 0 and referred to habitat that was either continuous or not (Epperson, 2003). Aspect was transformed as Roberts and Cooper (1989) TRASP (topographic radiation aspect index) using $AV = \cos(\text{aspect} - 30 \text{ degrees})$.

Distance Based Redundancy Analysis of RTC Mitochondrial DNA

DISTLM v.5 (Anderson, 2004) was used to test mitochondrial genetic distance ϕ (π) with geographic distance and environmental variables of slope aspect, and habitat fragmentation. Habitat fragmentation was represented as binomial 1 or 0 and referred to habitat that was either continuous or not (Epperson, 2003). Aspect was transformed as

Roberts and Cooper (1989) TRASP (topographic radiation aspect index) using $AV = \cos(\text{aspect} - 30 \text{ degrees})$.

Habitat Suitability and Estimate Number of Rufous Treecreepers

A georeferenced habitat suitability map was constructed, using ArcView GIS (Geographical Information Systems), and a botanical map of the Dryandra Woodlands (Coates, 1995). This habitat suitability map provides a measure of habitat that is sufficient to maintain populations of Rufous Treecreeper. It was then used in combination with a known territory size of 2.6 ha (Luck, 2001) and RAMAS GIS software (Akçakaya, 2002), to estimate the number of populations (groups) living within in the Dryandra Woodlands. Also using Luck's (2001) estimate size of each breeding group (up to 7 individuals), an estimate of the number of Rufous Treecreepers within the Dryandra woodlands was estimated.

Rainfall Data

Rainfall data collected by the Bureau of Meteorology (BOM, 2010) for the Wandering weather station, located approximately 20km away from the Dryandra woodlands. The effect of annual rainfall on tree canopy cover was investigated and rainfall was considered an indicator of climate change.

Rufous Treecreeper and Yellow-plumed Honeyeater Captures and Correlation

The capture- recapture data for the Rufous Treecreeper and Yellow-plumed Honeyeater was collected over 5 sampling occasions between 2003 and 2007. Data from all 5 sampling occasions were used to calculate apparent survival rates and only 3 single sampling occasions (collected during the same month of October, in each year 2003,

2004 & 2007), were used for the logistic regression of captures, foliage cover and rainfall. A Pearson Correlation (2-tailed test) was also conducted (SPSSv22).

Percentage Foliage Cover

Land satellite imagery for the Dryandra Woodlands was collected from the Department of the Environment and Conservation and a Projected Foliage Cover (PFC) index was calculated according to Zdunic, *et al.*, (2012). The PFC is the percentage of area which is covered by foliage. To prepare the 7 band Landsat TM imagery an index is applied to the imagery to provide a contrast in values between vegetation and other land types.

Index applied is $(\text{Band3} + \text{Band5}) / 2$, this index was developed in Land Monitor project for the south west of Western Australia. This index is scaled to 0-255 values, with lower values corresponding to greater vegetation cover. The linear regression between average index values at sites and observed PFC in 2005 was calculated to be $\text{PFC} = -0.65(\text{index value}) + 62.3$. Linear regression parameters were applied to the index values to transform them to PFC. The linear regression parameters are applied to all years of available calibrated imagery to provide a time sequence of PFC. Time series of PFC values were calculated for each 1 hectare sampling site within the Dryandra woodlands between 1988 and 2010. This provided an accurate and measurable description of the percentage tree canopy cover. The time series data from 2007 to 2010 was obtained from Behn (2011).

Regression of Rainfall, Species Captures and Canopy Cover

A regression analysis (GLM) was conducted to examine if canopy cover at each site within each year was a predictor of the number of captures per unit trapping effort for the Rufous Treecreeper and Yellow-plummed Honeyeater and also the total of the two

species. The number of captures in each case was log-transformed ($\log(x + 1)$) to stabilise the count data variance. In effect, a repeat measure analysis of covariance was applied since the captures at each site represented a repeated and inherently potentially correlated variable, across the years. Also, since only one rainfall measurement was available for the area of interest, the cover estimates were totalled across sites. Linear regressions were calculated for total cover using both current annual rainfall (anrain) and the previous years annual rainfall (panrain) as the independent variable. The mixed model module of the SPSS software package PASW Statistics v18 (SPSS Inc., 2009) was used for this analysis.

Apparent Survival Rates

Target species included the Rufous Treecreeper, Yellow-plumed Honeyeater and the Western Yellow Robin. They were sampled on five different occasions using mist nets, from a total of 8 sites within the Dryandra Woodlands. The software program MARK (White & Burnham, 1999) was used to analyse capture-recapture data and estimate the apparent survival rates, based on a capture history of gains and losses between sampling occasions. The Cormack-Jolly-Seber Model (CJS) was used to build a multinomial distribution of captures and recaptures for single aged, open populations. The assumptions of this model are that the capture and survival probabilities are identical for all individuals in the sampled population and the time between the sampling occasions is short, to minimise deaths, recruitment and movement out of the study area (Williams, *et al.*, 2001).

CHAPTER 3 - RESULTS

The Rufous Treecreeper microsatellite DNA analysis was conducted to find a population structure as well as a maximum dispersal distance within a continuous and fragmented habitat system. Mitochondrial DNA analysis investigated the spatial distribution of genetic diversity (differentiation) and made inference of the population range that existed prior to land clearing. Initial microsatellite amplification experiments were conducted on the Rufous Treecreeper, Yellow-plumed Honeyeater and Western Yellow Robins, using microsatellite primers designed for Australian passerines and others that amplified across-species (Appendix 1), but were not successful.

Amplification of Rufous Treecreeper microsatellite DNA was achieved with some adjustment to pre-existing microsatellite primers designed for the Brown Treecreeper (*Climacteris picumnus*). Mitochondrial primers were designed (de novo) from multiple copies of a section of the cytochrome b gene (*Cytb*), in closely related species which were accessed via GENBANK (NCBI). The Control Region (CR) was problematic, but eventually amplified in segments using previously published primers (Tarr, et al., 1995).

The Yellow-plumed Honeyeater and Western Yellow Robin genetic analysis was not completed. Considerable time and effort was taken in the laboratory testing primers and formulating PCR and sequence reactions, until eventually time, finances and sample DNA was exhausted. However, fragments of mitochondrial DNA sequences were successfully amplified for segments of the Cytochrome b and Control Region for these 2 species. As these mitochondrial DNA sequences have not been previously published, they do provide useful information for primer design in future studies. The test for Bottlenecked Populations (Cornet & Luikart, 1996) and Assignment Test (Pritchard, *et*

al., 2000) analysis requires microsatellite frequency data (F_{st} values) and because the microsatellite data set was relatively small, these software programs were not successful and not suitable for analysis. However a microsatellite pairwise comparison method, using various measures of genetic similarities (kinship values) and differences (genetic distances) between each pair of individuals (7 alleles per individual), it did achieve a measure of complexity that was useful for the following analysis.

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3.1 GENOTYPING AND DNA ANALYSIS

3.1.1 Microsatellite Primers

The microsatellite primers used in this study were based on primers designed for the Brown Treecreeper (BTC) by Doer, 2004 (unpublished). Initially, these 8 primers (Appendix 1) were used to amplify the Rufous Treecreeper DNA, then cloned (see Appendix 2) and sequenced (see Appendix 3). The resulting Rufous Treecreeper microsatellite sequence information was used to synthesise new primers to improve primer binding and specificity. The underlined sequences represent the BTC primers (Doer unpublished, 2004) and re synthesised primers (in red) for the Rufous Treecreeper (RTC).

```
TGGCTTCCCATTTTGGTTTACGGTGCAAACCCTCAGGACCCTTCACCTCCACCAGATGCTGACT
GTGATGATGATGATGATGATGATGATGATGATGACGAGCACCCCGGAGTTCCCCATCCCTTCGCACCTCC
CACCTCGCCCCCGCCGTGCTGGTTGGTGTCAGGCTTCCGATTTCT
```

Fig. 3.1.1a Rufous Treecreeper microsatellite 6 is 169 bases in length. The (TGA)₉ triplet repeat unit is highlighted, underlined sequences show original primers and red indicate new primer sequences.

RTC microsatellite sequences then were compared with the BTC microsatellite sequences using CLUSTAL W, V 1.83 software and were found to be on average, 97% similar. The Rufous Treecreeper microsatellite allele size variation is shown in blue.

```
BTC . 6      TGGCTTCCCATTTTGGTTTACGGTGC AAACCCTCAGGACCCTTCACCTCCACCAGATGCT
RTC . 6A    TGGCTTCCCATTTTGGTTTACGGTGC AAACCCTCAGGACCCTTCACCTCCACCAGATGCT
RTC . 6B    TGGCTTCCCATTTTGGTTTACGGTGC AAACCCTCAGGACCCTTCACCTCCACCAGATGCT
*****
BTC . 6      GACTGTGATGATGATGATGATGATGATGATGATGACGAGCAGCCCGGAGTTCCCCATCCCTTC
RTC . 6A    GACTGTGATGATGATGATGATGAT-----GATGACGAGCAGCCCGGAGTTCCCCATCCCTTC
RTC . 6B    GACTGTGATGATGATGATGATGATGATGATGATGACGAGCACCCCGGAGTTCCCCATCCCTTC
*****
BTC . 6      GCACCTCCCACCTCGCCCCCGCCGTGCTGGTTGGTGTCCAGGCTTTCGATTTCT 175
RTC . 6A    GCACCTCCCACCTCGCCCCCGCCGTGCTGGTTGGTGTCCAGGCTTTCGATTTCT 169
RTC . 6B    GCACCTCCCACCTCGCCCCCGCCGTGCTGGTTGGTGTCCAGGCTTT----- 166
*****
```

Fig. 3.1.1b A sequence comparison of microsatellite 6 with the Brown Treecreeper shows the RTC to be 96% similar to BTC.

3.1.2 Fragment Length Analysis of Microsatellite DNA

The microsatellite sequence length information was necessary for programming GeneMapper® software, which detects the sequence lengths of each allele. These electropherograms were produced by GeneMapper and show fragment length on the horizontal scale and peak intensity on the vertical axis. The highlighted peak shows the size of the allele followed by 2bp stutter peaks.

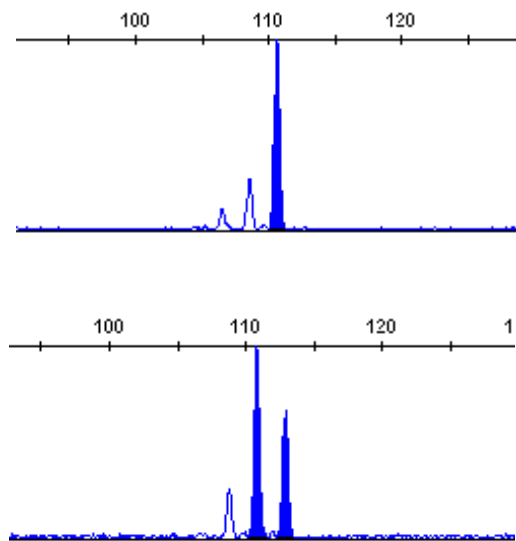


Figure 3.1.2a Electropherogram of locus 3 shows the first individual to be homozygous, with a single peak and allele size of 111 bp. The second individual is heterozygous with a double peak at 111 and 113 bp.

The electropherogram below shows an unusual allele pattern produced by primers for locus 5. This data set was omitted from analysis because it could not be scored. A singlet (homozygote) or doublet (heterozygote) peaks are normally expected.

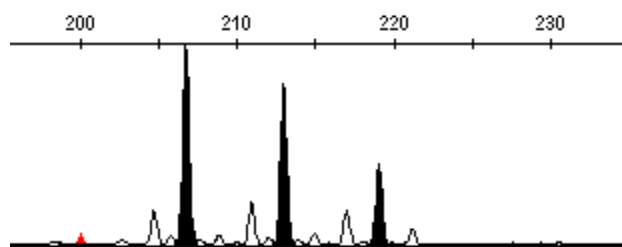


Figure 3.1.2b Electropherogram of locus 5 shows an allele pattern with a triplet peak of 207, 213 and 219 bp.

Locus 5 (See above Figure 3.1.2.b) was omitted from further analysis as the triplet allele pattern is beyond the expected homo/heterozygote patterns and cannot be scored. Three banded (tri-allelic) patterns are known to occur and are reproducible artefacts of the sample (Butler, 2005, Zamir et al., 2002).

Table 3.1.2 Shows primer sequences, fluorescent labels and allele size produced.

GeneMapper Allele Size of Rufous Treecreeper Microsatellite Loci

Locus	1	2	3	4	6	7	8
Repeat Motif	(ct)16 (cct)3 cc (at)3 (ac)2	(ca)3 ga (ca)3 (ca)2	(gt)10	(ca)10	(tga)9	(ca)3 (cg)3 (ca)10	(cg)10 ccc (tc)3
Fluor. Label	6FAM	VIC	6FAM	PET	PET	VIC	NED
Colour	blue	green	blue	red	red	green	black
Alleles (bp)	185 187 195 197 199 201 203 207	213 215 221 222 230	81 111 113	242 249 251 253 255	165 168 169 171 177 178 181	111 117 119 121 123	105 109 111 113
Homozygotes	185/185 199/199 203/203 187/187	230/230 213/213	111/111 113/113	251/251 253/253	165/165 168/168 169/169 171/171 178/178	119/119 121/121	109/109 111/111
Heterozygotes	195/197 199/201 203/207	213/230 213/221 215/230 221/230 222/230	81/111 111/113	251/253 249/253 242/251 249/251 249/255 251/255	165/168 168/171 165/171 168/177	111/119 117/119 117/121 119/121 119/123 121/123	105/109 109/111 109/113

There were occurrences of fragment size discrepancies between the cloned (sequenced) microsatellite size and the calculated size using GeneMapper software. For example, two fragment sizes of cloned microsatellite 6 was 175 and 166 (see Fig. 3.2.1b).

However, GeneMapper software calculated these fragments as 177 and 165. These types of fragment length discrepancies is because the PCR products have different mobilities (on a gel) and the sizing of DNA fragments by GeneMapper, are based on the mobility of a fragment and not specifically it's length in base pairs (Applied Biosystems, 2005).

3.1.3 Microsatellite Neutrality Test

DETSEL software (Vitalis, *et al.*, 2003) was used to determine neutrality for the Rufous Treecreeper microsatellite alleles. Figure 3.1.3 shows neutrality for microsatellite 2, where neutrality is demonstrated by a two dimensional contour graph with only 1 contour with a 95% probability. The intercept within the 95% probable region was derived from a neutral model according to Vitalis, *et al.*, (2001).

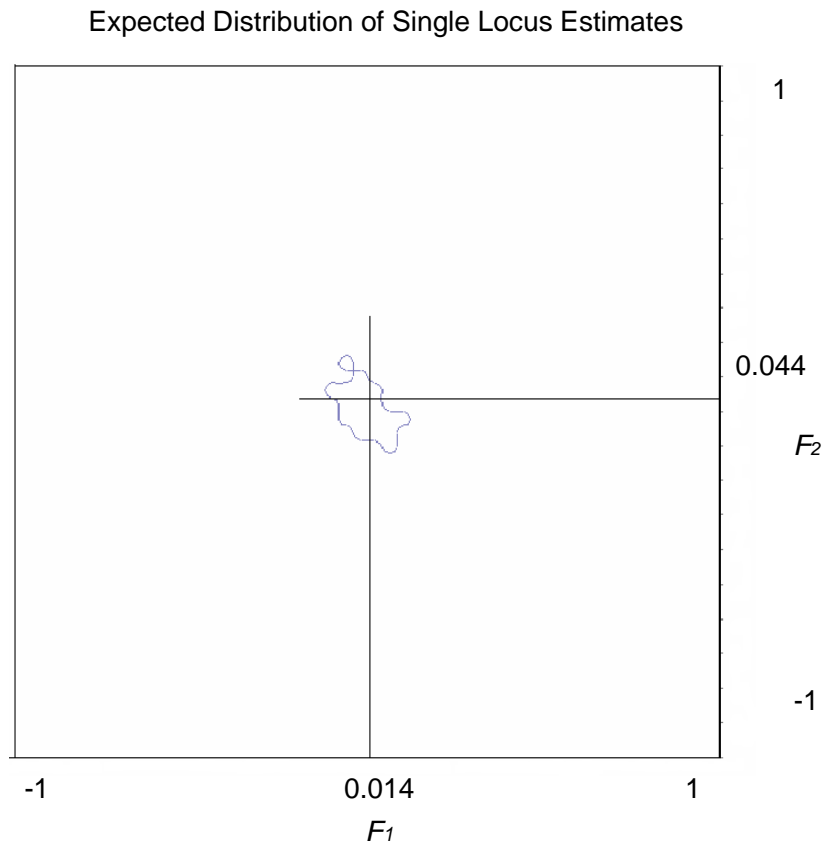


Figure 3.1.3 Contour graph shows 95% probable region of neutrality for locus 2.

3.1.4 Heterozygosity Excess and Hardy Weinberg Equilibrium

INEST 2.0 software (Chybicki, 2014) was used to perform a test for Heterozygosity Excess and a permutation test for Hardy-Weinberg Equilibrium (HWE). The data was analysed in 3 separate groups of total sampled Rufous Treecreepers (Table 3.1.4a), samples from just one year (Table 3.1.4b) and the Dryandra woodlands only (Table 3.1.4c). This was to examine whether the change in gene frequencies is more constant in a single year, or within a larger continuous population. Microsatellites 1, 4 or 5 infer a deficit of heterozygotes as $H(\text{obs})$ is less than $H(\text{exp})$ and therefore deviate from HWE in each separate analysis group. In the following Tables, if the observed F falls within the confidence interval, heterozygosity excess does not depart from zero (at the significance level of 0.05).

Table 3.1.4a Displays values for Heterozygote Excess and Permutation Test for all Rufous Treecreepers sampled at all sites over 4 years.

Total Rufous Treecreeper Populations - All Years									
Locus	NGen	Miss	NAll	Hobs	Hexp	Fis	Fobs	Fperm [0.025]	Fperm [0.975]
ms1	18	0.735	8	0.222	0.714	0.689	0.6889	-0.2444	0.2222
ms2	63	0.074	5	0.524	0.461	-0.136	-0.1357	-0.1702	0.1396
ms3	54	0.206	3	0.259	0.256	-0.014	-0.0135	-0.1583	0.2037
ms4	58	0.147	5	0.241	0.412	0.413	0.4135	-0.2149	0.1621
ms5	21	0.691	7	0.524	0.761	0.311	0.3115	-0.2519	0.1863
ms6	45	0.338	5	0.467	0.552	0.154	0.1539	-0.2087	0.1942
ms7	28	0.588	4	0.393	0.444	0.114	0.1142	-0.2884	0.2753

Table 3.1.4b Displays values for Heterozygote Excess and Permutation Test for Rufous Treecreepers sampled during 2003 only.

Rufous Treecreeper Populations – Single Year 2003									
Locus	NGen	Miss	NAll	Hobs	Hexp	Fis	Fobs	Fperm [0.025]	Fperm [0.975]
ms1	14	0.736	8	0.214	0.717	0.701	0.7011	-0.2952	0.2030
ms2	49	0.075	5	0.592	0.507	-0.168	-0.1682	-0.1682	0.1541
ms3	41	0.226	3	0.341	0.324	-0.055	-0.0549	-0.2056	0.2465
ms4	44	0.170	5	0.250	0.466	0.464	0.4639	-0.2185	0.2202
ms5	11	0.792	6	0.545	0.697	0.217	0.2174	-0.4348	0.2174
ms6	34	0.358	4	0.441	0.527	0.163	0.1632	-0.2831	0.2190
ms7	16	0.698	4	0.375	0.504	0.256	0.2560	-0.3640	0.2560

Table 3.1.4c Displays values for Heterozygote Excess and Permutation Test for Rufous Treecreepers within the Dryandra woodlands only.

Rufous Treecreeper Populations – Dryandra woodlands only									
Locus	NGen	Miss	NAll	Hobs	Hexp	Fis	Fobs	Fperm [0.025]	Fperm [0.975]
ms1	10	0.756	6	0.200	0.621	0.678	0.6780	-0.4492	0.1949
ms2	39	0.049	5	0.436	0.422	-0.032	-0.0323	-0.2145	0.1498
ms3	33	0.195	3	0.212	0.233	0.090	0.0900	-0.1700	0.2200
ms4	35	0.146	4	0.229	0.390	0.414	0.4140	-0.2452	0.1943
ms5	20	0.512	7	0.500	0.768	0.349	0.3489	-0.2371	0.2187
ms6	29	0.293	5	0.483	0.601	0.196	0.1964	-0.2628	0.2538
ms7	26	0.366	3	0.346	0.419	0.173	0.1730	-0.2865	0.2649

In the Test for Heterozygosity Excess NGen is the number of genotyped individuals, Miss is the observed proportion of missing genotypes, NAll is the number of observed alleles (excluding zeros), Hobs is the observed proportion of heterozygotes, Hexp is the expected proportion of heterozygotes and Fis is the inbreeding coefficient ($Fis = 1 - Hobs / Hexp$).

For the Permutation test for HW (based on heterozygosity excess, $Fis = 1 - Ho/He$) Fobs is the observed Fis, Fperm [0.025] is the 2.5% quantile of the permuted Fis (expected under HW), Fperm [0.975] is the 97.5% quantile of the permuted Fis (expected under HW) and based on 1000000 permutations.

Most genetic analysis methods are based on microsatellite allele frequencies and assume HWE to be present in the data set. However, failure to meet HWE is not typically grounds for discarding loci (Selkoe & Toonen, 2006) especially for approaches which are not based on specific assumptions of genetic equilibrium such as the detection of location and genetic shape of boundaries (Safner, et al., 2011).

3.1.5 Null Alleles and Inbreeding Coefficients of Microsatellites

A Bayesian approach for the simultaneous estimations of null alleles, inbreeding coefficients and genotyping failures was conducted with INEST 2.0 program (Chybicki, 2014). The software defines three different models n (null alleles), f (inbreeding) and b (genotyping failures). These models were tested to compare which type of model best fits the data. The model with the lowest Deviance Information Criterion (DIC) value outperforms the others. The b, nb and n models below (Table 3.5.1) show negative results with 0 values for Avg(Fi), HPDI and HPDh. The Avg(Fi) is the sample mean inbreeding coefficient and the HPDI and HPDh is the 95% highest posterior density interval. The nf, bf and nbf models show positive results with the nbf (null alleles, genotyping failures and inbreeding) model showing the lowest DIC value (Table 3.2.5).

Table 3.1.5 Highlighted values for the (nbf) null alleles, genotyping errors and inbreeding model for total Rufous Treecreeper populations.

Total Rufous Treecreeper Populations				
Model	Avg(Fi)	HPDI	HPDh	DIC
nbf	0.0562	0.0001	0.1586	1469.342
nf	0.1589	0.0576	0.2476	1538.181
b	0	0	0	1505.823
nb	0	0	0	1467.412
bf	0.2319	0.1505	0.3378	1488.402
n	0	0	0	1559.572

3.2 DNA Sequencing of the Mitochondrial Control Region

The sequencing of the control region was problematic and often resulted in a double stranded sequence that was unreadable (See chromatogram below). Despite this, a 981 bp DNA sequence of the control region of the Western Yellow Robin was constructed from 2 separate fragments of DNA.

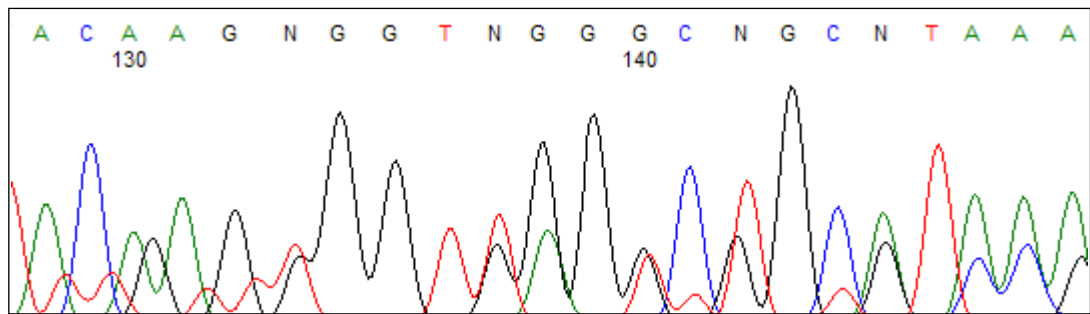


Figure 3.2a A Rufous Treecreeper DNA control region fragment produced by L436 and 12S primers.

```

CGGGGCTTAAACCTCCGTTTTCCATGGAGATGAGTTCCAGTACACCTTGCGAATTTACCCGC
GTCATAAGTTTCGCCCCACCTCCTAGGATATGTTATCTCCCTACAGCTTTCAAGTCCACCCAA
GCCAGAGGACCAGGTATCTATTAACGGTGCACCTCACGAGAACCGAGCTACTCAACGTCA
GTTATACCCTCGTTATTGGCTTCAAGGCCATACTTTCCCCCTACACCCTAGCCCAACTTGCTC
TTTTGCGCCTCTGGTTCCTATTTTCAGGGCCATAAATCTCCTGATTCCTTCTCAATTGCTCTTCA
CAGATACAAGTGGTTGGTCTGCATAAATCCTCCTTTTAACTCGTGATCGCGGCATCTGACCGT
TTTTCTCTTGTTTTCTTTCTGGGGTCTCTTCAATAAACCCCTTCAAGTGGTAGCAGGTGTTAT
CTTCTCTTGACATGTACATCATATGACATCCGAGCGGCCTCATCGCCCGCAGAGCTATCTA
AGTGTAAATGGTTTCGTTGGATAACCTGTGTCATACTTAGACTCTGATGCACTTTGCCCCATT
CATGAAACCCGCGCTGTTTACCTCTTGGGTACAGATGGTGTATGGTTGTGGGACATGACT
ATTTTTTCATGCAGTTCTAGGGACTTATAGTAAAACCCCTATTTTCACGCATTATTTGCGCAAT
TTTTCTTTTTTGTGTTGTCATTTTTTTGTTAACATAACAAAAAATTAACCGAACCTACCCTACA
TTGTCAAACCATTAATAATTCAAACTGTTTATGCACTTTCCACCTAAACACACATTACC
TTTCTTCATGACATTGGAACCAAACAAAAACACGGACACCACCTCACCCAACAAACCAGCA
AACCCCTACCCCATGCCCTTGTAGCTTACAACAAAGCATGGCACTGAAGATGCCAAGATGGC
CGTCATAAAACGCCCAAGGACAAAAGACTTAGTCCTAACCT

```

Figure 3.2b The mitochondrial control region of the Western Yellow Robin, 981bp.

3.2.1 DNA Sequencing of the Mitochondrial Cytochrome b Gene

The sequencing of the cytochrome b gene was carried out in two parts, using 4 different primers.

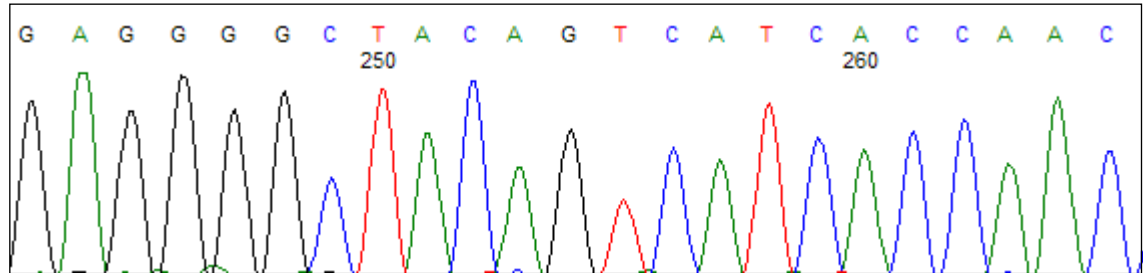


Figure 3.2.1a Chromatogram for part of Rufous Treecreeper Cytochrome b region of the mitochondria.

```
ACATCTGCCGAGACGTTCAATTCGGCTGATTAATCCGCAATCTCCATGCTAACGGAGCCTCT
ATGTTCTTCATCTGCATCTACCTACACATCGGCCGAGGCTTCTACTATGGATCCTACGCAAAC
AAGGAAACCTGAAACACCGGAGTCTCCTACTTCTCACCTTAATAGCAACAGCCTTCGTAGG
CTACGTA CTCCCCTGAGGACAAATATCATTCTGAGGGGCTACAGTCATCACCAACCTATTCT
CCGCTATCCCATACATCGGCCAAACCCTCGTAGAATGAGCTTGAGGAGGCTTCTCAGTAGAC
AACCCGACCTCACACGATTCTTTGCCCTCCACTTCTACTGCCATTTCGTAATCGCAGGACTC
ACCCTAGTCCACCTAACCTTCCCTACACGAAACAGGCTCCAACAACCCCTTAGGCATCCCCTC
AGACTGCGACAAAATCCCATTCCACCATAACACACCACAAAAGACATCTAGGATTCGCAC
TAATATTTGTCTCCTTGCATCACTCGCTTTATTCTCCCCAACCTGCTAGGAGACCCAGAAA
ACTTTACCCCGCTAACCCCTAGCCACACCTCCCCACATCAAACCAGAATGATACTTCTCTGT
TTGCCTACGCCATCCTGCGTTCCATCCCCAACAACTAGGAGGAGTC
```

Figure 3.2.1b Rufous Treecreeper cytochrome b region of the mitochondria, 671bp.

```
GGATGCGGCGAGGGCTAGGACTCCTCCTAGTTTGNNGGGGAGGGAACGCAGGATGGCGTAGG
CAAACAGGAAGTATCATTCTGGTTTGATGTGGGGAGGTGTGGCTAGGGGATTAGCGGGGGT
AAAGTTTTCTGGGTCTCCTAGCAGGTTTGGGGAGAATAAAGCGAGTGATGCAAGGAGGACA
AATATTAGTGCGAATCCTAGGATGTCTTTTGTGGTGTGGTATGGGTGGAATGGGATTTTGTG
GCAGTCTGAGGGGATGCCTAGGGGGTTGTTGGAGCCTGTTTCGTGTAGGAAGGTTAGGTGGA
CTAGGGTGAGTCTGCGATTACGAATGGCAGTAGGAAGTGGAGGGCAAAGAATCGTGTGAG
GGTCGGGTTGTCTACTGAGAAGCCTCCTCAAGCTCATTCTACGAGGGTTTGGCCGATGTATG
GGATAGCGGAGAATAGGTTGGTGTGACTGTAGCCCCTCAGAATGATATTTGTCCTCAGGGG
AGTACGTAGCCTACGAAGGCTGTTGCTATTAAGGTGAGAAGTAGGAGGACTCCGGTGTTCAG
GGTTTCCTTGTGGTGGTAGGATCCATAGTAGAAGCCTCGGCCGATGTGTAGGTAGATGCAGA
TGAAGAACATAGAGGCTCCGTTAGCATGGAGATTGCGGATTAATCAGCCGAATTGAACGTCT
CGGCAGATGTGGGCAACGGAGGCGAAGGCTAGGGAAAGTGTCTGCTGTGTAGTGTATAGCGA
GAA
```

Figure 3.1.4c Yellow-plumed Honeyeater cytochrome b region of the mitochondria, 742bp.

3.2.2 Analysis of Mitochondrial DNA Sequences

The sequencing of the control region and cytochrome b segments of mitochondrial DNA was synthesised in two fragments and the overlapping sequences were used to join the segments together. The isolation of mitochondrial DNA from whole genomic DNA, could have avoided cellular components from contaminating the sequencing reactions, especially of the control region.

Mitochondrial Gene Order

The Mitochondrial gene order was established in the Rufous Treecreeper.

Consideration also had to be given to 2 different orientations and four different gene regions. After some experimentation with a selection of primers, the second orientation of a cytochrome b - glutamic acid - ND₆ – control region – phenylalanine - 12S order was found. Amplification of the entire 4 segmented gene region was completed and the amplified fragments were separated by gel electrophoresis to check fragment size and sequenced. The size of target gene regions was referenced by using published sequences in GENBANK (NCBI). A final continuous DNA sequence was traced with multiple sequence alignments of each gene region, which contained regions of similarity in overlapping segments.

Control Region

Amplifying the control region was problematic, which resulted in what appeared to be sequences generated from a mixed template. Mitochondrial pseudogenes or numpts, were suspected to be the cause for the problems in sequencing the control region (Refer to section 1.5, p.45). Therefore, it is recommended to purify mitochondrial DNA from total DNA prior to sequencing to avoid the interference of pseudogene sequences being

produced in the reaction. White, *et al.*, (1998) provides a Density-Gradient Ultra-centrifugation Method which isolates pure mitochondrial DNA, prior to any sequencing reactions. Also, the results for this study may have improved if feather samples had been first processed using this method. However, a 981bp sequence of the control region was found for the Western Yellow Robin. Part of the cytochrome b gene was sequenced for the Rufous Treecreeper (671bp) and Yellow-plumed Honeyeater (742bp).

Cytochrome b Gene

Primers for cytochrome b (*cytb*) were designed from a collection 50 mitochondrial gene sequences from highly conserved gene regions (from within the same family group of species). They were located in GENBANK (NCBI) and aligned by a computer program BioEDIT (Hall, 1999). This allows a multiple sequence alignment showing consensus sequences within coding regions of a transcribed gene. Specific primer sets were then designed from the areas of high consensus which also flanked a variable region. This variable part of the gene containing a higher rate of mutations (synonymous) was selected as the targeted area for analysis.

The intention of designing new primers was to increase specific binding to template DNA. Designing a new set of primers (*de novo*), allows the faithful replication and prediction of the expected length of the DNA product. The Rufous Treecreeper mitochondrial Cytochrome b Gene (partial *cyb* sequence 671bp), was translated into a 255 amino acid sequence code and identified with Blast at NCBI database. The Conserved Domains data base produced a 100% alignment with cytochrome b segment of the Rufous Treecreeper with accession number AAB05474. Please see Appendix 6 for details. .

3.2.3 Detection of Natural Selection of Cytochrome b DNA

Detection of Natural Selection in mitochondrial DNA of partial cytochrome b (*cytb*) gene was conducted with the synonymous (silent) and non-synonymous base substitution rate method and the second method estimates any recombination events in the target gene to test for natural selection.

The rates of mutations in the Rufous Treecreeper *cytb* gene, were found to be under influence of natural selection. Since *cytb* is transcribed into a functional protein of the mitochondria, the specific amino acid combinations would have to be faithfully translated, avoiding a faulty functioning protein. Under this evolutionary pressure, the mutations that do occur in the functioning *cytb* gene are silent (non-lethal) mutations. These mutations are a form of redundancy of the genetic code (base substitutions), whereby an amino acid is specified by more than one codon. The test for neutrality for mitochondrial DNA using synonymous (silent) and non-synonymous base substitution rates using DnaSP software (Rozas, et al., 2003), was conducted with ten different haplotypes. For each haplotype there were 223 codons analysed from 669 sites. The average number of synonymous sites was 165.78 and 503.22 for the number of non-synonymous sites. The relative levels for these rates (synonymous < non-synonymous) indicate diversifying selection as the mode of selection for the Rufous Treecreeper *cytb* gene.

The second recombination event method was applied to the Rufous Treecreeper partial cytochrome b gene, where 671 sites and 13 polymorphic sites were tested. The number of pair wise comparisons analysed was 78, the number of sites with four gametic types: was 0 and the minimum number of recombination events (R_m) was 0. This test indicates there was no recombination found for the Rufous Treecreeper *cytb* gene.

3.3 SPATIAL GENETIC ANALYSIS OF MICROSATELLITE DNA

3.3.1 Genetic Diversity of Microsatellites

Analysis of allele variance (AMOVA) within and between Rufous Treecreeper populations was conducted with GenAleX 6 (Peakall and Smouse, 2006). AMOVA analyses partitioned variation according to correlations among genotypes rather than variation in gene frequencies. This analysis shows a higher genetic variance within populations (78%) than among populations (22%). The global F_{st} value is 0.218 with a probability value of 0.01. AMOVA infers there is less gene flow within populations and a higher amount of gene flow (less genetic variance) between populations.

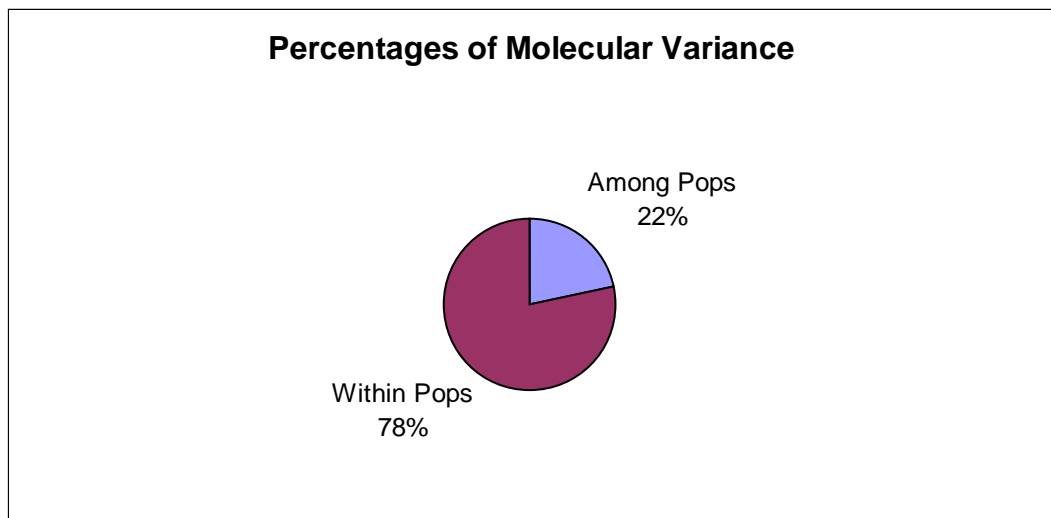


Figure 3.3.1 AMOVA analysis of Rufous Treecreeper microsatellite alleles.

3.3.2 Spatial Pattern of Microsatellites within Dryandra

INEST program (Chybicki, 2014) utilises a Bayesian Approach to find an Isolation by Distance (IBD) pattern for Rufous Treecreepers within the Dryandra woodlands. If the observed slope (Obs) lies within the bounds of [0.025] and [0.975] then there is no spatial genetic structure for IBD. The (Obs) is at the significance level of 0.05. There is no IBD for the Rufous treecreepers in Dryandra. The criterion for computing distance classes was equal pairs and the number of permutations was 999.

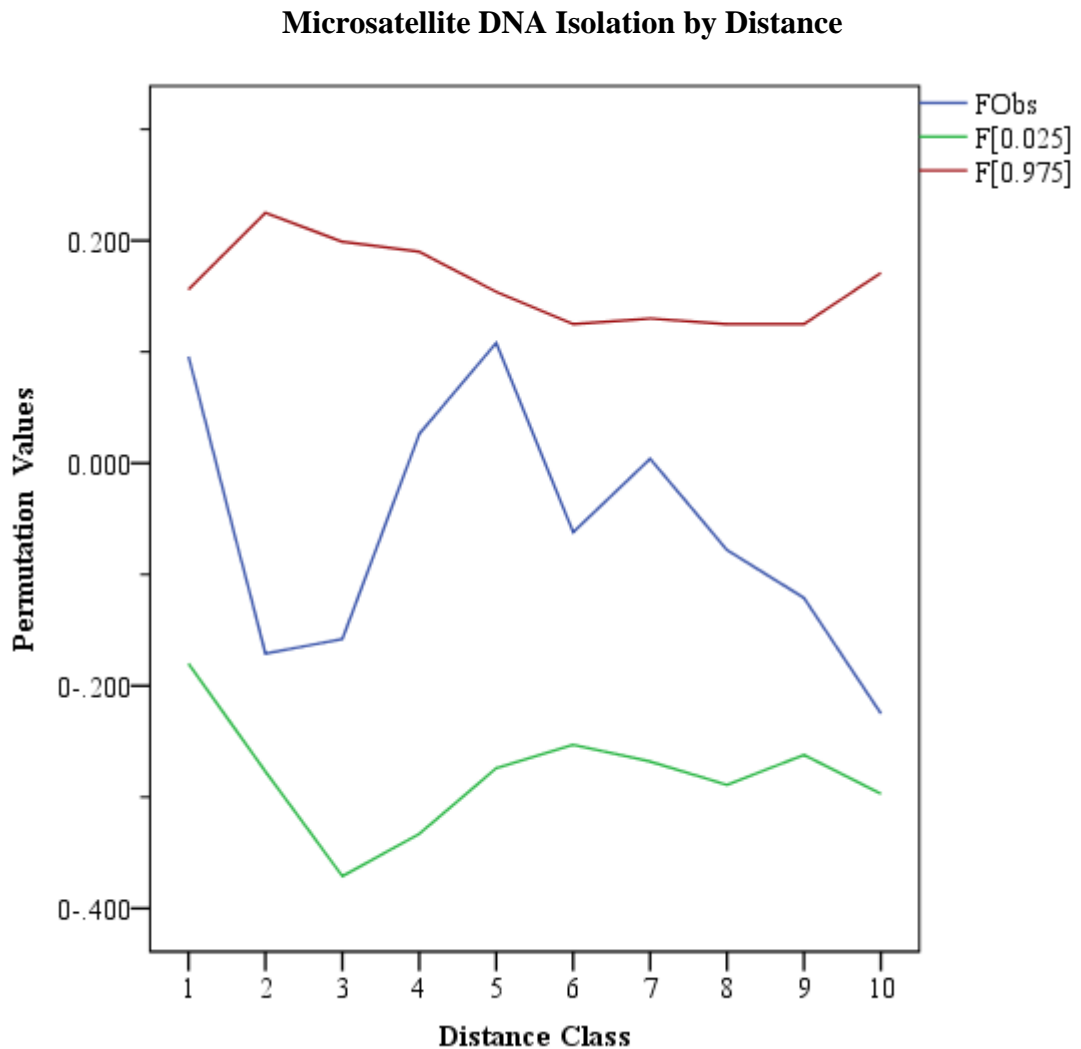


Figure 3.3.2 Fobs (blue line) shows no IBD across 10 distance classes spanning 25km.

3.3.2.1 Spatial Patterns of Regional Microsatellites

An Isolation by Distance (IBD) pattern for Rufous Treecreeper microsatellites was tested across a region of approximately 85kms, including the Dryandra woodlands. The observed slope (Obs) lies within the bounds of [0.025] and [0.975] and therefore there is no spatial genetic structure for IBD. The Rufous Treecreepers do not follow the Stepping Stone Model of population expansion. The (Obs) is at the significance level of 0.05 and the criterion for computing distance classes was equal pairs and the number of permutations was 999.

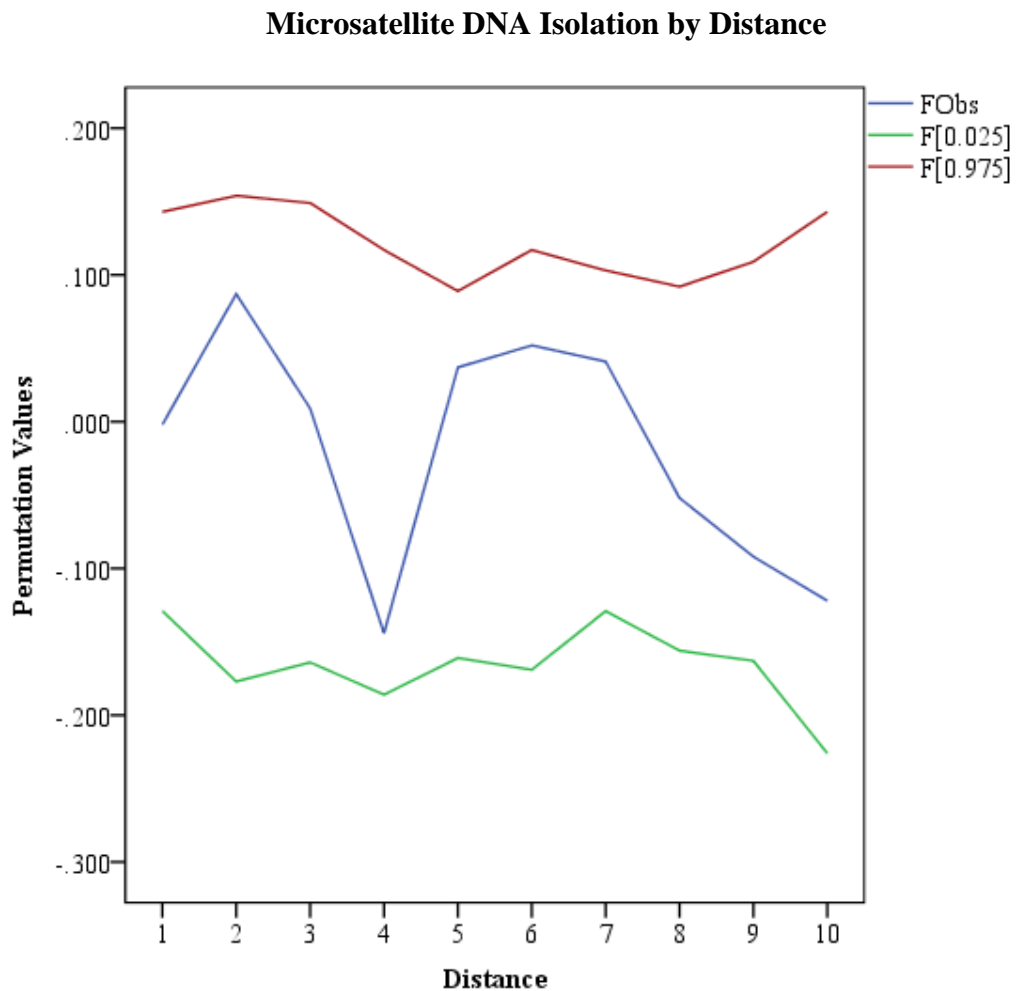


Figure 3.3.2.1 Permutation values of F Observed in between F[0.025] and F[0.975] shows no IBD, using 10 distance classes spanning approximately 85km.

3.3.3 Spatial Scale of Microsatellite Distances within Dryandra

AIS (Miller, 2005) software applies Nei's (1983) genetic distances to pairs of individuals rather than pairs of populations and plots these measures against geographical distances. The resulting Mantel Test shows a positive correlation between genetic distance and geographical distance, up to approximately 25kms. The genetic distances between Rufous Treecreepers appear to differentiate with distance across the Dryandra woodlands resulting in a positive, non-random genetic distribution. The regression coefficient (r) is 0.316. The probability of observing a correlation greater than or equal to observed data is 0.004 (less than 0.05) after 10000 replicates performed. The Mantel's Test did not produce significant results for regional RTC microsatellite DNA.

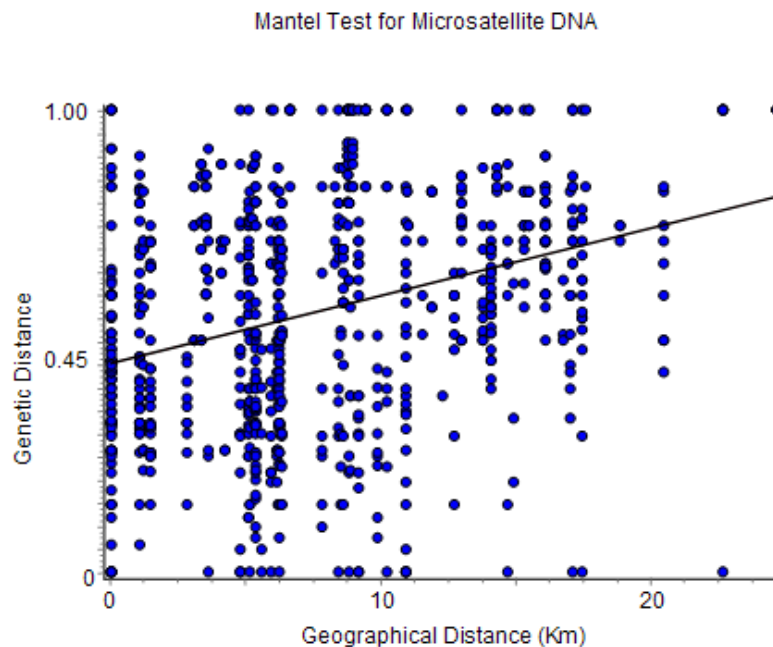


Figure 3.3.3 Mantel Test of microsatellite DNA showing an increase of genetic distance within an increase of geographical distance up to 25kms.

3.3.3.1 Spatial Scale of Regional Microsatellite Distances

The Spatial autocorrelation was conducted with AIS software (Miller, 2005) and shows a spatial scale of 10 distance classes across the Region. The curved line below shows that within four distance classes (up to 25 km), there is a strong genetic structure. However after 25 km, beyond the Dryandra woodlands there is no genetic structure and indicates a pattern of genetic discontinuity or variation across the landscape. A_y is the average genetic distance (grey line) between pairs of individuals, that fall between distance class y . The value of a_y is 0 when all individuals are genetically similar and 1, when all individuals are completely dissimilar (Miller, 2005). This analysis was performed with 10 unequal distance classes with equal sample sizes and 10 000 permutations. The full histogram: $V = 0.055$ with a probability of observing a random value of $V \geq$ observed V by chance: $P = 0.009$. V is an estimate of the P-value for each distance class, after Bonferroni corrections.

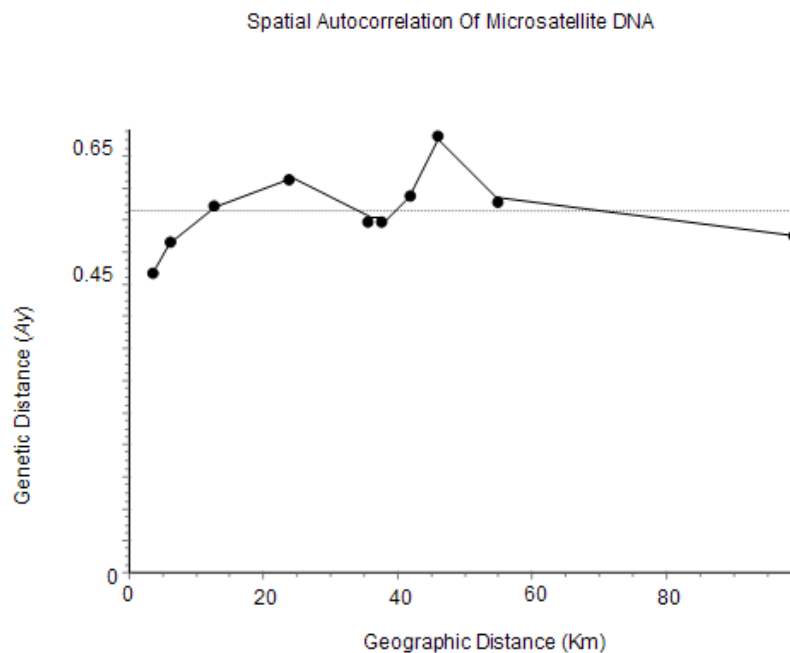


Figure 3.3.3.1 Spatial autocorrelation analysis of microsatellite DNA.

3.3.4 Spatial Distribution of Microsatellite Distances within Dryandra

The Landscape Interpolation Model of AIS (Miller, 2005) was used to measure the genetic distance (Nei's 1983) of individual Rufous Treecreepers across the landscape. Each individual RTC has x and y coordinates which are midpoints of a triangulation based connectivity network and the surface heights (z value), represent genetic distances. The z value is calculated using the inverse distance-weighted interpolation by Watson (1992) & Watson and Philips (1985) and is used to infer genetic distances at locations on a uniformly spaced grid overlaid on the entire sampled landscape (Miller, 2005). Within the Dryandra woodlands, genetic differentiation is greater on the western side and moving eastwards where genetic distances decline.

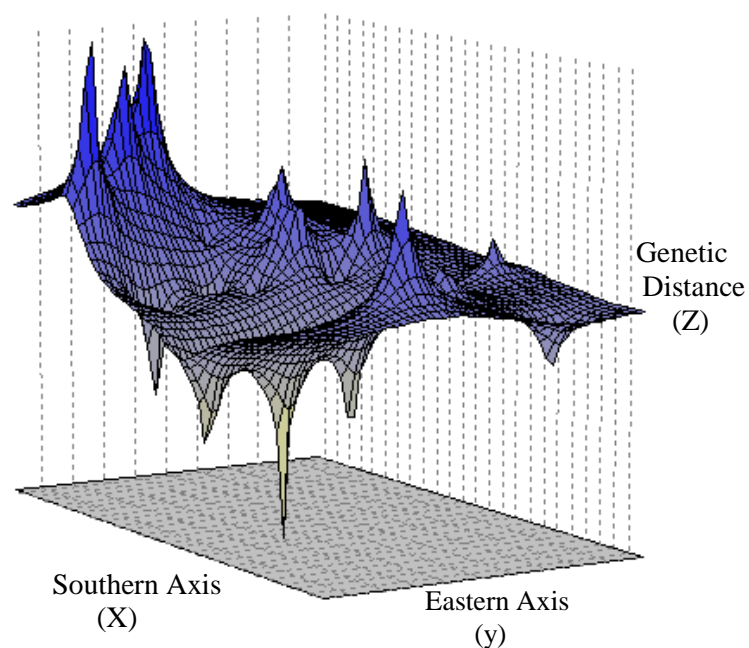


Figure 3.3.4 The distribution of microsatellite genetic distance estimates within the Dryandra woodlands.

3.3.4.1 Spatial Distribution of Regional Microsatellite Distances

Dryandra appears to have a very significant proportion of genetic differentiation compared to the regional area. The regional area spans approximately 100 kilometres and includes Boyagin Reserve to the north, Dryandra woodlands and Highbury Forest on the southern, Stratherne site to the east and Dongolocking in the south east. Each individual Rufous Treecreeper has x and y coordinates and the surface heights (z value), represent genetic distances. The region surrounding the Dryandra woodlands (apart from Highbury on the southern axis) appears to be more genetically similar and indicates the presence of gene flow between neighbours in these outer populations.

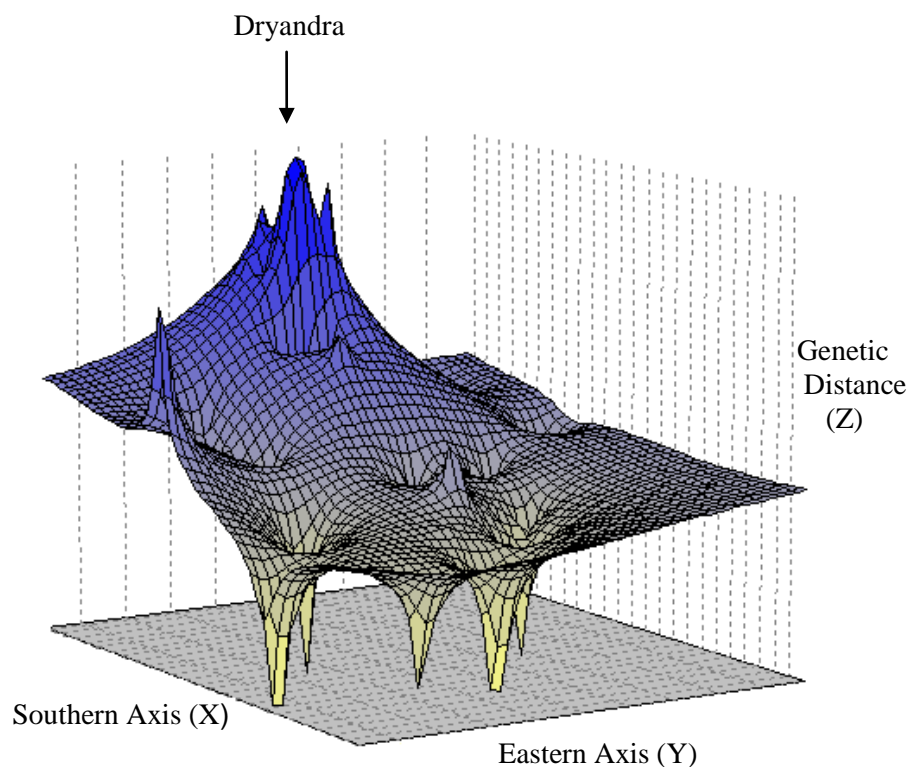


Figure 3.3.4.1 Distribution of RTC microsatellite with Nei's 1983 Genetic Distance estimates within the region

3.3.5 Population Structure of Microsatellites within Dryandra

The maximum difference Delaunay triangulation of RTC microsatellite DNA was conducted with AIS (Miller, 2005) software. The resulting diagrams represent the internal genetic boundaries of 12 populations within the fragmented Dryandra woodlands, based on maximum genetic differentiation. The 3 central populations reside in the centre of the largest continuous habitat and show distinct genetic neighbourhood boundaries.

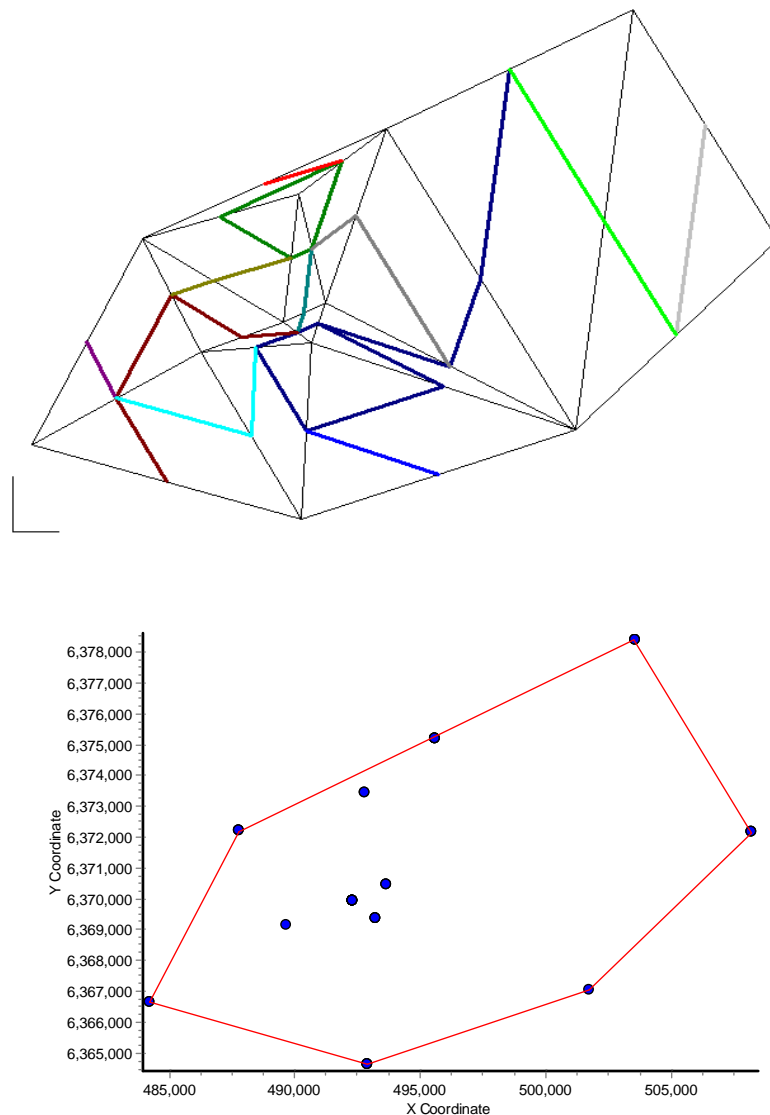


Figure 3.3.5 Delaunay triangulation of microsatellite DNA shows genetic boundaries between populations at different sampling sites within Dryandra. The 3 closest sites within the centre are Norn, Baaluc North and Baaluc South.

3.3.5.1 Population Structure of Regional Microsatellites

Approximate Bayesian Computation of Regional RTC Microsatellites was conducted with *DIYABC* (Cornuet, *et al*, 2013). Individuals were geographically measured from the centre of the Dryandra woodlands and grouped into distance class populations.

After many different types of distanced based population structures were tested, only two structures were found to fit the data set. These two structures represent a Stepping Stone and Continent Island model of population expansion.

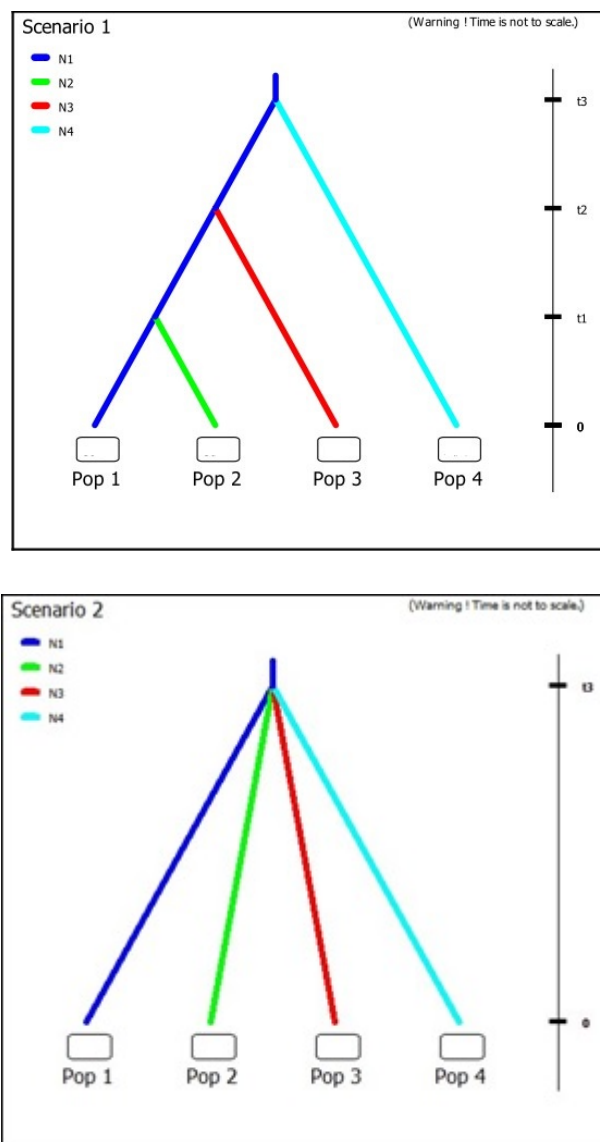


Figure 3.3.5.1a Population 1: 0-20 km; Dryandra woodlands, **Population 2:** 20-40 km; Commondine Reserve, Boyagin, Warren Highbury, **Population 3:** 40-60 km North Yilliminning. Birdwhistle Reserve, Narrakine Highbury and **Population 4:** 60-80 km; Dongolocking Reserve.

After pre-evaluation of each scenario using prior distributions the estimation of posterior distributions of parameters were made and a model checking procedure (or goodness of fit) was used to model parameter posterior combinations (Cornuet, *et al.*, 2013). A Principal Component Analysis (PCA) was produced to show the space of summary statistics using data sets simulated with the prior distributions of parameters, the observed data and sets from the posterior predictive distribution, all on each plane of the PCA.

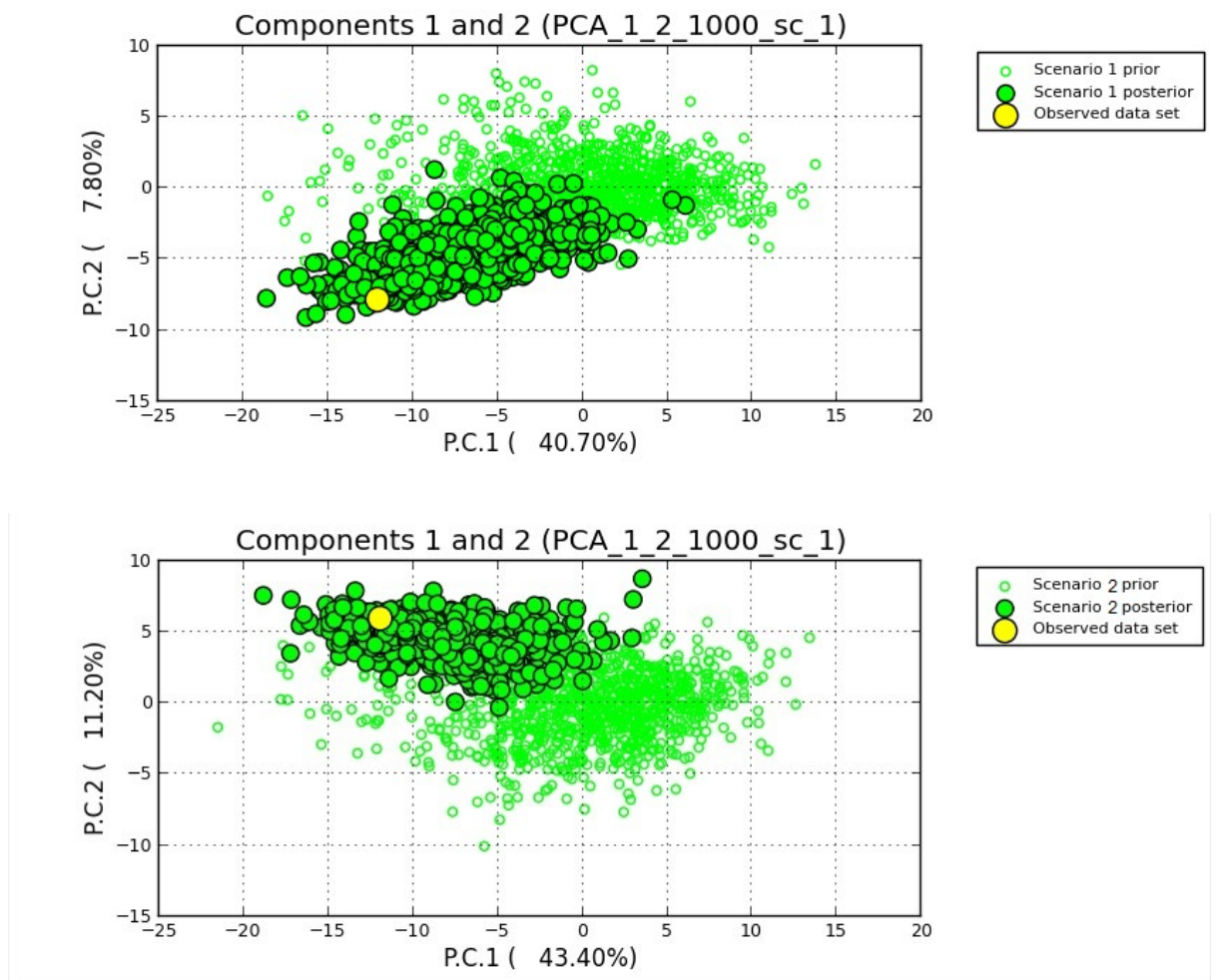


Figure 3.3.5.1b Comparison of PCA for scenario 1 and PCA for scenario 2.

Finally, a comparison of possible scenario choices was made by a computation of the posterior probabilities of each scenario. For finding the confidence in scenario choice, the number of simulated data sets was 1 000 000. Using the Direct Approach and Logistic Regression the number of selected data sets for each was 500, from a total of number of 10 000.

Table 3.3.5.1 shows the number of times each scenario has the highest posterior probability

	Scenario 1	Scenario 2
Direct Approach	142	358
Logistic Approach	241	259

The second scenario is the preferred choice with the highest values in both the Direct and Logistic Approach. On a regional scale, the Rufous Treecreeper appears to follow a Continent-Island population model of expansion. The majority of dispersal would most likely be emigration from the larger woodlands (Dryandra) to smaller remnant habitat and to a smaller extent, immigration from the smaller, more distant remnants back to Dryandra.

3.3.6 Dispersal Patterns of Microsatellites within Dryandra

Ritland's (1996) Kinship Coefficient is based on a Continuous Population Model and therefore, appropriate for assessing the smaller, neighbourhood structure within the Dryandra woodlands. The pairwise kinship Neighbour-Joining (NJ) tree of 37 RTC individuals shows 7 lineages made up from different individuals from different sampling sites. This indicates a pattern of dispersal between populations within Dryandra. However, the blue lineage contains individuals from only 3 sampling sites within an area of 1.7 km. These closely related individuals represent a genetic neighbourhood size (see Appendix 10 for phylogram with Kinship values).

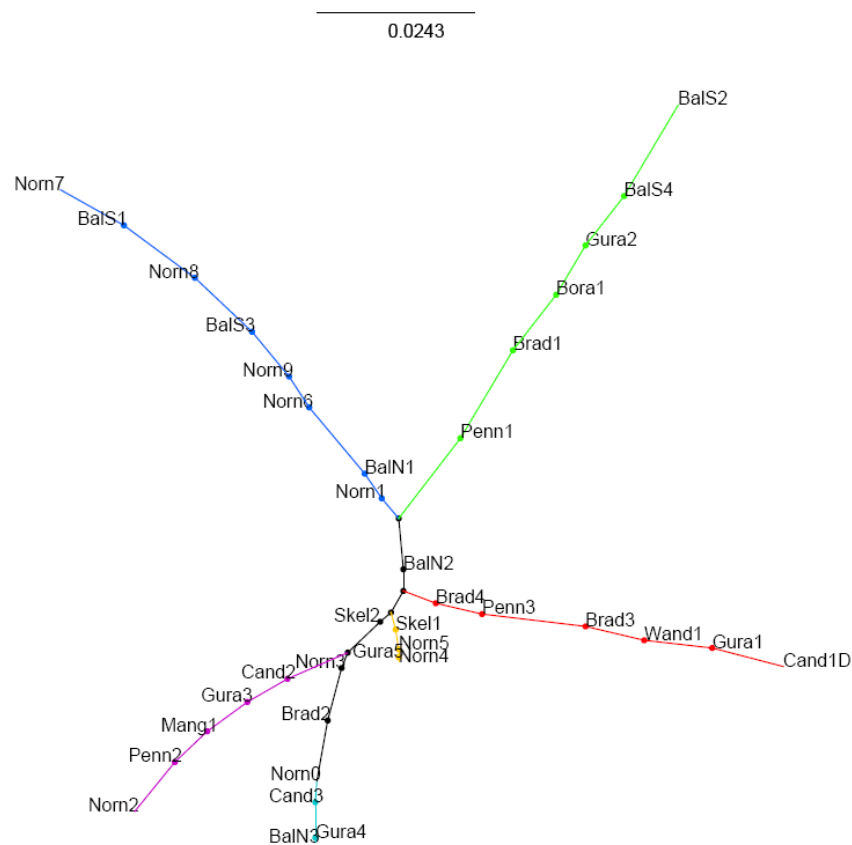


Figure 3.3.6 Radial Kinship Tree of RTC within the Dryandra woodlands. There is a mean pair-wise distance between nodes of 0.062, a variance of 0.001, total tree length of 0.318 and mean edge length of 0.004.

3.3.6.1 Dispersal Patterns of Regional Microsatellites

A spatial scale of similarity is shown by Loiselle (1995) Kinship radial tree. It shows 9 main lineages comprising 60 individuals arranged by pair-wise comparisons using the Neighbour Joining Tree method. Genetic similarity is shown by close proximity of nodes. Each individual RTC is shown as a node (coloured dot) and those from the Dryandra woodlands are shown as black dots. Individuals from the Dryandra woodlands appear to have diffused throughout the region, as they are found on every branch and show a significant amount of genetic relatedness with geographically distant individuals (see Figure 3.3.6.1a and b).

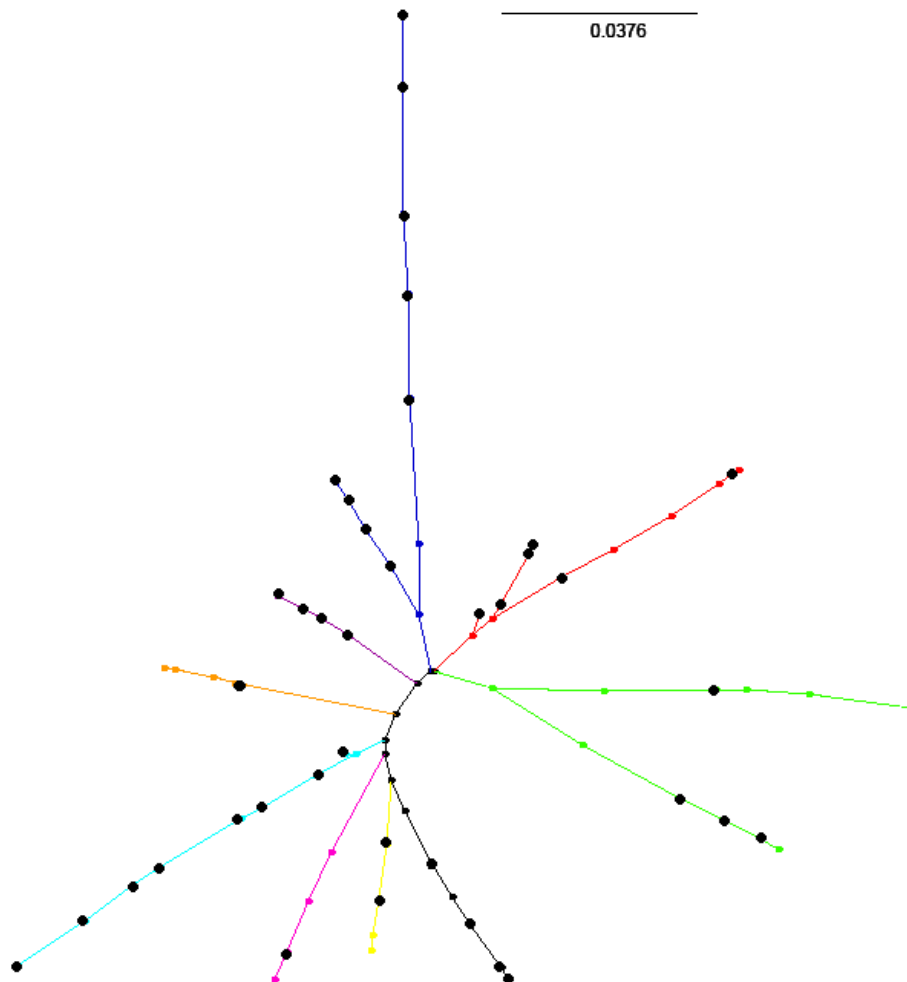


Figure 3.3.6.1 A radial tree of regional RTC microsatellite DNA. The total tree length is 0.731, has a mean pairwise distance between nodes of 0.094 and a mean edge length of 0.006.

The smallest genetic distance between any two individuals is North Yilliminning and Baaluc (South) in Dryandra (0.0019). The geographical distance between these two sites is 42.3 km.

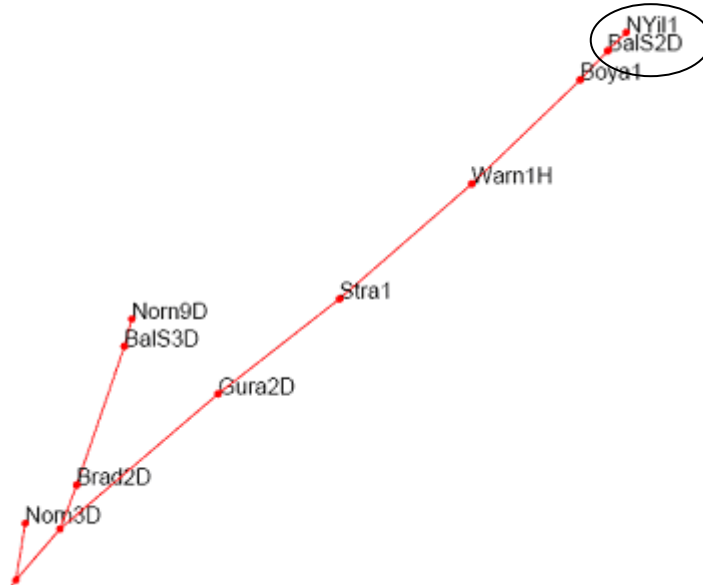


Figure 3.3.6.1a Red lineage from Loiselle Kinship tree showing smallest genetic distance between any pair of individuals.

The genetic distance between individuals from Dongolocking Reserve and Narrakine Block in Highbury is 0.0031, separated by a geographical distance of 48.6 km (yellow lineage).

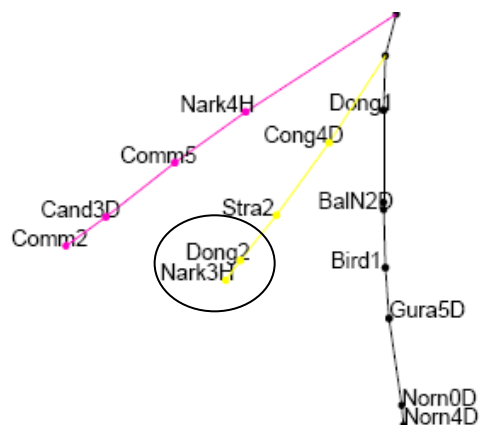


Figure 3.3.6.1b Yellow lineage from Loiselle Kinship tree shows smallest genetic distance with largest geographical distance between two individuals.

3.3.7 Sex-Biased Dispersal of the Rufous Treecreeper

The landscape interpolation maps show the distribution of genetic distances of the male and female Rufous Treecreeper. The males are evenly distributed across the region, but the females are less diverse within Dryandra and more highly divergent in outer-regional sites. This suggests that within Dryandra Woodlands where habitat is continuous, females are dispersing at a higher rate than in smaller and more isolated remnants, where habitat and nesting hollows are limiting.

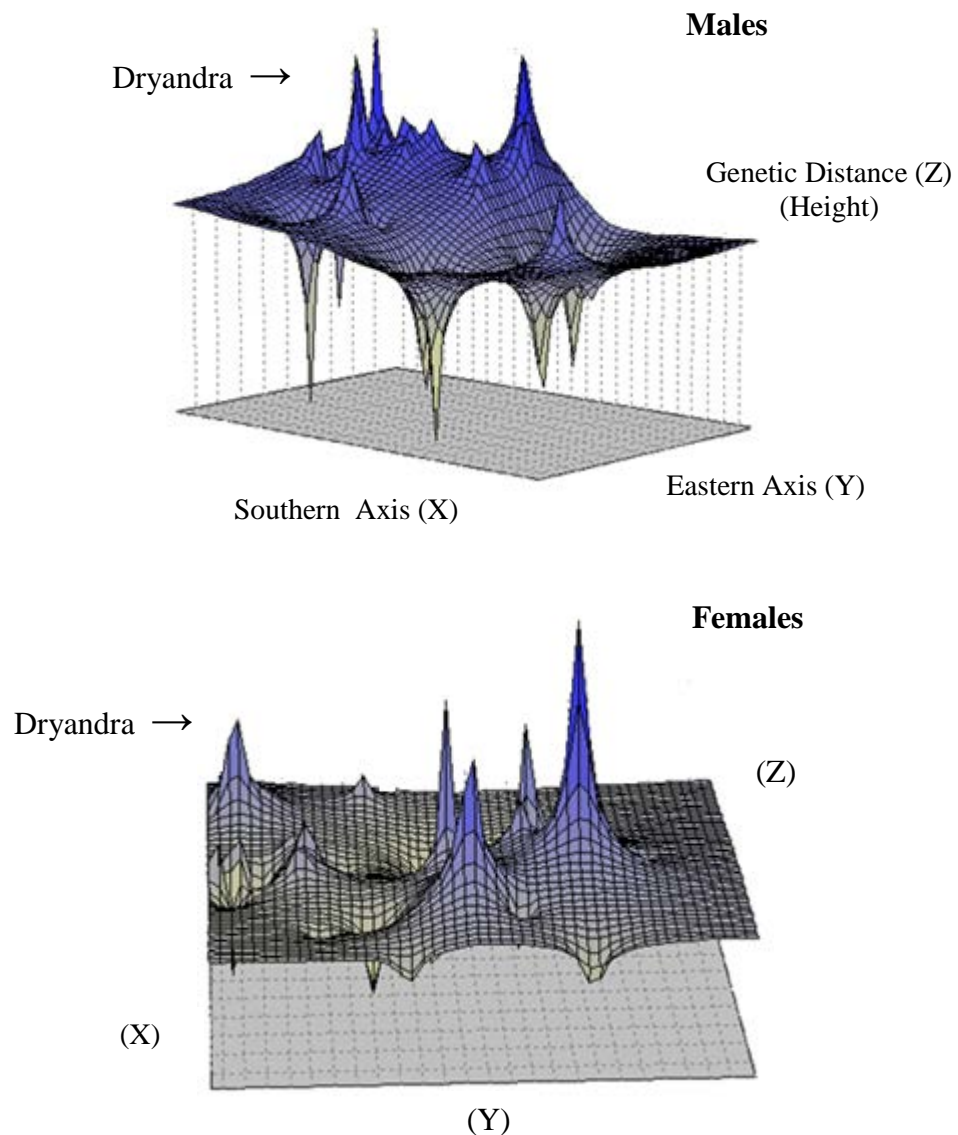


Figure 3.3.7 Male and Female genetic divergence estimates across the landscape.

3.4 SPATIAL GENETIC ANALYSIS OF MITOCHONDRIAL DNA

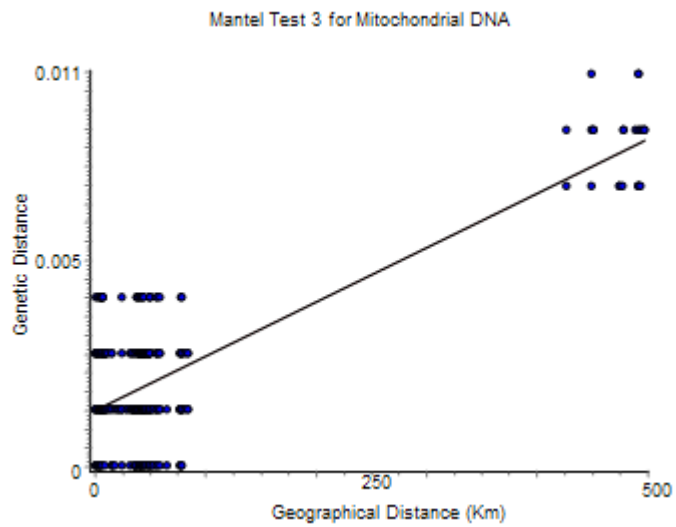
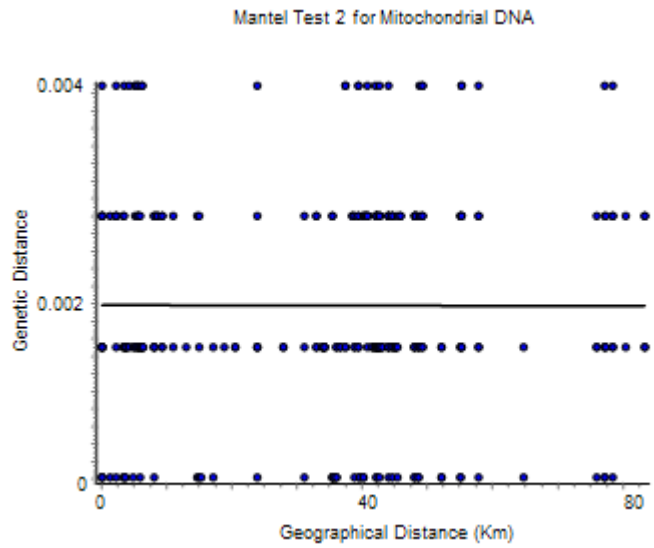
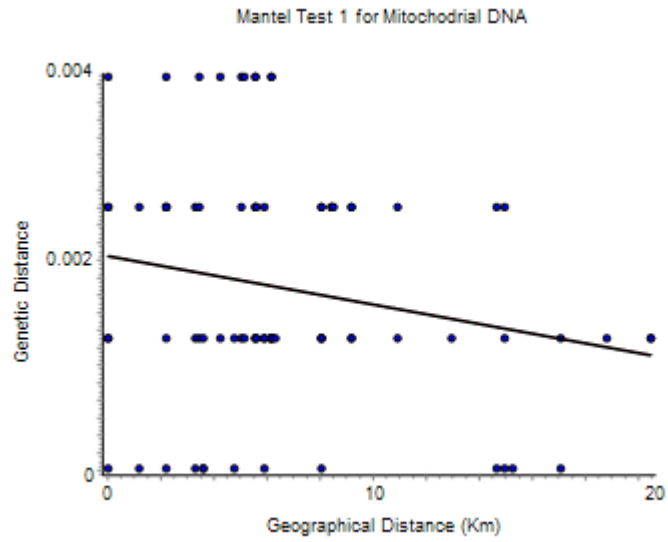
3.4.1 Spatial Scale of Mitochondrial DNA

The following graphs represent the correlation of genetic distance and geographic distance of mitochondrial cytochrome b DNA at three different distances of 20, 80 and 500 km. The sample and subsequent DNA sequence from the Norseman location (500km away from Dryandra) was obtained from the Birds and Mammals Museum, Victoria, Australia and is registered as B17991. The Mantel's Test shows the scale at which mitochondrial DNA significantly differentiates. It infers no historical population structure (dispersal patterns) at a distance of 500 km, indicating separate bioregions.

Mantel's Test 1 was conducted at a distance of approximately 20 km, within the Dryandra Woodlands. The correlation coefficient between genetic and geographical distances was $r = -0.198$. The probability of observing a correlation greater than or equal to observed: $P = 0.7822$ after 10000 replicates performed. This P value is not significant

The Mantel's Test 2 was conducted within the region, at a geographical distance of 80 km. The correlation coefficient was $r = -0.0031$. The probability of observing a correlation greater than or equal to observed: $p = 0.4875$, after 1000 replicates performed. This P value is not significant.

The Mantel's Test 3 was conducted across regions at a maximum geographic distance of approximately 500kms. The correlation coefficient $r = 0.7989$. The probability of observing a correlation greater than or equal to observed: $P = 0.0249$, after 10000 replicates performed. This P value is significant and shows a positive relationship between genetic and geographical distance of the RTC.



3.4.2 Spatial Distribution of Mitochondrial Divergence

Interpolation and Principal Component Analysis of Regional RTC Mitochondrial DNA was conducted with the Interpolation of PC1 scores of mitochondrial Genetic Distance ϕ (π), using Natural Neighbour technique (GIS). The red colour represents negative Natural Neighbour values of -4 (high genetic distances) through to blue, which represents positive Natural Neighbour values +4 (small genetic distances). Treecreepers found in areas containing low genetic distances (in blue) are most likely to have experienced a reduction in population size and have lost genetic variation in the mitochondrial DNA. The highest genetic distance estimates reside in the centre of the Dryandra woodlands and Strathern site in Wickepin (R) and North Yillimining Reserve (S).

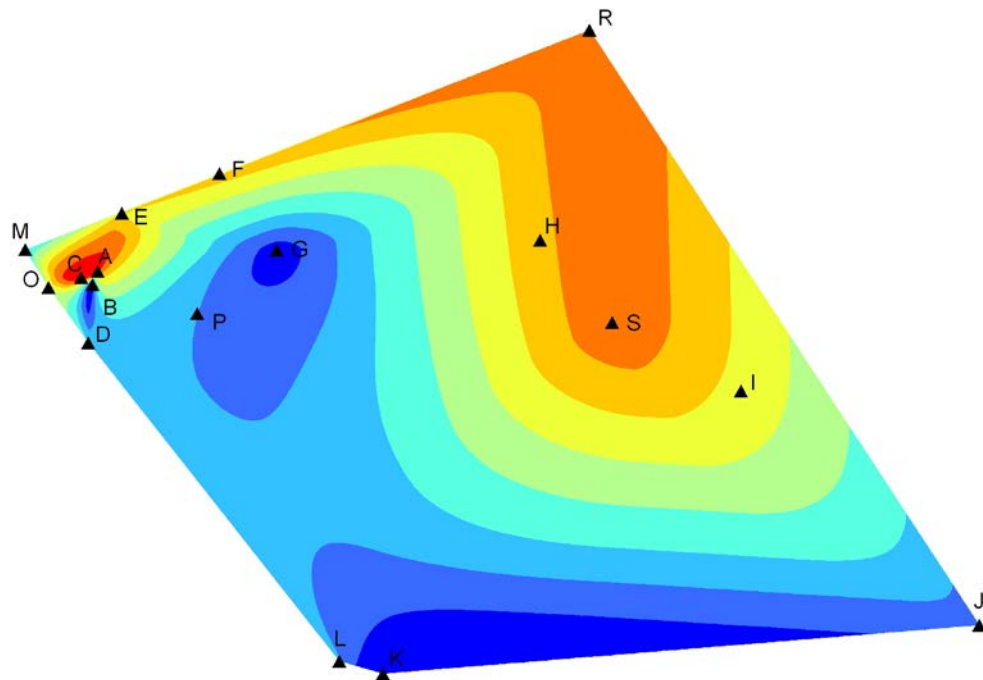


Figure 3.4.2 Natural Neighbour Interpolation map using ϕ (π) Values.

Haplotype Locations A: Baaluc Rd. North (D), B: Baaluc Rd. South (D), C: Norn Rd. (D), D: Bradford Block (D), E: Gura Rd. (D), F: Penny Block (D), G: Candy Block (D), H: Commondine Reserve, I: Birdwhistle Reserve, J: Dongolocking Reserve, K: Narrakine Block Highbury, L: Warren Rd. Highbury, M: Skelton Block (D), N: Wandering Rd. (D), O: Mangart Rd. (D), P: Borraning Block (D), R: Strathern Rd, S: North Yillimining Reserve (D)=Dryandra woodlands.

3.4.3 Phylogeography of Rufous Treecreeper Populations

Constructing a genealogy of Rufous Treecreepers gives insights into the past evolutionary processes of shared ancestry. The coalescence method of analysis was conducted using the highly conserved cytochrome b gene (*cytb*). The unrooted haplotype tree presents the central haplotypes (3&5) as the earliest ancestral haplotypes and the haplotypes positioned at the tips of the tree (Haplotypes 2,8,7 & 4,1,9,6) are the most recently mutated haplotypes. The outlier (Hap.0) from Norseman is situated approximately 500 km away and is more distantly related to all other haplotypes.

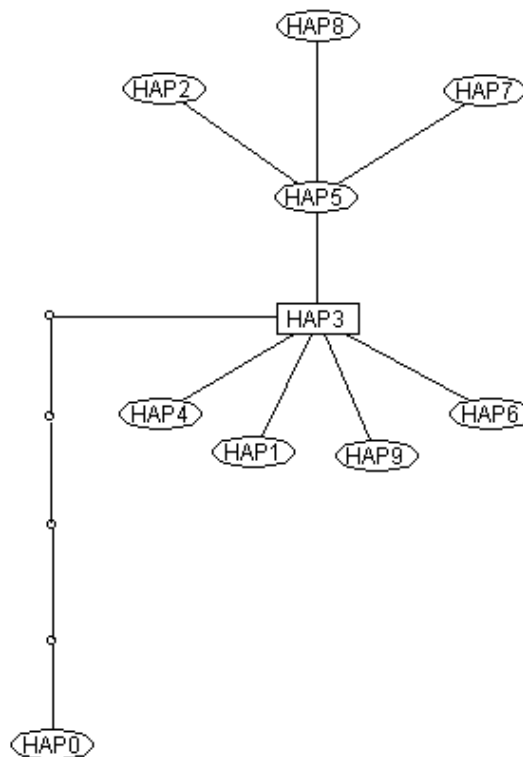


Figure 3.4.3 Coalescence of RTC mitochondrial DNA. Haplotype Identification: **Hap1-** Norn Rd. (D), **Hap2-** Norn Rd. (D), **Hap3-** Norn Rd. (D), Bradford Block (D), Baaluc Rd. Sth (D), Baaluc Rd. Nth (D), Knights Lane (D), Stratherne Rd. (Wickepin), Narrakine Block (Highbury) and Dongolocking Reserve, **Hap4-** Skelton Block (D), and Mangart Rd. (D), **Hap5-** Bradford Block (D), Stratherne Rd. (Wickepin) and Nth.Yilliminning Reserve, **Hap6-** Gura Rd.(D), **Hap7-** Baaluc Rd. Sth (D), **Hap8-** Stratherne Rd. (Wickepin), **Hap9-** Nth Yilliminning Reserve, Warren Rd. (Highbury) and Dongolocking Reserve, **Hap0-** Norseman., (D)-Dryandra woodlands

3.5 ECOLOGICAL NICHE, CLIMATE CHANGE AND POPULATION VIABILITY

The ecological niche of woodland avifauna was investigated utilising different methods. A distance based Multivariate Analysis was conducted on the Rufous Treecreeper microsatellite and mitochondrial DNA, with geographical variables such as slope, aspect as well as fragmented or continuous habitat. This study was conducted to find a relationship between abiotic landscape features and historical genetic divergence patterns. This link between a species and a habitat characteristic can help to explain the past distribution of a species or for planning where a species may be well adapted to in the future. Also, a habitat suitability map based on old growth *E.wandoo* forest was constructed from a vegetation survey of Dryandra (Coates, 1995) and GIS mapping techniques. Combined with known habitat requirements and demographic information from a previous study (Luck, 2002, 2001) an estimate of the number of Rufous Treecreepers that are able to reside within Dryandra woodlands was made based on habitat suitability. This model was intended as a management tool for monitoring number of declining populations or for future translocation and revegetation projects. Also, a further investigation into the impact of climate change on the forest habitat and Rufous Treecreepers was conducted by statistical analysis of rainfall data, percentage tree foliage cover and avifaunal captures. A linear regression was conducted on tree foliage cover and annual rainfall data and a logistic regression was conducted on species capture data and tree foliage cover. This analysis intended to find a relationship between all three variables. Population viability was assessed with an apparent survival rate from mist net capture recapture data and by comparing a survival rate from a previous study (Luck, 2000). Temporal changes in habitat quality (foliage cover) over the same time frame, was also compared.

3.5.1 Distance Based Redundancy Analysis of Rufous Treecreeper Microsatellite and Mitochondrial DNA

Mitochondrial cytochrome b sequences from RTC individuals at specific locations and microsatellite DNA were used in a Distance Based Redundancy Analysis, DISTLM v.5 (Anderson, 1994). Genetic distances were used as predictor variables, geographic distances as covariables and slope, aspect (direction of slope) and habitat fragmentation (continuous habitat or not) as response variables. The analysis was based on microsatellite (Nei's 1978) and mitochondrial phi (π) (Nei's 1987) genetic distances, and geographical Euclidean distances. The permutation of residuals (full model) and 9 999 permutations for each calculation were used. Mitochondrial genetic distance was found to be most significantly correlated to aspect combined with slope and explained 29.16% of the genetic differentiation. There was no correlation between microsatellite DNA genetic distance (Nei's 1972 Distance) and other tested Kinship estimates.

Table 3.5.1 A distanced based redundancy analysis results show mitochondrial distances are significant to response variables of slope and aspect.

	Aspect	Slope	Habitat Fragmentation	Slope & Aspect	Habitat Fragmentation & Aspect
Microsatellite DNA					
P value (permutation)	0.998	0.978	0.960	0.981	0.992
Proportion of variation explained %	74.30	87.02	54.64	86.97	63.50
Mitochondrial DNA					
P value (permutation)	0.049	0.084	0.086	0.042	0.054
Proportion of variation explained %	28.20	25.36	23.33	29.16	26.36

3.5.2 Habitat Suitability and Estimate Number of Rufous Treecreepers

The habitat suitability map of the Rufous Treecreeper within the Dryandra woodlands shows remaining *E. wandoo* forest in black. These areas have been identified as extremely important nesting habitat for the Rufous Treecreeper (Rose 1993). Also using information of the Rufous Treecreeper from a previous study (Luck, 2001, 2002) and RAMAS GIS (Akcakaya, 2002), it was estimated that the Dryandra contained enough suitable habitat for a maximum of 158 populations or 1 106 individuals.

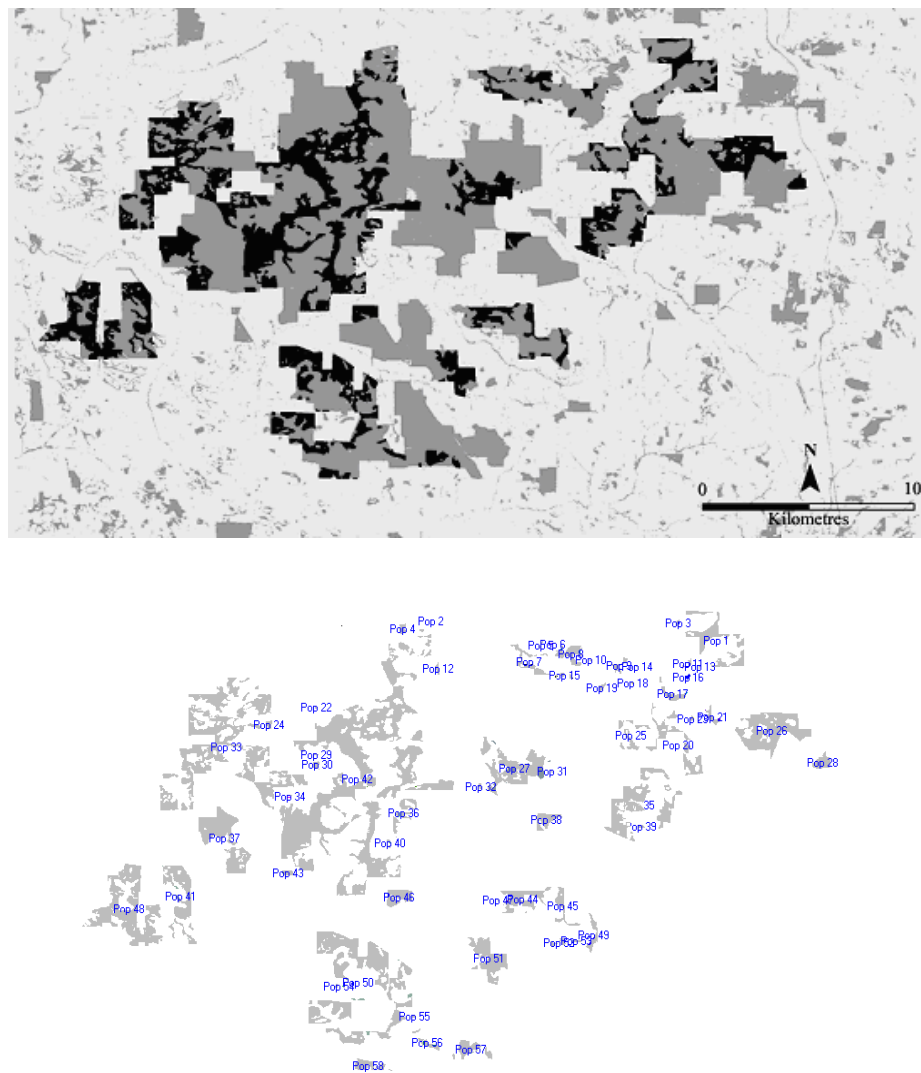


Figure 3.5.2 Habitat Suitability map (above) with an estimate of 158 maximum populations (groups) able to inhabit Dryandra (below).

3.5.3 Climate Change and Climatic Range

Situated approximately 20kms from the Dryandra Woodlands rainfall data from the Wandering weather station was used as a surrogate data set for the Dryandra woodlands.

It appears the extreme reductions in average rainfall coincide with El Niño climatic events which were during 2002/03, 2006/07 and 2009/10 (BOM, 2015). In 2010 the rainfall reached 277.4mm, which fell below the climatic range (350-1000mm) of *E.wandoo* (Zdunic, *et al.*, 2012 Yates, *et al.*, 2000). Using a long term average rainfall measurement of 488.93mm (BOM, 2010), trajectories to 2030 and 2070 were estimated from climate modelling that predict decreases of up to 20% and 60% respectively (CSIRO, 2005).

Annual Rainfall Measurements and Trajectories

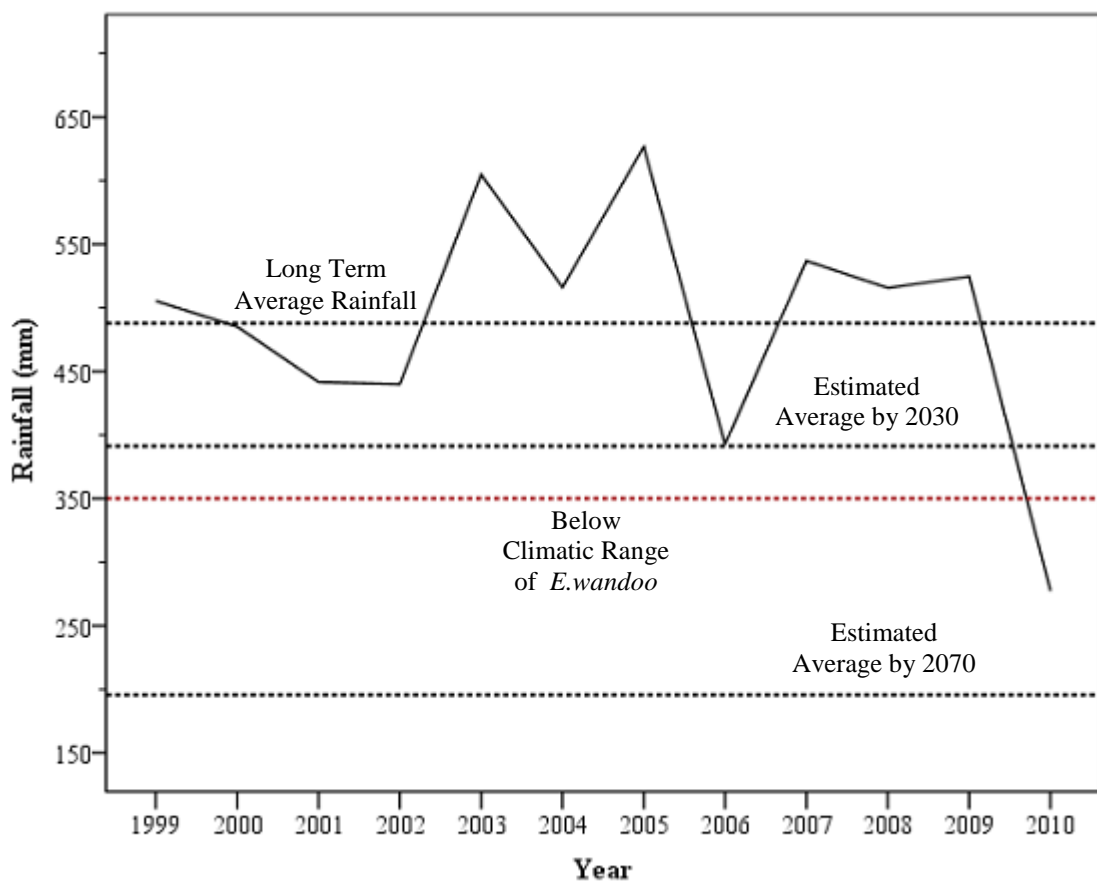


Figure 3.5.3 Annual rainfall measurements with long term averages and minimum climatic range (red line) of *Eucalyptus wandoo*.

3.5.4 Foliage Cover and Critical Threshold

The graph below shows the percentage foliage cover of *Eucalyptus wandoo* at each 1 hectare sampling site, within the Dryandra Woodlands. This data was obtained through land satellite imagery and converted into Projected Foliage Indices, or percentage foliage cover (Behn, 2011). During bird sampling period between 2003 and 2007, there appears to be a clear pattern of an increase in foliage cover, followed by a decrease in the following year. However, Skelton Block site 3 (shown in black) does not appear to recover foliage cover above 11.53%, after a reduction to 7.73% in 2003. This appears to be a critical threshold of foliage cover for this species within Dryandra.

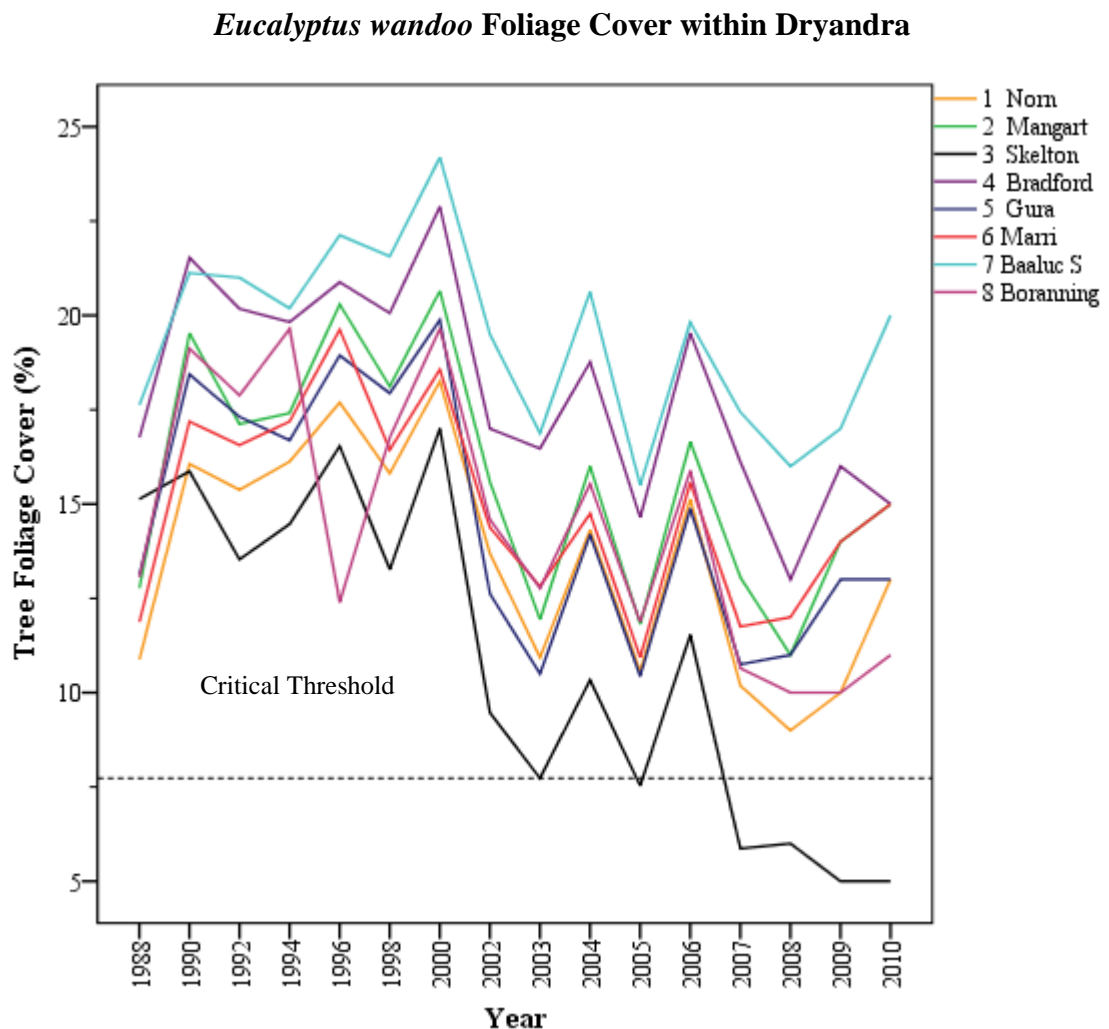


Figure 3.5.4a Percentage tree foliage cover of all 1 hectare sampling sites within the Dryandra woodlands.

3.5.5 Avifauna Captures

The capture re-capture data for the Rufous Treecreeper shows a total of 162 individuals encountered over 5 sampling times between 2003 and 2007. A recapture rate of 16.66% was found. RTC data shows highest populations in Norn and Brad, with Skelton and Baaluc S the only sites with no recaptures.

Table 3.5.5a Rufous Treecreeper captures and re-captures for the 8 sampling sites at the Dryandra woodlands

Location	Sampling Times (Encounters)					Captures	Recaptures	Total Individuals Captured
	Oct 2003	Sep 2004	Oct 2004	Nov 2004	Oct 2007			
Norn	8	10	1	5	5	29	4	25
Mangart	4	5	1	1	4	15	2	13
Skelton	2	1	5	0	10	18	0	18
Bradford	4	18	5	3	11	41	2	39
Gura	6	5	6	4	7	28	5	24
Marri	5	3	3	2	7	20	4	16
Baaluc S	3	0	3	1	1	8	0	8
Boranning	0	12	6	4	7	29	10	19
Total	32	54	30	20	52	188	27	162

The capture re-capture data for the Yellow-plumed Honeyeater shows a total of 216 individuals encountered over 5 sampling times between 2003 and 2007. A recapture rate of 12% was found. YPH data shows highest populations in Gura Rd where a flock of Honeyeaters were feeding on flowering Eucalyptus at the time of sampling.

Table 3.5.5b Yellow-plumed Honeyeaters captures and re-captures for the 8 sampling sites within the Dryandra woodlands.

Location	Sampling Times (Encounters)					Captures	Recaptures	Total Individuals Captured
	Oct 2003	Sep 2004	Oct 2004	Nov 2004	Oct 2007			
Norn	6	17	3	1	2	29	4	25
Mangart	2	20	4	3	4	33	4	29
Skelton	2	5	9	0	2	18	1	17
Bradford	8	17	6	7	9	47	11	36
Gura	3	16	42	4	8	73	3	70
Marri	6	5	1	4	11	28	1	27
Baaluc S	1	0	1	1	0	3	0	3
Boranning	0	8	3	0	0	11	2	9
Total	28	88	69	20	34	242	26	216

The complete species capture data set, from 5 sampling occasions between 2003 and 2007 across 8 sampling sites in the Dryandra woodlands, was assessed. A correlation of capture data shows a strong positive relationship between Rufous Treecreepers and Yellow-plumed Honeyeaters netted on each occasion. A Pearson Correlation (2-tailed test) was conducted (SPSSv22) and resulted in a correlation coefficient of 0.58 at a significance level of 0.01. Both species show a positive (non-random) relationship to each other across sampling sites and since the trapping effort at each site was consistent, the relationship between species captures at each site was significant.

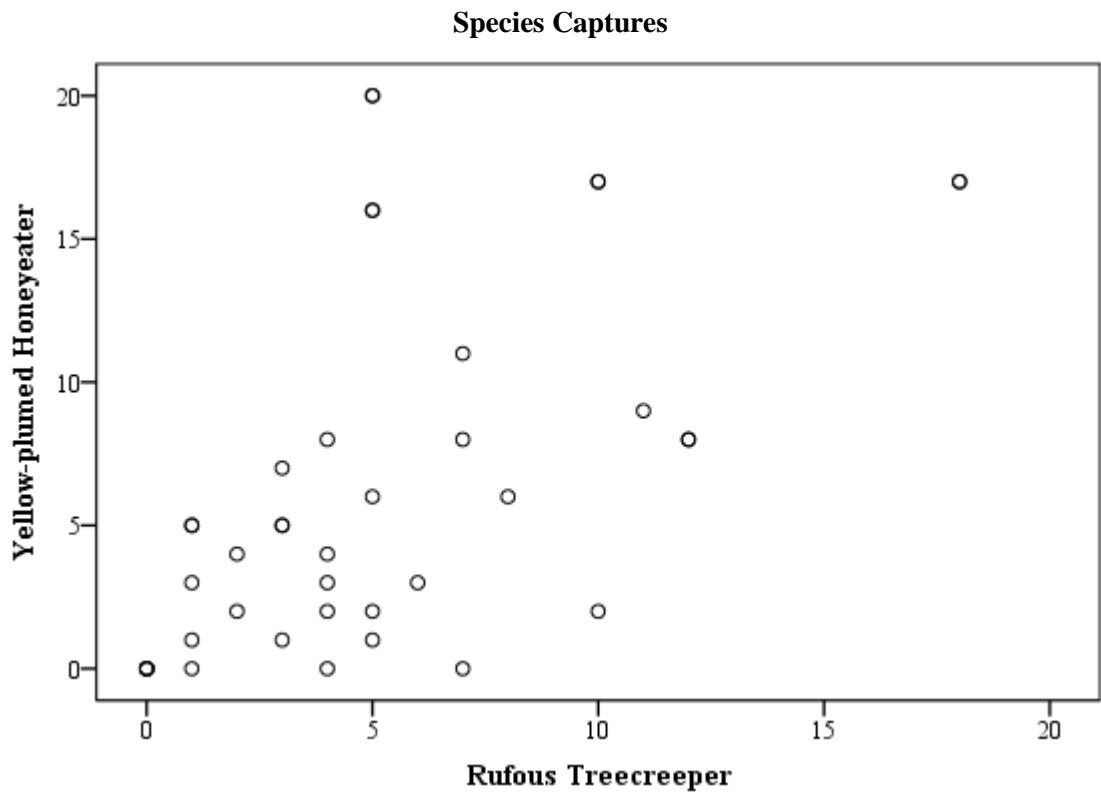


Figure 3.5.5 Scatter plot of Rufous Treecreeper and Yellow-plumed Honeyeater captures.

3.5.6 Regression of Rainfall, Foliage Cover and Captures

When combined species captures is plotted against rainfall and mean foliage cover, a pattern of increased bird captures with increased foliage cover and a decrease in captures with a decrease in foliage cover appears. Also during 2003 and 2007, an inverse relationship between rainfall and foliage cover was clearly observed.

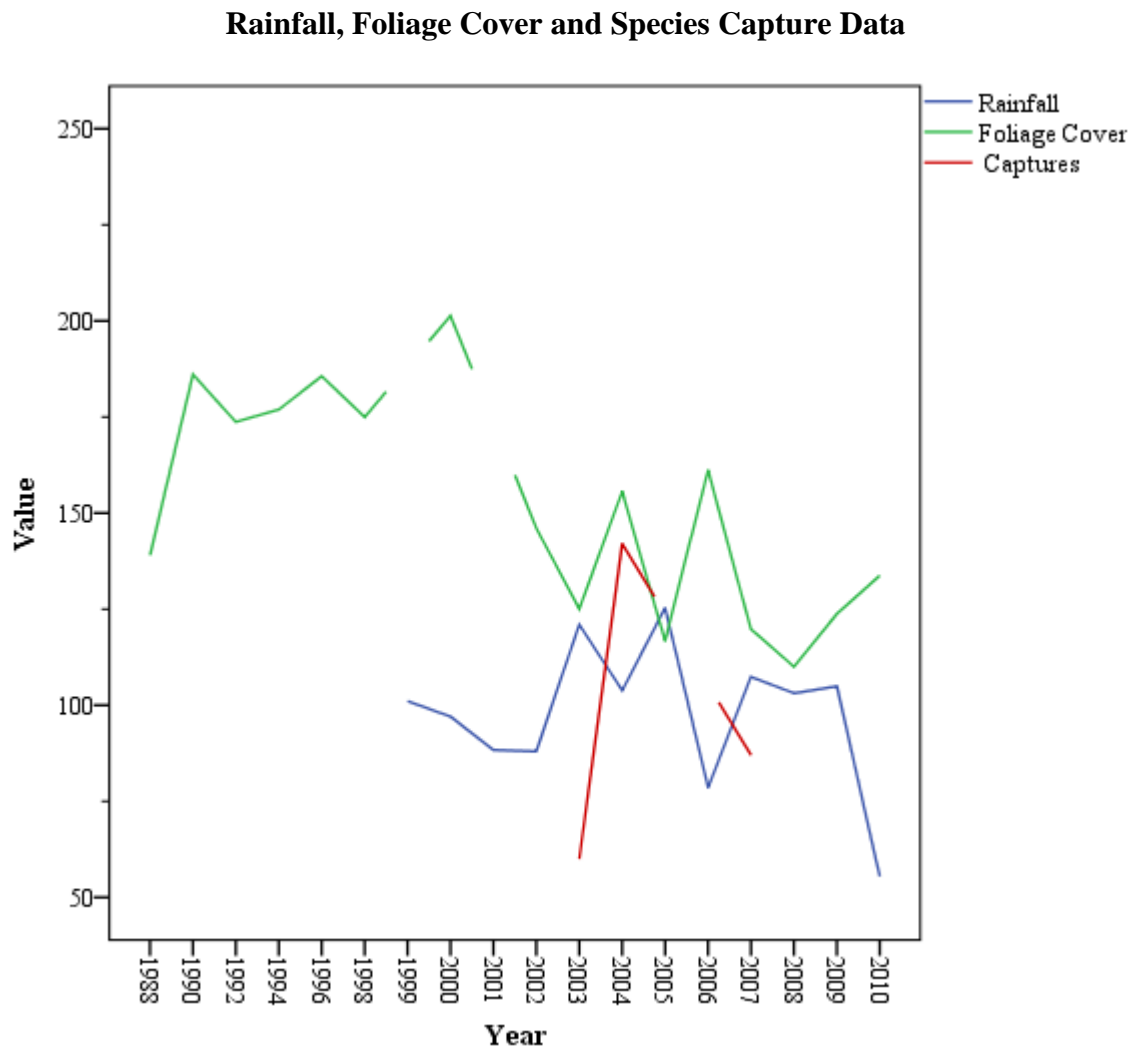


Figure 3.5.6a The Y axis value represents total RTC and YPH captures, rainfall data as 10^{-1} mm x 2 and % foliage cover as 10^{+1} , so that all three variables could be measured by the same scale.

A further analysis of species captures, foliage cover and rainfall was conducted. A logistic regression (General Linear Model) examined percentage foliage cover at each site, within each year (coveryear) as a predictor of the number of captures per unit trapping effort for the Rufous treecreeper (RTC) and Yellow-plumed Honeyeater (YPH) and also the total of the two species. In the case of LogYPH and the case of Log(YPH+RTC) coveryear was not a significant predictor. However, in the case of LogRTC, the coveryear (i.e. cover per site within each year) was a significant predictor ($p < 0.05$)

Table 3.5.6 Type III Tests of Fixed Effects. Shows significance of foliage cover as a predictor of RTC captures.

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	8.263	48.814	.000
coveryear	1	9.546	5.713	.039

Since for the RTC the variation in foliage cover between sites and years is potentially a critical determinant of variation in abundance as measured by the captures per unit trapping effort, the effect of annual rainfall on canopy cover was then investigated.

Linear regressions were calculated for total cover using both current annual rainfall (anrain) and the previous year's annual rainfall (panrain), as the independent variable.

The regression of current annual rainfall on total cover was not significant

($F_{1,6} = 3.385$, $P = 0.115$) whilst the regression coefficient of previous year's rainfall was ($F_{1,6} = 7.278$, $P = 0.036$).

Regression of Annual Rainfall and Foliage Cover

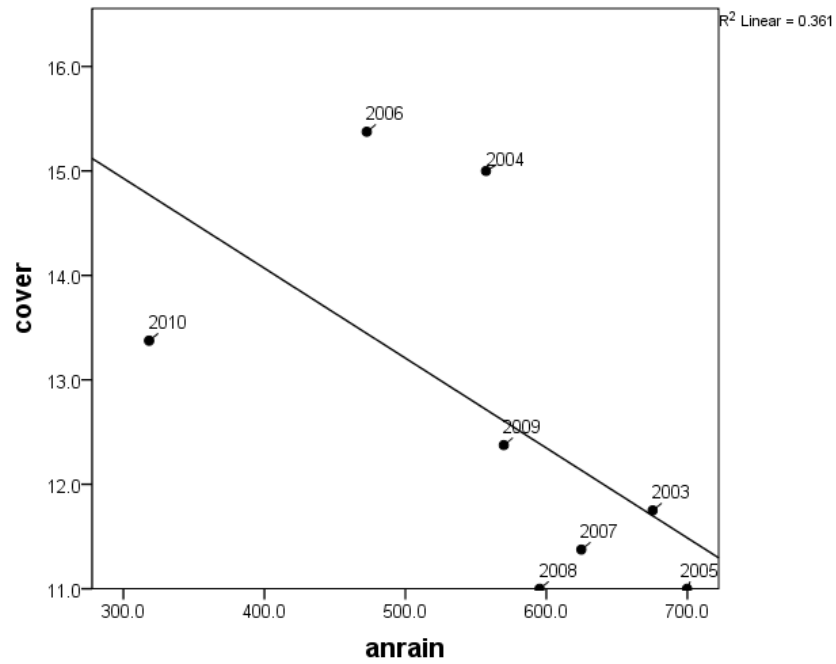


Figure 3.5.6b The regression line of total cover on current annual rainfall (anrain) shows the regression coefficient is not significant.

Regression of Previous Years Rainfall and Foliage Cover

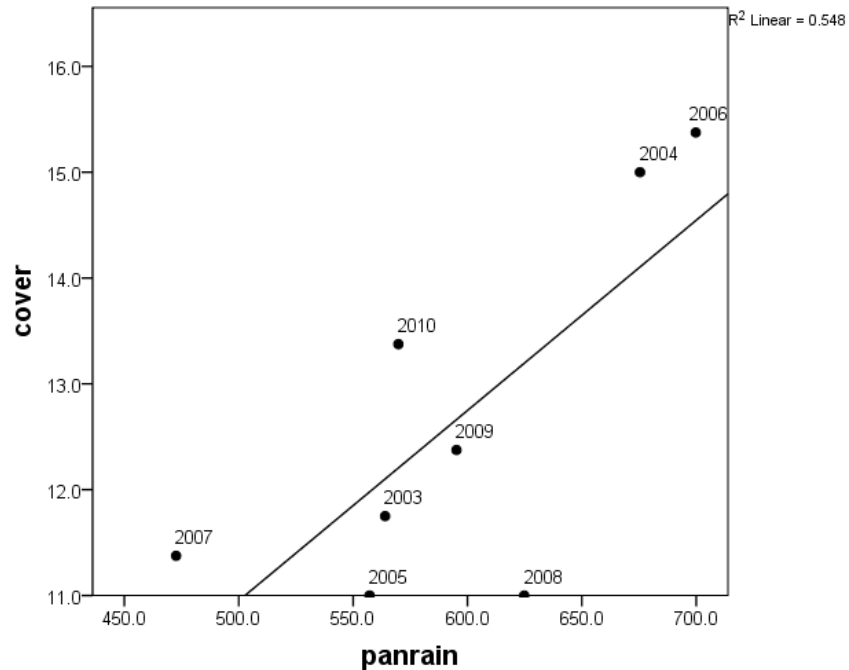


Figure 3.5.6c The regression line of total cover on the previous year's annual rainfall (panyear) shows the regression coefficient is significant.

3.5.7 Avifauna Viability Analysis

Population survival estimates were calculated with capture recapture data, using the MARK software program (White & Burnham, 1999). The Rufous Treecreeper and Yellow-plumed Honeyeater data was collected from the Dryandra woodlands over the total 5 separate sampling times or encounters during 2003, 2004 and 2007 (Tables 3.5.5a and 3.5.5b). The apparent survival rates of combined males and females were calculated to be 0.653 for the Rufous Treecreeper and 0.303 for the Yellow-plumed Honeyeater.

Table 3.5.7 Apparent Survival Rates of the Rufous Treecreeper and Yellow-plumed Honeyeater. The apparent survival estimate (Phi) is based on a constant survival rate and constant recapture population model, with a 95% confidence interval.

Species	Estimate (Phi)	Standard Error	Lower	Upper
Rufous Treecreeper	0.653	0.131	0.375	0.854
Yellow-plumed Honeyeater	0.303	0.086	0.162	0.493

Luck (2000) estimated the adult survival rate of the RTC within the Dryandra woodlands to be 0.77 (± 0.06) for primary males and 0.75 (± 0.05) for primary females. In this study (8 years later), an apparent survival rate of 0.65 (SE 0.13) was found for both male and female Rufous Treecreepers in Dryandra. Also, the average foliage cover across all sampling sites during the same time period had decreased 4.72% (from 17.62% to 12.90%). This infers that the Rufous Treecreeper is declining in response to a reduction in foliage cover and quality of habitat.

CHAPTER 4 - DISCUSSION

4.1 Spatial Analysis and Population Structure of Microsatellite DNA

The spatial genetics of Rufous Treecreeper (RTC) populations were assessed using geographic distance and various methods of microsatellite analysis. Since this investigation began, rapid developments in sequencing technology has increased and improved the speed and efficiency of genetic analysis (Sommer, *et al.*, 2013). However, some conventional methods of sequence and fragment analysis are still used and the Sanger Sequencing method is still preferred when using known, species specific microsatellite primers (Li, *et al.*, 2015). Next generation Sequencing is highly recommended for finding microsatellite loci across broad range of taxa (Gardner, *et al.*, 2011) and for the analysis of genetic diversity of populations (Davey & Blaxter, 2011). More recently, powerful Transcriptomics technologies such as DNA microarrays and RNA sequencing can measure genome wide gene expression in large numbers of individuals and can explore the specific genome basis of phenotypic variation and rapid response to environmental change (Alvarez, *et al.*, 2015).

Different spatial genetic models were applied to the DNA data used to measure how the Rufous Treecreeper populations are related and distributed within a continuous and fragmented habitat system. Dispersal patterns and processes that underlie population structure were investigated by measuring gene flow and by measuring the differences between and within populations. Wagner & Fortin (2013) suggest several levels of spatial analysis methods which are assessing data at localised sites, gene flow between sites, connectivity of a population with all other populations, identifying local

neighbourhoods and the delineation of genetic boundaries. This study as well as other similar genetic studies have investigated species spatial distribution and include song sparrows (Wilson, *et al.*, 2011), north American tree swallows (Stenzler, *et al.*, 2009), the northern spotted owl (Funk, *et al.*, 2008) and Florida scrub jays (Coulon, *et al.*, 2010).

4.1.1 Genotyping and DNA Analysis

Primarily, the quality of the microsatellite data was assessed and edited before it could be statistically tested and applied to population models. The fragment length analysis of microsatellite DNA discovered an unusual allele pattern with a triple peak for microsatellite 5 (Figure 3.1.2b). These peculiar patterns are possibly due to some kind of chromosome duplication of the locus, or a primer point mutation (Butler, 2005, Zamir *et al.*, 2002). Further investigations need to be conducted to resolve the tri-allelic pattern. This would involve conducting a Southern Blot Analysis, designing new primers or conducting a family (back-crossing) study. The family study can identify the parental genotypes and assortment in progeny. Also, sequence information generated by a cloning procedure would allow specific restriction enzyme sites to be chosen and digest products analysed to ascertain exact microsatellite copy numbers. Also, the sequence information can then be used to redesign new primers to bind at sites where there may be a single base mutation.

The Neutrality Test DETSEL (Vitalis, *et al.*, 2003) was applied to the data set. As DETSEL calculates neutrality based on gene frequency data, a reduced data set with possible null alleles only produced a positive result for locus 2 (Figure 3.1.3). As the neutrality test could not be completed on all Rufous Treecreeper (RTC) microsatellite

loci, therefore the results are inconclusive and the possibility of selection processes within the data remain. However, since the primers for the RTC were re-designed from the Brown Treecreeper primers (Doer, 2004, pers.com) which were tested for neutrality, it is highly probable the same neutral loci were targeted in the same genus.

Null Alleles and Inbreeding Coefficients for the Rufous Treecreeper microsatellite data population was tested using a Bayesian approach INEST 2.0 (Chybicki, 2014). The model that best fit the data was the genotyping failure, inbreeding and null allele model (Table 3.1.5). This shows that within the data set, there are some discrepancies between observed genotypes and true underlying genotypes (Wagner, et al, 2006). A genotype error of 1%, which is an uncommonly good value for most studies, can lead to a substantial number of incorrect multilocus genotypes, in a large data set (Selkoe & Toonen, 2006). Also, (Wagner, et al., 2006) found that ignoring null alleles in a study of striped hyenas found to underestimate the relatedness by 20%. Null alleles in data sets are common, widely acknowledged and documented by researchers, but options for dealing with them are limited, time consuming and expensive (e.g. redesigning new primers) (Wagner, *et al*, 2006). The presence of null alleles, genotyping failure and possible inbreeding infers some degree of error in the data set. Therefore, the magnitude of these effects in the experimental data cannot be assessed with any accuracy and a methodology for a proper solution has not been fulfilled.

The permutation test for Heterozygosity Excess and Hardy Weinberg Equilibrium (HWE) was conducted on the RTC microsatellite DNA and found 3 out of 7 loci deviated from HWE. Comparing the Total Rufous Treecreeper Populations (Table 3.1.4a) with a single year of captures (Table 3.1.4b) and the Dryandra Only sample

(Table 3.1.4.c) was to reduce variation in gene frequencies in the data set, but it did not improve results. A deficit of heterozygotes implies less gene flow received into Rufous Treecreeper populations that have become isolated. This deviation of HWE was suspected because of the Wahlund Effect. This occurs where habitat fragmentation creates isolation between other small populations and as these separate populations receive less external gene flow, it causes a sub-structure in the data set (Johnson, *et al.*, 2003). When genetic drift and gene flow are not in equilibrium, this violates a number of assumptions under HWE and for measuring gene flow with F - statistics (Johnson, *et al.*, 2003). However, failure to meet HWE is not typically grounds for discarding loci (Selkoe & Toonen, 2006). For example, Relatedness estimates can be utilised for further analysis and are independent of assumptions of HWE or Wright's Inbreeding Coefficient (Vekemans & Hardy, 2004).

4.1.2 Genetic Diversity

AMOVA shows a significant amount of the variance of RTC microsatellite DNA was explained from within populations (Figure 3.3.1). However when this variation is compared to the variation among all populations, the overall variation is reduced. This infers that gene flow (migration) is exceeding genetic drift and that the small, regionally fragmented populations are most probably dependent on migration as there is high levels of gene flow between them.

4.1.3 Spatial Patterns of Microsatellites

The Isolation by Distance (IBD) analysis for the Dryandra woodlands shows no relationship between genetic similarity and geographical distance (Figure 3.3.2). This is characterised by a non-linear relationship between pairwise kinship values (Ritland's ρ)

and geographical distance. In IBD a linear relationship between pairwise genetic distances and the logarithm of geographic distances, works best when the geographic distances are greater than the effective dispersal distances (σ) of the population (Rousset 1997, 2000). This is the most likely reason why no IBD was detected within the Dryandra woodlands. The dispersal distances of the Rufous Treecreepers exceed the geographical distance of the Dryandra woodlands. The pattern of RTC microsatellites shown across the region does not give a positive IBD result either (Figure 3.3.2.1). In recently isolated populations, the levels of gene flow (migration) are less likely to be in equilibrium with regional genetic drift and therefore do not conform to IBD (Johnson, *et al.*, 2003). This study shows that on a larger regional scale, populations residing in small fragmented remnants surrounding the Dryandra woodlands do not follow a gradual stepping stone model of population expansion.

4.1.4 Spatial Scale of Microsatellites

The Mantel Test based on Nei's (1983) genetic distance, shows a positive correlation between genetic distance and geographical distance within a range of approximately 25km (Figure 3.3.3). This analysis shows that there is RTC population differentiation across the Dryandra woodlands and that these populations are not one continuous or panmictic population. It appears that when populations are not under regional equilibrium (HWE) or when experimental data is random or sparse (non-parametric), genetic distance measures are more robust for detecting genetic variation across geographic distances than genetic kinship measures such as in IBD analysis.

Spatial Autocorrelation of RTC microsatellites across the region (Figure 3.3.3.1) describes genetic distance (A_y) as a function of geographical distance. A curve is

formed within the first 5 distance classes, reaching a maximum at approximately 30 km and is indicative of a significant genetic structure within the Dryandra woodlands. However, after approximately 30 km there is a random correlation over 6 distance classes and infers no genetic structure. The spatial correlogram shows a significant genetic structure where populations reside in a continuous habitat and genetic discontinuities in a highly fragmented habitat system, where dispersal is more likely to occur. Spatial Autocorrelation has been used to determine local genetic structure and dispersal in Superb Fairy-Wrens (Double, *et al.*, 2005) White-breasted Thrasher (Temple, *et al.*, 2006), analysis of the spatial structure of continuous populations (Wagner, *et al.*, 2005) and for populations that show Isolation by Distance (Hardy & Vekemans, 1999, Barbujani, 1987).

4.1.5 Spatial Distribution of Microsatellites

The spatial distribution of genetic differentiation of the Rufous Treecreeper (RTC) was conducted with the AIS program (Miller, 2005), which links inter-individual genetic distances to landscape coordinates with an interpolation procedure. The Landscape Interpolation map of the Dryandra woodlands (Figure 3.3.4), shows a clear pattern of higher genetic differentiation in the west and lower diversity in the east. The Landscape Interpolation of regional RTC microsatellites (Figure 3.3.4.1) spanning approximately 100km also shows the same pattern. The Dryandra woodlands contains the highest proportion of RTC genetic differentiation and declines in an easterly direction. This genetic pattern corresponds to increased fragmentation of the RTC habitat in an easterly direction. Also it shows where habitat is continuous there is more genetic differentiation and as fragmentation increases, genetic differentiation declines. In a similar study on the Threatened northern spotted owl, Landscape Interpolation was used to show that

landscape features (high elevation mountains and dry valleys) can strongly affect gene flow and genetic variation (Funk, *et al.*, 2008). However, a limitation of this technique is that although it identifies geographical areas of low and high inter-individual genetic divergence, it does not test the effects of these landscape features on the genetic structure within populations (Funk, *et al.*, 2008).

4.1.6 Population Structure of the Rufous Treecreeper

To investigate the genetic structure within the Dryandra woodlands, the Maximum Difference Delaunay Triangulation (Chapter 3.3.5) was used to detect genetic boundaries of populations with small geographical distances between them. The results from the Maximum Difference Delaunay Triangulation method show a genetic boundary between Norn and Mangart sites, separated by a geographical distance of only 1.3km. This method was useful for the detection of a structure (genetic boundaries) within a group of populations that reside geographically in close proximity to each other. Luck (2001) observed a social characteristic of the Rufous Treecreepers, in which they formed co-operatively breeding groups. This explains the genetic barrier that exists between breeding individuals from different family groups, living in close proximity to each other. They may not breed with each other, but they do benefit from living next to each other by relaxing territory defence to non breeders during breeding times, to help feed other nestlings (Luck, 2001). Other studies have used this method for genetic boundary detection in spotted owls (Funk, *et al.*, 2008), pumas (Safner, *et al.*, 2011), and in humans (Manni, *et al.*, 2004).

The structure of regional populations of Rufous Treecreeper microsatellite DNA was based on a past history of populations Approximate Bayesian Computation model and

conducted with *DIYABC* (Cornuet, *et al*, 2013). Populations were grouped into 4 distance classes of 0-20, 20-30, 40-60 and 60-80 km, from a sampling site in the centre of the Dryandra woodlands. The Stepping Stone Model and the Continent Island Model of population expansion were the two most likely evolutionary scenarios that best fit the data (Figure 3.3.5.1). These models were compared by a direct and logistic approach and resulted in the preference of the Continent Island population Model, with the higher posterior probability (Table 3.3.5.1).

The study demonstrates the microsatellite population structure resembles the Continent Island Model. Although the Continent Island and Island –Mainland metapopulation models both describe a mainland that provides a source of colonists to nearby populations of varying size and isolation (Attiwill and Wilson, 2003), the Continent Island Model also assumes reciprocal gene flow having a negligible effect on the allele frequency in the source population (Hedrick, 2000). This study shows at different distances up to 80km, the regional Rufous Treecreeper populations all share a common and recent ancestor from Dryandra. Also, it cannot be assumed that some individuals do not return to Dryandra, as the Rufous Treecreepers are known cooperative breeders that belong to family groups (Luck, 2001). Other studies that have used Bayesian modelling for the analysis of genetic population structure and differentiation between populations include Pritchard, *et al.*, 2000, Corander, *et al.*, 2008 and Guillot, *et al.*, 2005.

4.1.7 Dispersal Patterns of the Rufous Treecreeper

Ritland's (1996) Kinship Coefficient was used to resolve the genetic dispersal patterns within Dryandra because it is based on a continuous population model and suitable for

the main block of Dryandra. A Neighbour Joining tree was constructed with the Ritland's Kinship coefficient values and shows a mixed population made up of seven distinctive lineages (Figure 3.3.6). Individuals from Gura site appear to be the most widely distributed, followed by Norn and Bradford sites. Since individuals from these sites are the most widely distributed across Dryandra, they are most likely to have a higher rate of breeding success. The blue lineage has exclusive members consisting of three adjacent populations at Norn, Baaluc (south) and Baaluc (north). The geographic distances between them are Norn to Baaluc (south) is 2.6 km, Norn to Baaluc (north) is 2.9 km and Baaluc (south) to Baaluc (north) is 1.07 km. The genetic similarity of these neighbours, form an exclusive lineage with close kinship values. The Rufous Treecreeper populations within the central Dryandra woodlands appear to be structured into a collection of discrete family groups sometimes forming larger genetic neighbourhoods of up to approximately 3km in distance (Figure 3.3.5 and 3.3.6). In a demographic study of the Rufous Treecreeper in Dryandra, Luck, (2001), found the social organisation of the Rufous Treecreeper was based on neighbourhoods of interacting territories.

The Loiselle (1995) Kinship Coefficient was used to measure the spatial scale of inheritance throughout the regional area because it is similar to the product of Moran's *I* Spatial Autocorrelation Statistic (Vekemans & Hardy, 2004), but also it summarises the strength of kinship between pairs of individuals, as a function of their geographical distance (Arnaud, et al., 2001). Therefore this model is useful for resolving dispersal patterns in spatially discontinuous populations, over a large geographical area. The range of distances between sampling sites in this study vary between approximately 1.5 km and 85 km. The constructed Loiselle (1995) Kinship Neighbour Joining tree (Figure

3.3.6.1) shows individuals from the Dryandra woodlands, in each of the 9 main lineages, with Norn Rd and Gura Rd individuals being the most widely spread (Figure 3.3.6.1). Dispersal patterns from Ritland's Kinship Tree, also confirms that the Gura site is a major source population within the Dryandra woodlands.

The spatial autocorrelation of microsatellites results show that after a distance of approximately 30 km, there is a loss of genetic structure (Figure 3.3.3.1). This suggests that beyond the Dryandra woodlands, there is less genetic differentiation and a higher proportion of dispersal. The Loiselle Kinship analysis method is useful because it can not only detect the smallest genetic distances between individuals, for example 0.0019 from Dryandra and North Yilliminning (42.3km) (Figure 3.3.6.1a) and 0.0031 for Dongolocking and Narrakine (48.6 km) (Figure 3.3.6.1b), but it is especially useful for finding the exact geographical locations of dispersal. Based on bird banding studies, data suggests that the Rufous Treecreeper have a movement ability of 12 km (BTO, 2014 and ABBBS, 2014). However the results from this study show that Rufous Treecreepers are able to disperse across a range of up to approximately 50kms within a highly fragmented habitat system.

A bias towards female dispersal was found within Dryandra but not in the smaller outer remnant habitats. This can be seen in the interpolation map of the distribution genetic distances for females (Figure 3.3.7). The regional sites show the highest levels, or peaks (z value) of genetic distance, whereas within the Dryandra woodlands genetic divergence is very low, even when compared to the males in Dryandra. Luck, (2001) also found the dispersal of juveniles to be female biased in a demographic study in Dryandra. This study found the unidirectional movement of gene flow from the

Dryandra woodlands out to smaller, more isolated habitat that follows a Continent Island Population model of population expansion (ABC Model, Figure 3.3.5.1 and Figure 3.3.6.1). Therefore the Rufous Treecreepers in Dryandra appear to be a highly organised collection of populations that constantly replenish themselves (Ritland's Kinship coefficient, Figure 3.3.6) and provide a source for other smaller populations throughout the region. However the majority of dispersal of males and females that re-populate other smaller and isolated habitats, would not all become breeders because of limited nesting hollows, higher competition and lower reproductive success in these remnants (Luck, 2001). Therefore the increased the genetic divergence estimates of females in these outer remnants, is most probably because of habitat saturation.

4.2 Spatial Analysis and Population Structure of Mitochondrial DNA

A total of 25 individual Rufous Treecreeper cytochrome b sequences (761bp) were grouped into 10 different haplotypes for analysis (Appendix 5). Since mitochondrial DNA is highly conserved, it shows an adherence to the genetic patterns of ancestral DNA (Avise, 2000). Therefore it was this region that was used to find the historical range of this species, prior to land clearing. The geographical distribution of mitochondrial DNA was undertaken by a Mantel's Test, spatial interpolation of genetic distances, a genealogy based on coalescence and an assessment of geographical distribution of haplotypes. The preservation of genetic diversity assessed by mitochondrial haplotypes is essential for maintaining long term evolutionary potential of the species.

4.2.1 Spatial Analysis of Mitochondrial Genetic Distances

The Mantel Test of RTC mitochondrial DNA (part cytochrome *b* gene), was measured by three different geographic distance to identify correlates of genetic distance (Chapter 3.4.1). At a 20 km range a negative regression was found ($r = -0.198$) and at 80 km, the regression line is neither positive nor negative (-0.003). Using an outlier sample from Norseman, 500 km away from Dryandra the Mantel Test shows there is a strong positive correlation (0.798) only at a much greater distance. Therefore the Mantel's Test detected at least 2 different bioregions of this species within a range of 500kms.

The Landscape Interpolation and Interpolation of PCA scores using the Nearest Neighbour method (GIS), provides a geographical assessment of the distribution of mitochondrial differentiation across the landscape (Figure 3.4.2). Mitochondrial DNA using genetic distance ϕ (π) shows very high genetic differentiation in the Dryandra woodlands, Stratherne (Wickepin) and North Yilliminning sites and low differentiation for regional populations in Highbury, and Dongolocking sites (Southern Region). It is highly likely that these southern populations have experienced a vast reduction in population size during broad scale land clearing events of the past, which has reduced the genetic differentiation in remaining populations. The long term consequences of this contraction of population size and range, has most likely reduced the genetic diversity of the once continuous populations in this area. Miller, *et al.*, (2006) in a phylogeographic study of Red Tree Voles, also used this landscape interpolation method to find discontinuities of genetic variation which corresponded to separate groups of haplotypes that were distinct from others.

4.2.2 Phylogeography of Rufous Treecreeper Populations

Coalescence of RTC mitochondrial DNA, cytochrome b sequences was constructed to assess the genealogies of the Rufous Treecreeper populations (Figure 3.4.3). An additional haplotype 0 from Norseman approximately 500km from Dryandra was included as an outlier for the rest of the data set. As expected this haplotype (Norseman) shows the greatest evolutionary distance from all other haplotypes. Basically, the tips of the coalescent tree organises haplotypes into groups that share a common ancestry with the earliest ancestors, which are located at the centre of the tree. The results of this study show haplotype 5 as the genealogical ancestor to clades 2, 7 and 8 and haplotype. Haplotype 3 is the most commonly shared haplotype and is genealogical ancestor to clades 4, 1, 6, 9 and 0. This method has been widely used for genealogy studies including Alexe, *et al.*, (2008) to find a mitochondrial phylogeny of clades in human populations.

In order to assess the historical range prior to land clearing, different haplotypes were grouped into their geographical locations (Table 3.4.3). The geographical distribution of the most widely distributed haplotype (Hap 3) has a geographical range from Dryandra to Dongolocking. This infers that prior to land clearing the Rufous Treecreeper in this region had a continuous habitat that facilitated a dispersal range of approximately 85 km. Pariset, *et al.*, (2011), discovered mitochondrial genetic patterns in sheep that reflected old migrations that occurred in historical times. Also, Deiner *et al.*, (2011) in a mitochondrial phylogeographic study of the Little Shrike-thrush (*Colluricincla megarhyncha*) and thirty sub-species, found that dispersal distance and range size are positively correlated across lineages. This study also discovered a significant rare ancestral haplotype (Hap 5) from the Dryandra and Yilliminning sites.

Haplotypes 3 and 5 are extremely important for the long term conservation of genetic diversity in this region (Figure 3.3.4 and Table 3.3.4).

4.3 Ecological Niche, Climate Change and Viability of Avifauna

Based on Ecological Niche Modelling, abiotic preferences of a species were investigated with a distance-based Redundancy Analysis (db-RDA) and found mitochondrial diversity to be significantly linked to slope and aspect (Table 3.5.1). The distribution of RTCs within the Dryandra woodlands was mapped according to nesting sites provided by old growth wandoo forest. This habitat requirement was mapped as an indicator of Habitat Suitability and applied with other parameters to estimate a maximum number of populations within Dryandra. This study also assessed the impacts of climate change on rainfall patterns, habitat quality and the viability of Rufous Treecreeper (RTC) and Yellow-plumed Honeyeater (YPH) populations within Dryandra. A combination of annual rainfall data, remote sensing data of tree foliage cover and bird survival estimates based on capture-recapture data was used to conduct the analysis.

4.3.1 Distance Based Redundancy Analysis

Initially RTC microsatellite data was applied to a distance-based Redundancy Analysis (db-RDA) using Kinship Coefficients of Loiselle (1995) and Ritland (1996) and Genetic Distances of Nei (1972, 1978) and Rousett (2000). There was no correlation found between RTC microsatellite DNA and slope aspect, percentage foliage cover or connectivity of habitat within Dryandra. However, the db-RDA did find significant relationship of mitochondrial DNA using Genetic Distance measure ϕ (π) (Table 3.5.1). Rufous Treecreepers are dependent on old growth *Eucalyptus wandoo* (Rose,

1993, Luck, 2000) and since *E. wandoo* are associated with yellow duplex soils and a slope landform (Yates, *et al.*, 2000), slope and aspect do explain a geophysical preference by *E. wandoo* that is also linked to historical divergence patterns of the Rufous Treecreeper. Since slope and aspect are linked to the Rufous Treecreeper's mitochondrial divergence estimates, these elements may have caused changes in the genome (adaptive traits) which are linked to specific habitat conditions. Therefore they may also play an important role in planning future habitat such as building revegetation corridors as well.

Distance base Multivariate Analysis in ecological studies has been criticised for confounding location and dispersal effects of multi species or community data (Warton, *et al.*, (2012). However this study involved the analysis of a single species only and was based on the potential location of past populations rather than present dispersal patterns. Also, other methods of multivariate analysis include Jombart, *et al.*, (2010), Jombart, *et al.*, (2009) and Jombart, *et al.*, (2008). These multivariate methods were not suitable for this study as they either require large data sets or assign individuals into clusters to reveal only the spatial genetic patterns of populations. However by using a distance based Redundancy Analysis (db-RDA), it examines how much of the variation in one set of variables explains the variation in another set of variables and is therefore better suited to assessing a combination of different ecological elements with genetic patterns.

4.3.2 Habitat Suitability and Estimate Number of Rufous Treecreepers

Habitat Suitability map was combined with species territory size and demographic data to model the total population size in the Dryandra woodlands. Since old growth

Eucalyptus wandoo is important for providing nesting hollows for the Rufous Treecreeper (Luck, 2001 & Rose, 1993), mature wandoo forest was selected as an indicator of habitat preference. The distribution of *E.wandoo* was then mapped from a vegetation survey of the Dryandra woodlands (Coates, 1993) and GIS techniques. A Habitat Suitability map was modelled on this requirement and with a parameter of 2.6 ha territory size (Luck, 2002), applied to RAMAS software (Akcakaya, 2002) which calculated an estimate of 158 Rufous Treecreeper populations in Dryandra (Figure 3.5.2). Also since each cooperatively breeding group (population) has a maximum population size of 7 individuals (Luck, 2001), then there are approximately 1 106 individuals that reside within the Dryandra woodlands. This modelling provides a more specific distribution of Rufous Treecreepers in the area and more accurate description of the total population size. In a study on woodland fragmentation in south eastern Australia, remnant size and habitat complexity was found to affect the composition and distribution of woodland birds (Watson, *et al.*, 2005).

4.3.3 Climate Change and Climatic Range

The impact of climate change on the wandoo woodlands and the viability of avifauna was assessed using annual rainfall measurements, remote sensing information of tree foliage cover and avifauna survival rates calculated from mist net capture-recapture data. Plotted annual rainfall data between 1999 and 2010 shows there was an overall declining trend in rainfall and years of extremely low rainfall coinciding with El Niño events of 2002/03, 2006/07 and 2009/10 (Figure 3.5.3). Also in 2010 for the first time since records began, the annual rainfall measurement of 277.4mm (BOM, 2015) fell below the minimum climatic range (350mm) of *Eucalyptus wandoo* (Zdunic, *et al.*, 2012, Yates, *et al.*, 2000). Based on climate modelling (CSIRO, 2005) the predicted

reduction rainfall by 20% by 2030 (average 391.15mm) and 60% by 2070 (average 195.57mm), will negatively impact this species by inducing a permanent state of drought.

4.3.4 Foliage Cover and Critical Threshold

Data obtained from Landsat remote sensing was converted into percentage foliage cover for each 8 (1 hectare) sampling site between 1988 and 2010 within the Dryandra woodlands. During sampling times of 2003 and 2007, the graph shows a declining trend with a fluctuating pattern of an increase in foliage cover, followed by a decrease in the following year (Figure 3.5.4a). The Skelton site (3) shown in black, does not appear to recover foliage cover beyond 11.53% after a reduction to 7.73% in 2003. This indicates a critical threshold of percentage tree canopy cover for the Eucalyptus wandoo in Dryandra. Since Bennet & Radford (2005) claim a threshold of 10% tree cover is a point at which a major change or collapse occurs in Australian woodland bird communities, the Dryandra woodlands are approaching a dangerous tipping point. The results from this study indicate that the impact of climate change is occurring rapidly and that it has the potential to cause ancient forests and ecosystems to collapse and become extinct.

4.3.5 Avifauna Captures

Raw capture data for both species was initially assessed by plotting a comparison of captures on each occasion, for each species (Figure 3.5.5). Both species show a significant correlation to each other across sampling sites and confirmed that species captures were not random events. The Yellow-plumed Honeyeater capture data does show a greater range in number of captures and appear to fluctuate more than Rufous

Treecreepers (Tables 3.5.5a and 3.5.5b). The most successful year for capture of target species was 2004, followed by 2007 and the least number was caught in 2003. Over 3 sampling occasions, Bradford site (4) netted the greatest number of target species.

Bradford also has an abundance of developing and mature *E.wandoo* trees and diverse under story plant communities. Skelton (3) and Marri site (6) and Baaluc south site (7) netted the least number of birds. (Also see Appendix 11).

From this study, it was found that banded or marked individual Rufous Treecreepers from one site were not netted or caught at other study sites within Dryandra. In another study conducted on the Rufous Treecreeper in the Dryandra woodlands, Luck (2001) did find RTC dispersals between territories in and from outside study sites, but they only occurred when a vacancy became available through a disappearance of a primary male or female. RTC family groups are highly conserved through a strict helping and replacement mechanism that serves to conserve their integrity within each territory (Luck, 2002).

This study did find evidence of genetic dispersal within Dryandra and up to 48kms across regional sites (Chapter 3.3.6. and 3.3.6.1). Although these genetic signatures do provide evidence of individuals migrating within the Dryandra woodlands, this behaviour cannot be common or widespread as Mantel's Test (Chapter 3.3.3), Delaunay Triangulation analysis (Chapter 3.3.5) and Spatial Autocorrelation (Chapter 3.3.3.1), show. These methods all detect significant genetic differentiation between populations in close proximity to each other. Also if there was open migration between populations within Dryandra many banded birds from some sites could be netted at others, but this did not occur. These study observations and results suggest that the Rufous Treecreeper

has a complex set of breeding behaviours (Luck, 2001) that have a strong tendency towards natural selection.

For the following analysis of Regression, species capture data taken from 3 sampling occasions of 2003, 2004 and 2007 at the same time (October) each year, to maintain a temporal consistency of annual variables. The extra 2 sampling occasions during 2004 were omitted from the data set (Table 3.5.5a & 3.5.5b). Also, as there are only 3 sampling occasions for species captures, it is difficult to infer any tendency of relationship with a small number of sampling occasions

4.3.6 Regression of Rainfall, Foliage Cover and Captures

To examine the relationship between rainfall patterns, foliage cover and species captures, a logistic regression (GLM) was applied to the data. Foliage cover at each site (within each year) was tested against of the number of captures (per unit trapping effort) of Rufous Treecreeper, Yellow-plummed Honeyeater captures and the total of the two species. Tree foliage cover within each year was found to be a significant predictor for Rufous Treecreepers only (Table 3.5.6). Luck (2002) found a positive correlation between the probability of Rufous Treecreeper occurrence and wandoo tree canopy density. Also, Pearman (2002) found the species richness of primary forest birds to be statistically related to the percent canopy cover of primary forest and Bennet and Radford (2005) found a disproportionate rapid loss the decline in species richness of Australian woodland birds with a low proportion of tree cover.

To investigate the temporal response of foliage cover to annual rainfall (Figure 3.5.6a), a linear regression was calculated for total canopy cover using both current annual

rainfall (anrain) and the previous year's annual rainfall (panrain) as the independent variable. The regression of current annual rainfall on total cover was not significant whilst the regression of previous year's rainfall was (Figure 3.5.6b and Figure 3.3.6c). Therefore, the results from this study confirm that there is a delayed response of foliage cover to previous year's rainfall and that foliage cover is a predictor of RTC captures.

A possible explanation for this delay in growth of foliage cover could be the survival drought strategy of the wandoo trees (Batini, 2004), which enable them to store moisture for longer periods and increase their foliage cover in years that experience a reduction in rainfall. Also, the *E.wandoo* may respond to a possible seasonal growth pattern or cycle that span several years (Batini, 2004). Therefore the percentage canopy cover of these trees do not show an immediate response to rainfall patterns within the same year, as shown between the fluctuations during 2003 and 2007 (Figure 3.5.6a).

4.3.7 Avifauna Viability Analysis

The apparent survival rates, for the Rufous Treecreeper and Yellow-plumed Honeyeater within the Dryandra woodlands, between the years 2003 and 2007 were calculated to be 0.653 (SE 0.13) and 0.303 (SE 0.08) (Table 3.5.7), using MARK software program (White & Burnham, 1999). Since the Rufous Treecreeper is a resident species, and has a maximum movement ability of 12 km (BTO, 2014 and ABBBS, 2014) and the Yellow-plumed Honeyeater is an uncommon resident (Saunders & Ingram, 1995) with a greater movement ability of up to 555 km (ABBBS, 2014), these two species have differences in their dispersal range, residency status and habitat preferences, each species displays a different response to the Dryandra woodlands. This is also reflected in the data of mist net captures and the survival rates for each species (Table 3.3.5a and Table 3.3.5b). The

apparent survival rate of 0.303 for the Yellow-plumed Honeyeater (YPH) indicates a temporary emigration pattern. Therefore, alternate population models may be needed to describe the number of individuals visiting these woodlands on a yearly basis (Williams, *et al.*, 2002).

During 2003 and 2007 the Rufous Treecreeper in the Dryandra woodlands was found to have a combined male and female survival rate of 0.65 ((Table 3.5.7) with the MARK program (White & Burnham, 1999). Since the equilibrium adult survival rate has a value of 1 (Krebs, 1994), these results indicate there is no 1:1 replacement and the population is in decline. By comparison, between 1997 and 1999 adult survival rates for Rufous Treecreepers within Dryandra was 0.76 (Luck (2001) calculated using the computer program CONTRAST. Reasons for the discrepancies between the two survival rates may result because of differences in experimental sampling regimes or by using different computer software programs to compute data. However, the more likely reason is because the Rufous Treecreepers within the Dryandra woodlands are continuing to decline.

Comparison of the two survival rates shows there is a reduction of 0.11 within an 8 year period. Since the life span of Treecreepers is about 8 years (BTO, 2014 and ABBBS, 2014), the survival rate has declined within a single generation. Also, during sampling times of Luck's (2000) study, average percentage tree foliage cover data across all sites between 1996 and 2000 was 18.72%. During this study between 2003 and 2007 the average foliage cover was 13.56 %. This study shows the 0.11 decrease in survival rate followed a 5.16 % decrease in mean foliage cover (from 18.72% to 13.56%). In another study conducted on Scarlet Robins and Eastern Yellow Robins in eastern

Australia, it was found that in order for these birds to produce enough young and to maintain a stable population, adequate foliage density was critical to prevent nesting and fledgling predation (Debus, 2006). This study concludes that *E.wandoo* (foliage cover), is not only a significant predictor in determining the presence of Rufous Treecreepers within the Dryandra woodlands (Table 3.5.6), but it also impacts the short term survival and long term viability of this focal species.

CHAPTER 5 – CONCLUSION

The south west of western Australia (SWWA) is one of 34 global biodiversity hotspots; rich in endemic species with over 4000 plant, 100 vertebrate species and simultaneously impacted by vast stretches of agricultural land known as the wheatbelt (WWF, 2014, Bradshaw, 2012). Since European settlement in the SWWA, over 93% of the native vegetation, including 97% of the York gum, wandoo and salmon gum woodlands have been cleared for agriculture (Saunders, 1989). This broad scale clearing has led to the extinction of many flora and fauna (Yates, *et al.*, 2000). Currently the west Australian wheatbelt is dominated by a mosaic of arable fields, pastures and salt pans, with thousands of small remnants of native vegetation scattered across the landscape (Saunders, *et al.*, 1993). Part of this investigation assessed the impact of habitat loss and fragmentation on gene flow and population structure in a focal species, within a scale of approximately 100km. By detecting and understanding restrictions to gene flow, this information is intended to improve the management of species by identifying habitats for either conserving genetic variation or required for population connectivity (Safner, *et al.*, 2011).

5.1.1 Genotyping and DNA Analysis

The microsatellite data of the Rufous Treecreeper did contain some null alleles and genotyping failures and therefore infers a degree of error in the experimental data set. However, the magnitude of these effects cannot be assessed with any accuracy and although some degree of errors in data sets are widely acknowledged and documented by researchers, options for dealing with them are limited, time consuming and expensive (Wagner, *et al.*, 2006). Since this investigation began, rapid developments in DNA and RNA sequencing technology has increased and improved the speed, accuracy

and resolution of genetic analysis. Next generation Sequencing has become the preferred method for finding microsatellite loci (Gardner, *et al.*, 2011) and for population genetic studies (Davey & Blaxter, 2011).

There was no equilibrium between gene flow and genetic drift in the microsatellite data set. Deviation of Hardy Weinberg Equilibrium was suspected because of the Wahlund Effect and implies a deficit of heterozygotes in the data set. This occurs where habitat fragmentation creates isolation between small populations and they become genetically fragmented (Frankham, *et al.*, 2002). As habitat size decreases and the distance between habitat remnants increases, the populations appear more homogenous across large distances (Frankham, *et al.*, 2002). AMOVA shows genetic variation to be higher within populations (78%) than among populations (22%) (Figure 3.3.1). Therefore the majority of genetic divergence patterns within remnant habitat, is repeated among most other remnants throughout the region. A negative Isolation by Distance at both small scale (Dryandra up to 28kms) and large scale (region up to 100kms), both confirm there is a significant degree of dispersal taking place throughout the region on a larger scale (Figure 3.3.2 and 3.3.2.1).

5.1.2 Spatial Scale of Microsatellites

One of the main challenges of conservation plans is to be able to identify the spatial scales at which species are able to disperse and then restore the ecological processes that promote species viability (Luque *et al.*, 2012). A Mantel's Test using (Nei's 1983) genetic distance found a correlation with geographical distance up to 28km and the Rufous Treecreepers in Dryandra, did not belong to one panmictic population (Figure 3.3.3). The Spatial Autocorrelation analysis detected a genetic structure between

populations up to a distance of 30 km, but after this distance there was no correlation. This infers a genetic discontinuity beyond the Dryandra woodlands, where dispersal is more likely to occur. The spatial distribution of microsatellite Nei's (1983) genetic distances also shows increased levels of divergence for the Dryandra woodlands and then genetic distances appear to decline as the distance between smaller, more fragmented habitat increases, in an easterly direction (Figure 3.3.4.1).

5.1.3 Population Structure of Microsatellites

The genetic structure of Rufous Treecreeper populations within Dryandra was resolved using the Maximum Difference Delaunay Triangulation method (Figure 3.3.5). The resolution of a collection of 3 adjacent populations (Norn, Baaluc North and Baaluc South) in the centre of the woodlands presented the same 3 populations that form a genetic neighbourhood, using Ritland's (1996) Kinship Coefficient (Figure 3.3.6). Luck (2001) observed co-operative breeding behaviour of Rufous Treecreepers, whereby territory defence was relaxed during the breeding season to allow non-breeders or helpers that fed nestlings from adjacent groups. This explains how the co-operative breeding behaviour of the Rufous Treecreeper forms genetic neighbourhoods, as seen in this analysis and Ritland's (1996) Kinship Coefficient analysis.

A Bayesian approach using *DIYABC* (Cornuet, *et al*, 2013), was able to resolve the regional structure of populations at distances of 20 – 80 km from central Dryandra (Figure 3.3.5.1a). This analysis shows that despite different distances from Dryandra, the most recent common ancestor originated from Dryandra. Therefore, the Stepping Stone Model (scenario) of population expansion was rejected and the Continent Island

Model best fit the data with the highest posterior probability was selected (Table 3.3.5.1).

5.1.4 Dispersal Patterns of the Rufous Treecreeper

To determine Rufous Treecreeper migration patterns, Kinship Neighbour Joining Trees were constructed for both local and regional distances. Ritland's (1996) Kinship Coefficient, based on a continuous population model, found the Norn and Gura populations to be the most widely spread (Figure 3.3.6). This analysis also detected a genetic neighbourhood consisting of Norn, Baaluc north and Baaluc south sites, which also supports the findings of the Delaunay Triangulation method (Figure 3.3.5). As the Loiselle's (1995) Kinship Coefficient summarises the strength of kinship between pairs of individuals as a function of geographical distance, it was selected for the regional analysis of dispersal. Results show individuals from the Dryandra woodlands in every branch of Loiselle's (1995) Kinship tree that spans the entire region. The individuals from Dryandra appeared to follow the Continent-Island Model of population expansion (Figure 3.3.6.1) and this observation is also supported by the Bayesian analysis (Table 3.3.5.1).

The closest genetic distance between individuals was found to be 0.0019 at a geographical distance of 43 km (Dryandra-North Yillimining sites) and secondly 0.0031 between Dongolocking and Narrakine (Highbury), separated by 48 km. This implies that in spatially discontinuous habitat surrounded by a landscape of mainly cleared agricultural land, the Rufous Treecreeper is able to disperse and breed across these distances. However, according to bird banding data the Rufous Treecreeper has a maximum movement ability of only 12 km (BTO, 2014 and ABBBS, 2014). Therefore,

genetic kinship analysis proves to be a more accurate method for detecting actual dispersal distances, rather than what can only be observed.

There evidence of a female biased dispersal pattern, where genetic divergence patterns are high in regional areas and low within the Dryandra woodlands (Figure 3.3.7). The males appear evenly distributed across the region. Breeding females outside the Dryandra woodlands found in small, fragmented habitat show high divergence estimates most likely because of limited nesting hollows, higher competition and lower reproductive success in these smaller remnants (Luck, 2001). Although these small and isolated populations have a higher risk of extinction, they also possess a high degree of adaptive genetic diversity and are therefore considered to be a high priority for conservation (MacDonald, 2002 & Sherwin, *et al.*, 2000).

5.1.5 Spatial Analysis of Mitochondrial DNA

Part of preserving the evolutionary processes responsible for adaption, is to conserve genetic diversity (Avice, 1994, Crandall, *et al.*, 2000). By comparing the differences or mutations in genotypic data of individuals and populations, the estimate of divergence can be made and is called a Genetic Distance (Kalinowski, 2002). Mantel's Test found no correlation between (Nei's, 1983) genetic distance and geographical distance at $\leq 80\text{km}$, but when an outlier sample from Norseman 500 km from Dryandra was included in the analysis, a significant amount of genetic divergence was found (Chapter 3.4.1). This is consistent with a natural range of a species where geographic distance acts as a barrier to dispersal (vicariance) and indicates at least 2 different biogeographic regions of this species, within a 500km distance.

The Interpolation and Principal Component Analysis of mitochondrial genetic distance ϕ_i , shows with increasing geographical distance in an easterly and southerly direction from Dryandra, the genetic variation in mitochondrial DNA declines in the same direction (Figure 3.4.2). The highest divergence patterns were found in Dryandra, North Yilliminning, Commondine Reserve and Wickepin sites. Populations with low divergence patterns in the Dongolocking, Highbury and east of Dryandra sites, would have most likely belonged to a larger population (gene pool), prior to land clearing. This relationship between genetic divergence and evolutionary time implies population separation times (Avice, 2000).

5.1.6 Phylogeography of Rufous Treecreeper Populations

The preservation of genetic diversity based on evolutionary and geographical origins was the premise for mapping the historical range of Rufous Treecreepers, prior to population separation and land clearing. Since suitable habitat is critical for the persistence of many forest species (Saunders, 2005, Luck, 2002, Bennet & Radford, 2005), restoration of existing habitat by the creation of vegetation corridors between extant endemic vegetation communities as a model (Hobbs, 2002) has proven to be successful in the past (CSIRO, 2009, Saunders, 1989, Beier & Noss, 1998 and Hass, 1995). However prior to planning vegetation corridors, re-construction of a species phylogeography would be useful to provide a guide to its natural range, prior to land clearing. Secondly, it would be useful to be able to identify rare haplotypes and link them to geographical areas of high conservation value. This genetic approach to landscape restoration is extremely important for the maintenance of genetic diversity and long term evolutionary potential of a species.

The genealogy study based on coalescence and species geographical distribution of mitochondrial haplotypes showed haplotype 3 to be the most commonly shared haplotype and both 3 and 5 to be the genealogical ancestors to all other clades (Figure 3.4.3). These haplotypes originated from sites in Dryandra, North Yillimining, and the southern populations of Dongolocking and Narrakine in Highbury. Also the maximum geographical distribution of haplotype 3, infers a historical range of approximately 85km (Table 3.4.3).

In conclusion of the genetic analysis of the Rufous Treecreeper, there are several important populations to prioritise for conservation purposes. The most important populations reside within the Dryandra woodlands and in particular the central populations. Not only do these populations contain the vast majority of genetic diversity, but they are also responsible for replenishing other populations on smaller remnants and have a maximum dispersal ability of up to 50km. Other sites selected for high genetic diversity are North Yillimining, Commondine Reserve and Wickepin (Strathern site) populations. Also a priority are the Dongolocking and Narrakine (Highbury) populations that have been separated from the northern populations and are most likely remnants of a larger southern population that once existed prior to land clearing.

5.2.1 Ecological Niche and Habitat Suitability

To investigate some of the Rufous Treecreeper's ecological requirements, a distanced based redundancy analysis and a habitat suitability study were conducted. The distanced based redundancy analysis found slope and aspect explained 29.16% ($p=0.04$) of the genetic variation ϕ (π) in the mitochondria. This analysis seeks to explain

adaptive traits and changes in the genome that are linked and a respond to specific habitat conditions. Therefore the geophysical elements are not only linked to the Rufous Treecreeper's mitochondrial divergence estimates, but may also play a role in future habitat planning based on a species ecological niche characteristics. The Habitat Suitability map (Figure 3.5.2) shows a vastly reduced habitat size when compared to the whole reserve area and provides a more accurate distribution of Rufous Treecreepers within Dryandra. Based on habitat suitability and combined with information of breeding group size and territory size from a previous study (Luck, 2002, 2001), it was estimated that 158 Rufous Treecreeper populations and approximately 1 106 individuals resided within the Dryandra woodlands. The information from this study can be used in species monitoring and recovery plans and to link habitat characteristics with species requirements and possible adaptive traits.

5.2.2 Climate Change and Climatic Range

The impacts of climate change was measured by annual rainfall data collected from Wandering weather station (BOM, 2011), 20km from the Dryandra woodlands. Rainfall data between 1999 and 2010 follows a declining trend (Figure 3.5.3), with dramatic changes in rainfall coinciding with El Niño Southern Oscillation Events (BOM, 2011). Also in 2010 for the first time since records began, the annual rainfall measurement of 277.4mm (BOM, 2015) fell below the minimum climatic range (350mm) of *Eucalyptus wandoo* (Zdunic, *et al.*, 2012 Yates, *et al.*, 2000). Climate models predict an increase in the frequency of El Niño Southern Oscillation Events (Riseby, *et al.*, 2009) and hotter and dryer climatic conditions for this region (CSIRO, 2005, IOCI, 2002). This will most likely negatively impact these forests by inducing a

permanent state of drought, increase the risk of high intensity wild fires (Gill, 2001) and inevitably increasing the risk of extinction.

5.2.3 Foliage Cover and Critical Threshold

The Dryandra woodlands in its present condition will not be able to continue to tolerate the impact of extended droughts (Veneklaas & Manning, 2007). For this reason the long term impact of climate change on the wandoo woodlands in Dryandra was assessed using remote sensing data of percentage tree foliage cover. Data obtained from remote sensing was converted into percentage foliage cover for each 8 (1 hectare) sampling site between 1988 and 2010. The Skelton site (3) shown in black (Figure 3.5.4a), does not appear to recover foliage cover beyond 11.53% after a reduction to 7.73 in 2003. This indicates a critical threshold of percentage tree canopy cover for the Eucalyptus wandoo in Dryandra. Since Bennet & Radford (2005) claim a threshold of 10% tree cover is a point at which a major change or collapse occurs in Australian woodland bird communities, the Dryandra woodlands are approaching a dangerous tipping point.

To observe the impact of declining rainfall patterns on *E.wandoo* forests and Rufous Treecreepers a multivariate analysis was conducted. A linear regression found a significant ($p = 0.036$) relationship between previous year's rainfall and percentage foliage cover. A delayed response to rainfall is explained by the defence mechanisms of *E.wandoo* that provide this species with drought tolerance (Batini, 2004) and which may also include a growth pattern that spans many years. A logistic regression analysis (GLM) found foliage cover within the same year to be a significant predictor ($p = 0.039$) of Rufous Treecreeper captures. Therefore declining rainfall patterns and tree canopy cover have a direct impact on the abundance and viability of Rufous

Treecreepers. These study results infer the impact of climate change is occurring rapidly and has the potential to cause ancient wandoo forests and ecosystems to collapse and become extinct.

5.2.4 Avifauna Viability Analysis

Between the years 2003 and 2007 the apparent survival rates for the Rufous Treecreeper and Yellow-plumed Honeyeater within the Dryandra woodlands, were calculated to be 0.653 (SE 0.13) and 0.303 (SE 0.08) (Table 3.5.7). Since the Rufous Treecreeper and the Yellow-plumed Honeyeater have differences in their dispersal range, residency status and habitat preference, each species displays a different response to the Dryandra woodlands. By comparison, between 1997 and 1999 adult survival rates for Rufous Treecreepers within Dryandra was 0.76 (Luck (2001) and show the Rufous Treecreepers within the Dryandra woodlands are continuing to decline.

Comparison of the two survival rates shows there is a reduction of 0.11 within an 8 year period, or within a single generation. This study shows the 0.11 decrease in survival rate followed a 5.16 % decrease in mean foliage cover during sampling times. This study concludes that *E.wandoo* (foliage cover), is not only a significant predictor in determining the presence of Rufous Treecreepers within the Dryandra woodlands (Table 3.5.6), but it also impacts the short term survival and long term viability of this focal species (Table 3.5.7).

5.3 Management Recommendations

It is highly likely that the Dryandra woodlands in its present condition will not be able to continue to tolerate the impact of longer drought periods (Veneklaas & Manning,

2007 & Batini, 2004). Therefore, urgent management recommendations include the revegetation and restoration of existing reserves, introduction of low intensity fire regimes and building vegetation corridors between isolated remnants (Jurskis, 2005, Thiele, *et al.*, 2000). The rehabilitation of native vegetation using endemic species, rather than introduced species is critical to the sustainable recovery of entire ecosystems (Kimber, *et al.*, 1999). Also Luck (2002) suggests that reducing major threats to Rufous Treecreeper population viability include replacement of older trees owing to poor seed replacement and that management must involve ensuring regeneration of endemic species and maintaining important old growth forests for nesting hollows.

Climate change is predicted to reduce the reproductive success of populations already fragmented by habitat loss and will probably further reduce the viability of those populations (Chambers, *et al.*, 2005). Therefore, some species will inevitably be lost while others will have to move to other locations because of climate change (NBS, 2009). Managing Rufous Treecreeper distributions would involve facilitating dispersal by building vegetation networks in a south west direction towards higher rainfall areas (Chambers, *et al.*, 2005 & Brereton, *et al.*, 1995). It is predicted that the decline of woodland bird species will continue unless appropriate habitat conservation strategies are applied (Watson, *et al.*, 2005).

The International Union for the Conservation of Nature (IUCN) has categorised the Rufous Treecreeper in the list of Threatened Species, as of least concern; in which species are known to be widespread and abundant (IUCN, 2011). The findings of this study found no evidence that the Rufous Treecreeper is abundant either in small fragmented habitats or within Dryandra, the largest woodlands in the wheatbelt. Based

on the status of the Rufous Treecreeper found in this study, it is recommended to have this species categorised as Vulnerable due to the high risk of extinction in the medium term or Near Threatened, as it is highly likely that this species will be qualified for a higher threatened priority in the near future (IUCN, 2011).

The Environmental Protection Authority's (EPA) State of the Environment Report (2007), found that Western Australia has 362 threatened plants, 199 threatened animals and 69 threatened ecological communities (Watson 2007). As recovery plans have only been developed for less than one-third of these threatened species and ecological communities (Watson 2007), it is evident that too little has been done to prevent the inevitable decline towards extinction of many endemic species. The Commonwealth Government of Australia has released a Nationwide Biodiversity Strategy for 2010-2030, but the primary legislation for the protection of biodiversity in Western Australia is the *Wildlife Conservation Act 1950* (Wylykino et al., 2010). The Conservation Council of Western Australia has criticised this Act as extremely outdated and does not provide adequate legislative basis for conservation of biodiversity (Wylykino et al., 2010).

In response, the state Department of Parks and Wildlife responsible for conserving biodiversity in Western Australia aims to pass a new *Biodiversity Conservation Bill 2015*. However, this bill has been criticised by WWF Australia and the Environmental Defenders Office (EDO) for giving the Government significant decision making power with no input required from independent bodies and therefore should be abandoned (Webber, 2016). Also, there is a broad and unfettered discretion that resides with the Minister for Environment or within the CEO of the Department of Parks and Wildlife

over every decision regarding the listing, delisting, identification of habitat, species, communities, recovery plans, interim recovery plans and biodiversity management plans (Pearlman, 2016).

The impact of these legal implications and intensifying environmental pressures will most likely continue the degradation of the remaining habitat which many species are critically dependant on for food, breeding sites and shelter (Saunders, 2005). In the long term, this will most likely lead to more extinctions and eventually ecosystem collapse. Therefore community involvement, continuing research, the implementation of scientific management strategies and a legitimate workforce are urgently needed to combat the deteriorating conditions of the west Australian wheatbelt. On reflection of our current ecological crises, I would like to end my thesis with a poignant quote, “Our lives begin to end the day we become silent about the things that matter.”

- *Martin Luther King Jr.*

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APPENDIX 1

PRIMERS

Microsatellite Primers designed for the Brown Treecreeper

Cpi 1F: ACAGAGCAGTTGGAGACCGT
 Cpi 1R: GGATACTGCCATGTCCAA
 Cpi 2F: GGAAACCCCCGCAGACAC
 Cpi 2R: CAGCAGGTCCTTCAGGAGAC
 Cpi 3F: GGAGGGATTGCTGTCTTGAA
 Cpi 3R: AAGGCATCACCTCACCAGAG
 Cpi 4F: AGAGCAGGGTCTTCCACAGA
 Cpi 4R: GCCTGGTCCCTCATGTCTAA
 Cpi 5F: CCTGCTGAAGTGCCCTACTC
 Cpi 5R: GATGACCCCAAGGTCTCCATA
 Cpi 6F: TGGCTTCCCATTTTGGTTTA
 Cpi 6R: AGAAATCGGAAAGCCTGGAC
 Cpi 7F: CCTTCAAATCCAAAACCA
 Cpi 7R: TAAAGTTTTGTGCGAG
 Cpi 8F: TTAAGGGATTTGGATGGCAG
 Cpi 8R: GATTGGATGGCAG

Mitochondrial Primers

Control Region

L537: CCTCTGGTTCCTCGGTCAG
 H774: CCATACGCCAACCGTCTC
 L436: TCTCACGAGAACCGAGCTAC
 HCR1: GAATAATTTGTAAAATGTAGGGAT
 H739: CAAGGGTTGCTTATTTCTCGTG
 L537: CCTCTGGTTCCTCGGTCAG
 H1248 (phe) CATCTTCAGTGTCATGCT
 L16743(glu) TTCTCCGAGATCTACGGCCT
 12S CACTGGRDCGCGGATACTTGCATG
 ND₆ GTTGGGYTTRTRTTGGRGG

Cytochrome B

F54 TCT CGC TAT ACA CTA CAC AGC
 R496 TTA GGT GGA CTA GGG TGA GTC
 F392 GAG CTT GAG GAG GCT TCT CAG
 R858 GCG GAA GGT TAT TGA GCG TTG
 CytB CAA CGC TCA ATA ACC TTC CG

PRIMER TRIALS

Australian Passerine Primers					
Species	locus	Acc. No	Repeat Motif	Primer Sequence 5'– 3'	Reference
Superb Fairy Wren	McyU8	U82392	(aaag) ^{>50}	F:cccaatggtgatgaaagtcc R:acattagtctcccttttttcc	Double, <i>et al.</i> , 1997.
	McyU4	U82388	(gt) ²⁶ at(gt) ³	F:ataagatgactaaggctctggtg R: tagcaattgtctatcatggttg	
	McyU2	U82386	(gt) ¹² gg(gt) ¹³	F:ctaagccctgagagggtg R:gcaaaagaggaaccaacaagc	
Bell Miner	BMC4	AF005378	(ca) ²³	F:gataggagactgagactgtccc R:ttctgaagggttagtacagacc	Painter, <i>et al.</i> , 1997.
Red Capped Robin	Pgm1	AY289550	(cttt) ³ t(cttt) ²⁰	F:tttactgttagcagaatgg R:tttcacaattttgtcatagggc	Dowling, <i>et al.</i> , 2003.
	Pgm2	AY289551	(tc) ⁵ (cttt) ⁶ ctgt(cttt) ² ccttcttctttt	F:tcctgtacaaaactaatgagg R:gtctcaccacaccttatgc	
	Pgm5	AY289554	(gt) ² tt(gt) ¹⁶	F:agtgtgaactgggagacc R:aacctgtcctctctctcc	
White Eyes	ZL 38	AF076672	(gt) ¹⁷		Dehnigan <i>et al.</i> , 1999.
Microsatellite Primers that show Cross-Species Amplification					
Swallow	HrU2	X84087	(ga) ⁶ (gt) ⁷ (tg) ³	F:catcaagagaggatggaaagagg R:gaaaagattattttcttctccc	Primmer, <i>et al.</i> , 1995.
	HrU5	X84090	(t) ¹⁰ (gc) ¹⁷	F:tcaacaagtgtcattaggttc R:aacttagataaggaagtatat	
	HrU6	X84091	(aag) ¹⁷ (ag) ² (aaag) ²	F:gctgtgtcattttacatgag R:acagggcagtggtactctgc	
	HrU7	X84092	(a) ⁴ (c) ³ (aaacc) ² (aac) ³	F:gattcacagtagacaatg R:gatcactatgagtctctggaa	
The Reed Bunting	Escu2	X77078	(ta) ⁹ (tg) ³⁰	F:tacagcaaagcggaaactg R:tgggcaaagatatgggaaga	Hanott, <i>et al.</i> , 1994
	Escu6	X77082	(ca) ¹⁵ cg(ca) ¹⁰ gta(ca) ³	F:catagtgtgcctgctagg R:gcaagtgtccttaattttgg	
Yellow Warbler	Dpu05		(gaaga) ²¹	F:ggctgtgctctgtatgg R:tctgaatattgaacgccta	Dawson, <i>et al.</i> , 1997
	Dpu15		(ac) ¹² at (ac) ²	F:ggctgcaaactcattattctc R:attgagtctgtcaggccag	
	Dpu16		(ac) ¹² (gc) ⁴ acgc(gc) ²	F:acagcaaggtcagaattaaa R:aactgtgtgtctgagcct	

APPENDIX 2

GEL PHOTOGRAPHS

Microsatellite DNA

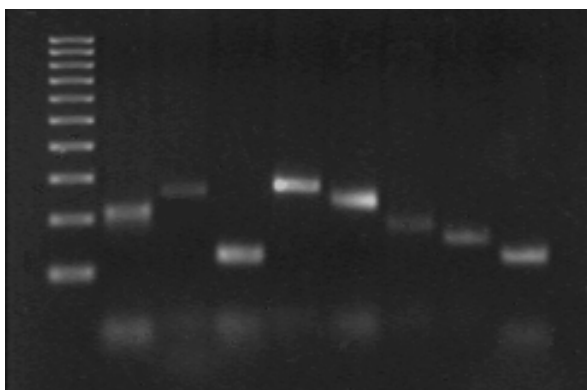


Figure a. Agarose gel showing 8 microsatellite DNA's from the Rufous Treecreeper. Lane 1. 100bp ladder, lanes 2-9 PCR products from primers Cpi 1-8 (Doher, 2004).

Microsatellite Clones

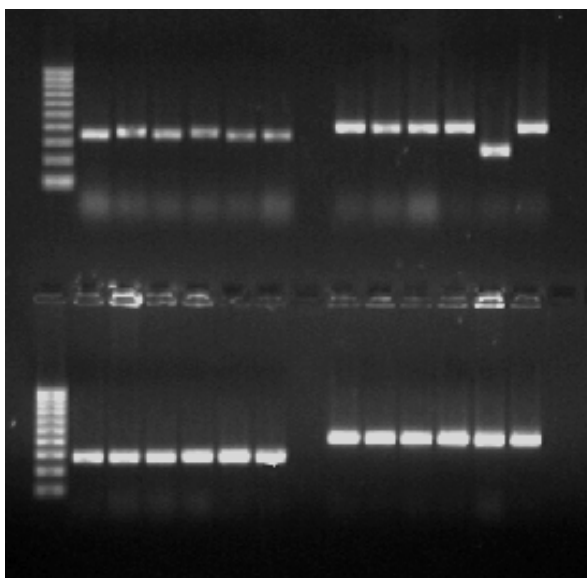


Figure b. Agarose Gel of Transformant DNA with micro-Satellite inserts. Top Row: Lane 1. DNA 100bp ladder, lane 2-7 Cpi 1, lanes 8-14 Cpi 2, Bottom Row: Lane 1. DNA 100bp ladder, lanes 2-7 Cpi 3, lanes 8-14 Cpi 4.

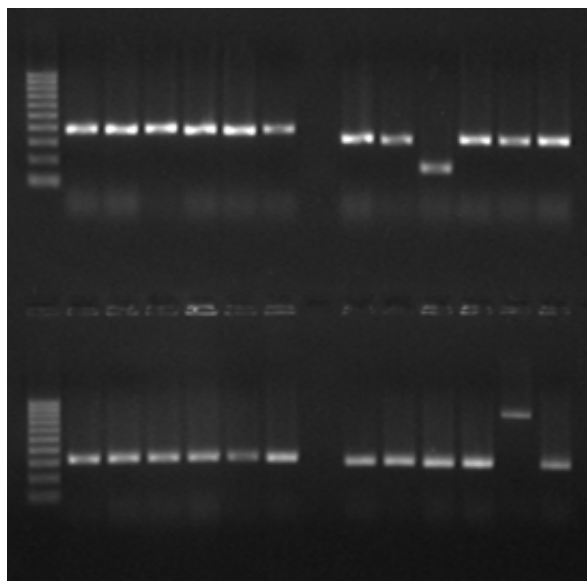


Figure c. Agarose Gel of Transformant cells with micro-Satellite inserts. Top Row: Lane 1. DNA 100bp ladder, lane 2-7 cpi 5, lanes 9-14 cpi 6, Bottom Row: Lane 1. DNA 100bp ladder, lanes 2-7 cpi 7, lanes 9-14 cpi 8.

Control Region

Photograph shows the testing of various primers on Rufous Treecreeper and Western Yellow Robin DNA.

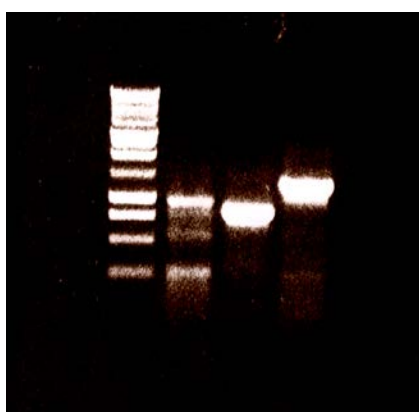


Figure d. Agarose gel of control region of control region of mitochondrial DNA. Lane 1. 1,500bp ladder with 100bp increments. First visible band equivalent to 300bp. Lane 2. RTC showing 3 fragments created with L436 and 12S primers. Lane 3. WYR showing one fragment of 500bp created with same primer. Lane 4. WYR showing one fragment of 750bp created with L16743 and 12S primers.

APPENDIX 3

MICROSATELLITE DNA

Modification of Microsatellite Primers

Underlined DNA sequences represent show show original Brown Treecreeper (BTC) primer sequences, red shows new Rufous Treecreeper primers and grey shaded area represents Rufous Treecreeper microsatellite sequences.

Climacteris Rufous Microsatellite 1 (199 bases)

Microsatellite Repeat (CT)₁₆ (CCCT)₃ CC(AT)₃ (AC)₂ (AT)₉

TTCCACAGAGCAGTTGGAGACCGTGAGTGAGGAACAACCTGCTCTCAAGTATTACC
CAAATGTTTCCTATATCTTTCTGCTTCTTAATCCTTCACCTCTCTCTCTCTCTCTCTCT
CTCTCTCTCTCTCCCTCCCTCCCTCCATATATACACATATATATATATATATATATTTCAG
TAGACTT**TGTTGGACATGGCAGTATCC**

Climacteris Rufous Microsatellite 2 (233 bases)

Microsatellite Repeat (CA)₃ GA(CA)₃ GA(CA)₂ CC(CA)₄ GA(CA)₂

GGAAACCCCGCAGACACGCACACAGACACACAGACACACCCACACACAGACAC
ACCCACACACCATAACAACAGCCCTGACCTGAGCATTGGATCTGTCTCGCCCCC
CATGCGGTTCTCAGCACGCTCACCTGCCGGGGGAGCGGAAGGAAAAGGTGGGAGA
GTGGCGTGGGGGGCTCAAACATCAACCAAGGAGGTGAGCCGTGTGCAG**GTCTCCT**
GAAGGACCTGCTG

Climacteris Rufous Microsatellite 3 (107 bases)

Microsatellite Repeat (GT)₁₀

GAGAGTGGAGGGATTGCTGTCTTGAAACATTTTGGATATGTGTGTGTGGTGTGTG
TGTGCGTGTGTAGGAAGGGGCAGGTCAGGCTTTG**CTCTGGTGGTGATGCCTT**

Climacteris Rufous Microsatellite 4 (250 bases)

Microsatellite Repeat (CA)₁₀

AAGAGCAGGGTCTTCCACAGAGCCACTCCCATCCCAGCACAATGTGTTGCCCTTT
GGGGACCTCCAGCAGACCCCTCAGCATCTATTTCCAAGGCACCACGCCATCCAAAC
CATCCCTTTGAGCAACACCTGCAGGACTGGATGCTCCTGGGATGGACACACACACA


```

RTC . 7      CCTTCCAAATCCAAAAACCAAATCCCATCACGCCAAAGATCGGGGCAATACTTTTATACC
BTC . 7      CCTTCCAAATCCAAAAACCAAATCCCATCACGCCAAAGATCGGGGCAATACTTTTATACC
*****
RTC . 7      TCTGAATGCTAACGGTGCCTTTTAAACAACCTTACTGCACACAC-GCGGCGCACACACA
BTC . 7      TCTGAATGCTAACGGTGCCTTTTAAACAACCTTACTGCACACACACGCGGCGCACACACA
*****
RTC . 7      CACACACACACACTCGCACAAAACCTTTA 146
BTC . 7      CACACACACACACTCGCACAAAACCTTTA 148
*****

```

RTC microsatellite 7 shows 98.6% similarity to BTC.

```

RTC8A      AAGGGATTTGGATGGCAGAAAGTGCCTCTCTCTCTCTCTCTCTC-CCTCTCTCTG
BTC . 8      AAGGGATTTGGATGGCAGAAAGTGCCTCTCTCTCTCTCTCTCTCTCCTCTCTCTG
RTC8B      AAGGGATTTGGATGGCAGAAAGTGCCTCTCTCTCTCTCTCTCTC----CCTCTCTCTG
*****
RTC8A      TAACTCTCCTGAGACCCGGGGCTGCCATTGGGCAGTCAGTCACTGCAATCCGCCTACT--
BTC . 8      TAACTCTCCTGAGACCCGGNGCTGCCATTGGGCAGTCAGTCACTGCAATCCGCCTACTTT
RTC8B      TAACTCTCCTGTGACCCGGGGCTGCCATTGGGCAGTCAGTCACTGCAATCCGCCTACT-
*****
114
118
112

```

RTC microsatellite 8 shows 94.2% similarity to BTC.

APPENDIX 4

MITOCHONDRIAL DNA

Western Yellow Robin Control Region

The partial control sequence for the Western Yellow Robin was most closely aligned to *Manucodia chalybata* control region (AY597012.1), complete sequence.

```
# Aligned_sequences: 2
# 1: WYR
# 2: M.CHALYBATUS
# Matrix: EDNAFULL
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 1485
# Identity:      736/1485 (49.6%)
# Similarity:    736/1485 (49.6%)
# Gaps:          620/1485 (41.8%)
# Score: 2381.0
```

```
WYR          1 ----- 0
M.CHALYBATUS 1 TCTAACTACCCCCCCTCCCCCCCACATTTCCATGTTTATAACATGGC 50
WYR          1 ----- 0
M.CHALYBATUS 51 TTTAGGGTATGTATTTCTTGCATACAATTATTGTCCACATCAGACACTA 100
WYR          1 ----- 0
M.CHALYBATUS 101 AATCAATGCAGTATATTCACATAACTAGTAATGCCAATCCTAACCAAAC 150
WYR          1 -----CGG---GGCTTAAACCTCC-- 16
M.CHALYBATUS 151 TCAAATATCACAGCCCATAACGATGCATAACGGACAGG--TAACCCTCCA 198
WYR          17 -----GTT----TTCCAT----- 25
M.CHALYBATUS 199 GGCACATCCTTGTTTCAGGTACCATAACAACCCAAATGATCCTACCTAACG 248
WYR          26 -----GG-----AGAT-GAG-----TTCC--AGTAC---- 43
M.CHALYBATUS 249 ACAGCGGGACAAGCGTCACCCAAGATCGGAGACTGTTTCCCTA-TACATAA 297
WYR          44 -----ACCTTGCGA-----ATTTACAC--- 59
M.CHALYBATUS 298 CACCCACAGACCATACGAGGAATATCTATCCACCCCAATGAATTCACAAA 347
WYR          60 -CGCGTCATAAGTTTCGCCCCACCTCCTAGGATATGTTATCT--CCCTAC 106
M.CHALYBATUS 348 TC----CATACGTTTCG-CCCACCTCCTAGGCAAAG--ACCTTCACCAAC 390
WYR          107 AGCTTTC AAGTCCACCCAAGCCAGAGGACCAGGT CATCTATTAACGGTGC 156
```

M. CHALYBATUS	391	440
WYR	157	ACCTCACGAGAACCGAGCTACTCAACGTCAGTTA--TACCCTCGTTATTG	204
M. CHALYBATUS	441	485
WYR	205	----GCTTCAAGGCCATACTTTCCCCCTACACCCTAGCCCAACTTGCTCT	250
M. CHALYBATUS	486	535
WYR	251	TTTGCGCCTCTGGTTCCTATTTTCAGGGCCATAAAT--CTCCTGATTCCTT	298
M. CHALYBATUS	536	583
WYR	299	CTCAATTGCTCTTTCACAGATACAAGTGGTTGGTCTGCATAAATCCTCCTT	348
M. CHALYBATUS	584	631
WYR	349	TTAACTCGTGATCGCGGCATCTGACCGTTTTTCCT---CTGTGTTTCTTT	395
M. CHALYBATUS	632	678
WYR	396	CTGGGTCTCTTCAATAAACCCCTTCAAGTGCGTAGCAGGTGTTATCTTCC	445
M. CHALYBATUS	679	728
WYR	446	TCTTGACATGTACATCATATGACATCCGAGCGGCCTCATCGCCCGCAGAG	495
M. CHALYBATUS	729	776
WYR	496	CTATCTAAGTGTAATGGTTTCGTTGGATAACCT-GTCGCATACTTAGACT	544
M. CHALYBATUS	777	822
WYR	545	CTGATGCACTTTGCCCCATTCAT----GAAACCCGCGCTGTTTACCTCT	590
M. CHALYBATUS	823	868
WYR	591	TGGGTACAGATGGTGTATGGTTGTGGGACATGACTATTTTTTCATGC-	639
M. CHALYBATUS	869	916
WYR	640	-AGTTCTAGGGACTTATA-GTAAAACCCCTATTTACGCATTATTTGCGC	687
M. CHALYBATUS	917	955
WYR	688	AATTTTTCTTT--TTTGTGTCATTTTTTTGTTAACATAACAAAAAAT	735
M. CHALYBATUS	956	997
WYR	736	TAACCGAACCTA-----CCCTACATTGTCCAAACCATTAAT	771
M. CHALYBATUS	998	1047
WYR	772	AATTCATCAAACCTGTTTATGCACT-----TTCCACCTA--	804
M. CHALYBATUS	1048	1091
WYR	805	----AACACA-----CATTACCTTTCTTC-----	824
M. CHALYBATUS	1092	1141
WYR	825	-----ATGACA-----TTGGAAC-----CAAACAA	844
M. CHALYBATUS	1142	1191
WYR	845	A-----AACAA-----CGGACACCACCTCAC	864

M. CHALYBATUS	1192				. .		1237
		ACCACCCTTCAACAAACATTTAATATGACTTACCCCAGAAATCA----				AC	
WYR	865	CC-----	AACAAACCAGCAAAC	-----			881
M. CHALYBATUS	1238		. ..				1287
		CCACAACAACAAAAACAAAAACAAAAACAAAAACAAAAACAAAA					
WYR	882	-----		-----	CCCTACCC	--ATGCCCT	897
M. CHALYBATUS	1288	AACAAACAAAAACAAAAACAAAAACAAAAACAC-			1336
WYR	898	TGTAGCTTACAA-	CAAAGCATGGCACTGAAGATGCCAAGATGGCCGTCAT				946
M. CHALYBATUS	1337		.				1369
		TGTAGCTTACAATCAAAGCATGACACTGAAGAT-----					
WYR	947	AAAACGCCCAAGGACAAAAGACTTAGTCCTAACCT				981	
M. CHALYBATUS	1370	-----					1369

APPENDIX 5

MITOCHONDRIAL cytochrome b

Yellow-plumed Honeyeater Cytochrome b

Query sequence belongs to the Yellow-plummed Honey-eater and subject sequence belongs to the Rufous Treecreeper *Climacteris rufa*. This species was the most similar (98%) to all other control region sequences in GENBANK.

[gi|1477744|gb|U58501.1|CRU58501](#) *Climacteris rufa* cytochrome b (cyt b) gene, mitochondrial gene
 encoding mitochondrial protein, partial cds
 Length=924
 Score = 1323 bits (716), Expect = 0.0
 Identities = 734/743 (98%), Gaps = 1/743 (0%)
 Strand=Plus/Minus

```

Query 1   GGATGCGGCGAGGGCTAGGACTCCTCCTAGTTTG-NGGGGAGGGAACGCAGGATGGCGTA
59      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 795  GGATGCGGCGAGGGCTAGGACTCCTCCTAGTTTGTGGGGATGGAACGCAGGATGGCGTA 736
Query 60  GGCAAACAGGAAGTATCATTCTGGTTTGATGTGGGGAGGTGTGGCTAGGGGATTAGCGGG
119     ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 735  GGCAAACAGGAAGTATCATTCTGGTTTGATGTGGGGAGGTGTGGCTAGGGGGTTAGCGGG 676
Query 120 GGTAAAGTTTTCTGGGTCTCCTAGCAGGTTTGGGGAGAATAAAGCGAGTGATGCAAGGAG
179     ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 675  GGTAAAGTTTTCTGGGTCTCCTAGCAGGTTTGGGGAAAATAAAGCGAGTGATGCAAGGAG 616
Query 180 GACAAATATTAGTGCGAATCCTAGGATGTCTTTTGTGGTGTGGTATGGGTGGAATGGGAT
239     ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 615  GACAAATATTAGTGCGAATCCTAGGATGTCTTTTGTGGTGTGGTATGGGTGGAATGGGAT 556
Query 240 TTTGTCGCAGTCTGAGGGGATGCCTAGGGGGTTGTTGGAGCCTGTTTCGTGTAGGAAGGT
299     ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 555  TTTGTCGCAGTCTGAGGGGATGCCTAGGGGGTTGTTGGAGCCTGTTTCGTGTAGGAAGGT 496
Query 300 TAGGTGGACTAGGGTGAGTCCTGCGATTACGAATGGCAGTAGGAAGTGGAGGGCAAAGAA
359     ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 495  TAGGTGGACTAGGGTGAGTCCTGCGATTACGAATGGCAATAGGAAGTGGAGGGCAAAGAA 436
Query 360 TCGTGTGAGGGTGGGTTGTCTACTGAGAAGCCTCCTCAAGCTCATTCTACGAGGGTTTG
419     ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 435  TCGTGTGAGGGTGGGTTGTCTACTGAGAAGCCTCCTCAAGCTCATTCTACGAGGGTTTG 376
Query 420 GCCGATGTATGGGATAGCGGAGAATAGGTTGGTGTGACTGTAGCCCTCAGAATGATAT
479     ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 375  GCCGATGTATGGGATAGCGGAGAATAGGTTGGTGTGACTGTAGCCCTCAGAATGATAT 316
Query 480 TTGTCTCAGGGGAGTACGTAGCCTACGAAGGCTGTTGCTATTAAGGTGAGAAGTAGGAG
539     ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 315  TTGTCTCAGGGGAGTACGTAGCCTACGAAGGCTGTTGCTATTAAGGTGAGAAGTAGGAG 256
Query 540 GACTCCGGTGTTCAGGTTTCCTTGTTCGCTAGGATCCATAGTAGAAGCCTCGGCCGAT
599     ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 255  GACTCCGGTGTTCAGGTTTCCTTGTTCGCTAGGATCCATAGTAGAAGCCTCGGCCGAT 196
Query 600 GTGTAGGTAGATGCAGATGAAGAACATAGAGGCTCCGTTAGCATGGAGATTGCGGATTAA
659     ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 195  GTGTAGGTAGATGCAGATGAAGAACAGAGAGGCTCCGTTAGCATGGAGATTGCGGATTAA 136
Query 660 TCAGCCGAATTGAACGTCTCGGCAGATGTGGGCAACGAGGCGAAGGCTAGGGAAGTGTC
719     ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 135  TCAGCCGAATTGAACGTCTCGGCAGATGTGGGCAACGAGGCGAAGGCTAGGGAAGTGTC 76

Query 720 TGCTGTGTAGTGTATAGCGAGAA 742
      ||||||||||||||||||||||
Sbjct 75  TGCTGTGTAGTGTATAGCGAGAA 53
  
```

Rufous Treecreeper Cytochrome b

Query sequence belongs to Rufous Treecreeper, from the Dryandra woodlands.and was matched by 99% to the Rufous Treecreeper cytochrome b sequence in GENBANK..

[|1477744|gb|U58501.1|CRU58501](#) *Climacteris rufa* cytochrome b (cyt b) gene,
mitochondrial gene
encoding mitochondrial protein, partial cds
Length=924
Score = 1206 bits (653), Expect = 0.0
Identities = 665/671 (99%), Gaps = 0/671 (0%)
Strand=Plus/Plus

```

Query 1 ACATCTGCCGAGACGTTCAATTCGGCTGATTAATCCGCAATCTCCATGCTAACGGAGCCT 60
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 107 ACATCTGCCGAGACGTTCAATTCGGCTGATTAATCCGCAATCTCCATGCTAACGGAGCCT 166
Query 61 CTATGTTCTTCATCTGCATCTACCTACACATCGGCCGAGGCTTCTACTATGGATCCTACG 120
      || ||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct 167 CTCTGTTCTTCATCTGCATCTACCTACACATCGGCCGAGGCTTCTACTATGGATCCTACG 226
Query 121 CAAACAAGGAAACCTGAAACACCGGAGTCCTCCTACTTCTCACCTTAATAGCAACAGCCT 180
      ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct 227 CAAACAAGGAAACCTGAAACACCGGAGTCCTCCTACTTCTAACCTTAATAGCAACAGCCT 286
Query 181 TCGTAGGCTACGTACTCCCCTGAGGACAAATATCATTCTGAGGGGCTACAGTCATCACCA 240
      ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct 287 TCGTAGGCTACGTACTCCCCTGAGGACAAATATCATTCTGAGGGGCTACAGTCATCACCA 346
Query 241 ACCTATTCTCCGCTATCCCATACATCGGCCAAACCCCTCGTAGAATGAGCTTGAGGAGGCT 300
      ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct 347 ACCTATTCTCCGCTATCCCATACATCGGCCAAACCCCTCGTAGAATGAGCTTGAGGAGGCT 406
Query 301 TCTCAGTAGACAACCCGACCCTCACACGATTCTTTGCCCTCCACTTCCTACTGCCATTTCG 360
      ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct 407 TCTCAGTAGACAACCCGACCCTCACACGATTCTTTGCCCTCCACTTCCTATTGCCATTTCG 466
Query 361 TAATCGCAGGACTCACCCCTAGTCCACCTAACCTTCTACACGAAACAGGCTCCAACAACC 420
      ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct 467 TAATCGCAGGACTCACCCCTAGTCCACCTAACCTTCTACACGAAACAGGCTCCAACAACC 526
Query 421 CCTTAGGCATCCCCCTCAGACTGCGACAAAATCCCATTCCACCCATACCACACCACAAAAG 480
      || ||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct 527 CCCTAGGCATCCCCCTCAGACTGCGACAAAATCCCATTCCACCCATACCACACCACAAAAG 586
Query 481 ACATCCTAGGATTCGCACTAATATTTGTCCTCCTTGCACTACTCGCTTTATTCTCCCCAA 540
      ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct 587 ACATCCTAGGATTCGCACTAATATTTGTCCTCCTTGCACTACTCGCTTTATTTTCCCCAA 646
Query 541 ACCTGCTAGGAGACCCAGAAAACCTTTACCCCCGCTAACCCCTAGCCACACCTCCCCACA 600
      ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct 647 ACCTGCTAGGAGACCCAGAAAACCTTTACCCCCGCTAACCCCTAGCCACACCTCCCCACA 706
Query 601 TCAAACCAGAATGATACTTCTGTTTGCCCTACGCCATCCTGCGTTCCATCCCCAACAAAC 660
      ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct 707 TCAAACCAGAATGATACTTCTGTTTGCCCTACGCCATCCTGCGTTCCATCCCCAACAAAC 766
Query 661 TAGGAGGAGTC 671
      ||||||| ||
Sbjct 767 TAGGAGGAGTC 777

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RTC Cytochrome b multiple Alignment

```

70      80      90      100     110     120     130     140     150     160     170     180
Norn 1D  CCCCATGTTCTTCATCTGCATCTACCTACACATCGGCCGAGGCTTCTACTATGGATCCTACGCAACCAAGGAAACCTGAACACCCGGAGTCCCTCTACTTCTCACCTTAATAGCA
Norn 2D  .....
Norn 3D  .....
Skel 1D  .....
Skel 2D  .....
Brad 1D  .....
Brad 2D  .....
Gura 1D  .....
Mang 1D  .....
Baal 1D  .....
Baal 2D  .....
Baal 3D  .....
Stra 2  .....
Stra 3  .....
NYil 1  .....
NYil 2  .....
NarkH 1 .....
NarkH 2 .....
WarnH 1 .....
Donq 1  .....
Donq 2  .....
RTC U58501 .....C.....A.....
    
```

```

290     300     310     320     330     340     350     360     370     380
Norn 1D  ATCGGGCAAACCCCTCGTAGAATGAGCTTGAGGAGGCTTCTCAGTAGACAACCCGACCCCTACACAGATTCCTTGCCCTCCACTTCCACTGCCATTCGTATCGCAGGACTCACC
Norn 2D  .....
Norn 3D  .....
Skel 1D  .....
Skel 2D  .....
Brad 1D  .....
Brad 2D  .....
Gura 1D  .....
Mang 1D  .....
Baal 1D  .....
Baal 2D  .....
Baal 3D  .....
Stra 1  .....
Stra 2  .....
Stra 3  .....
NYil 1  .....
NYil 2  .....
NarkH 1 .....
NarkH 2 .....
WarnH 1 .....
Donq 1  .....
Donq 2  .....
RTC U58501 .....C.....T.....
    
```

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430     440     450     460     470     480     490     500     510     520     530     540
Norn 1D  CCCCATTAGGCATCCCCTCAGACTGGGACAAAATCCCATCCACCCATACCACACCACAAAAGACATCCTAGGATTCGCCTATATATTTGTCCCTCCCTGCATCACFCGGTTTATCT
Norn 2D  .....C.....
Norn 3D  .....C.....
Skel 1D  .....
Skel 2D  .....
Brad 1D  .....
Brad 2D  .....
Gura 1D  .....
Mang 1D  .....
Baal 1D  .....
Baal 2D  .....G.....
Baal 3D  .....
Stra 1  .....
Stra 2  .....
Stra 3  .....
NYil 1  .....C.....
NYil 2  .....
NarkH 1 .....
NarkH 2 .....
WarnH 1 .....C.....
Donq 1  .....
Donq 2  .....C.....
RTC U58501 .....C.....T.....
    
```

```

560     570     580     590     600     610     620     630     640     650     660     670
Norn 1D  CTAGGAGACCCGAAAACCTTACCCCGCTAACCCCTAGCCACACCTCCCCACATCAAACAGAAATGATACTTCTCTGTTGCTAGCCATCCCTGGTTCCATCCCAACAAC
Norn 2D  .....T.....
Norn 3D  .....
Skel 1D  .....
Skel 2D  .....
Brad 1D  .....T.....
Brad 2D  .....
Gura 1D  .....
Mang 1D  .....A.....
Baal 1D  .....
Baal 2D  .....T.....
Baal 3D  .....T.....
Stra 1  .....T.....
Stra 2  .....T.....C.....
Stra 3  .....
NYil 1  .....
NYil 2  .....T.....
NarkH 1 .....
NarkH 2 .....
WarnH 1 .....
Donq 1  .....
Donq 2  .....
RTC U58501 .....
    
```

APPENXDIX 6

RTC Cytochrome b protein DNA Sequence Translation

>RTC Norn 1

```

1      CTT CGC CTC CGT TGC CCA CAT CTG CCG AGA CGT TCA ATT CGG CTG   45
1      L  R  L  R  C  P  H  L  P  R  R  S  I  R  L   15
46     ATT AAT CCG CAA TCT CCA TGC TAA CGG AGC CTC TAT GTT CTT CAT   90
16     I  N  P  Q  S  P  C  *  R  S  L  Y  V  L  H   30
91     CTG CAT CTA CCT ACA CAT CGG CCG AGG CTT CTA CTA TGG ATC CTA  135
31     L  H  L  P  T  H  R  P  R  L  L  L  W  I  L   45
136    CGC AAA CAA GGA AAC CTG AAA CAC CGG AGT CCT CCT ACT TCT CAC  180
46     R  K  Q  G  N  L  K  H  R  S  P  P  T  S  H   60
181    CTT AAT AGC AAC AGC CTT CGT AGG CTA CGT ACT CCC CTG AGG ACA  225
61     L  N  S  N  S  L  R  R  L  R  T  P  L  R  T   75
226    AAT ATC ATT CTG AGG GGC TAC AGT CAT CAC CAA CCT ATT CTC CGC  270
76     N  I  I  L  R  G  Y  S  H  H  Q  P  I  L  R   90
271    TAT CCC ATA CAT CGG CCA AAC CCT CGT AGA ATG AGC TTG AGG AGC  315
91     Y  P  I  H  R  P  N  P  R  R  R  M  S  L  R   105
316    TTC TCA GTA GAC AAC CCG ACC CTC ACA CGA TTC TTT GCC CTC CAC  360
106    F  S  V  D  N  P  T  L  T  R  F  F  A  L  H   120
361    TTC CTA CTG CCA TTC GTA ATC GCA GGA CTC ACC CTA GTC CAC CTA  405
121    F  L  L  P  F  V  I  A  G  L  T  L  V  H  L   135
406    ACC TTC CTA CAC GAA ACA GGC TCC AAC AAC CCC TTA GGC ATC CCC  450
136    T  F  L  H  E  T  G  S  N  N  P  L  G  I  P   150
451    TCA GAC TGC GAC AAA ATC CCA TTC CAC CCA TAC CAC ACC ACA AAA  495
151    S  D  C  D  K  I  P  F  H  P  Y  H  T  T  K   165
496    GAC ATC CTA GGA TTC GCA CTA ATA TTT GTC CTC CTT GCA TCA CTC  540
166    D  I  L  G  F  A  L  I  F  V  L  L  A  S  L   180
541    GCT TTA TTC TCC CCA AAC CTG CTA GGA GAC CCA GAA AAC TTT ACC  585
181    A  L  F  S  P  N  L  L  G  D  P  E  N  F  T   195
586    CCC GCT AAC CCC CTA GCC ACA CCT CCC CAC ATC AAA CCA GAA TGA  630
196    P  A  N  P  L  A  T  P  P  H  I  K  P  E  *   210
631    TAC TTC CTG TTT GCC TAC GCC ATC CTG CGT TCC ATC CCC AAC AAA  675
211    Y  F  L  F  A  Y  A  I  L  R  S  I  P  N  K   225
676    CTA GGA GGA GTC CTA GCC CTC GCC GCA TCC GTC CTA GTC CTC TTC  720
226    L  G  G  V  L  A  L  A  A  S  V  L  V  L  F   240
721    CTC GTG CCC TTC CTA CAC AAA TCG AAA CAA CGC TCA ATA ACC TTC  765
241    L  V  P  F  L  H  K  S  K  Q  R  S  I  T  F   255
766    CGC   768
256    R

```

```

LRLRCPHLPRRSIRLINPQSPC*RSLYVLHLHLPTHPRLLLLWILRKQGNLKHRSPPPTS
HLNSNSLRRLRTPLRNTNIIILRGYSHHQPIILRYPIHRPNPRRMSLRFSVDNPTLTRFFA
LHFLLPFVIAGLTLVHLTFLHETGSNNPLGIPSDCDKIPFHPYHTTKDILGFALIFVLL
ASLALFSPNLLGDPENFTPANPLATPPHIKPE*YFLFAYAILRSIPNKLGGVLALAASV
LVLFLVPFLHKSKQRSITF

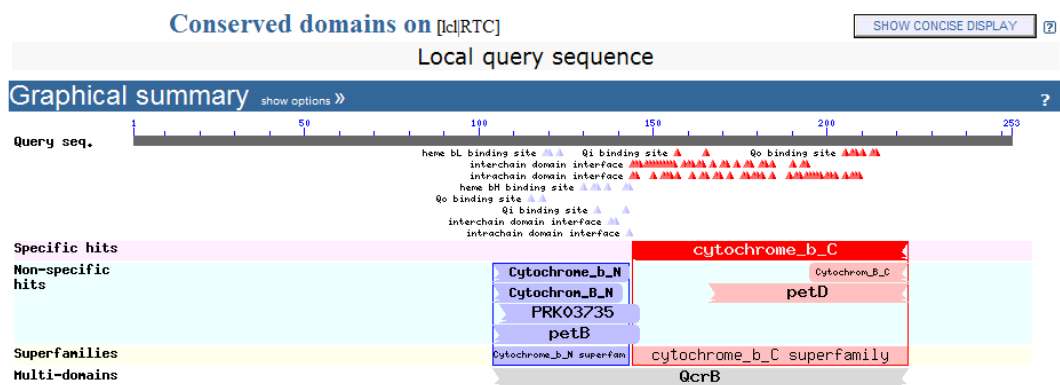
```

RTC Cytochrome b protein Sequence Identification

GenBank: AAB05470.1

cytochrome b

Features	Sequence
LOCUS	AAB05470 308 aa linear VRT 02-AUG-1996
DEFINITION	cytochrome b.
ACCESSION	AAB05470
VERSION	AAB05470.1 GI:1477745
DBSOURCE	locus CRU58501 accession U58501.1
KEYWORDS	.
SOURCE	mitochondrion <i>Climacteris rufa</i> (rufous treecreeper)
ORGANISM	Climacteris rufa



APPENDIX 7

PCA of RTC mitochondrial (*cytb*) DNA Genetic Distance (Phi)

File: C:\Documents and Settings\sara\Desktop\PRIMER\Cyt B Phi.pri
 Sample selection: All
 Variable selection: All

Eigenvalues

PC	Eigenvalues	%Variation	Cum.%Variation
1	4.72	36.3	36.3
2	2.32	17.8	54.1
3	1.50	11.6	65.7
4	1.08	8.3	74.0
5	0.95	7.3	81.3

Eigenvectors

(Coefficients in the linear combinations of variables making up PC's)

Variable	PC1	PC2	PC3	PC4	PC5
Norn	0.137	0.149	-0.138	0.407	-0.810
Caen	-0.294	-0.435	-0.208	-0.231	-0.106
Cong	-0.270	0.345	-0.372	0.017	-0.105
Gura	-0.263	-0.023	0.025	0.654	0.377
Mang	-0.278	-0.393	-0.222	-0.334	-0.231
BaalS	-0.434	0.051	-0.130	0.113	-0.081
BaalN	0.016	0.346	-0.386	-0.225	0.274
Knight	-0.344	-0.056	0.002	0.033	0.041
Wick	-0.015	0.366	-0.441	-0.070	0.076
Yilli	-0.084	0.440	0.349	-0.391	-0.125
Narrak	-0.310	0.008	-0.096	0.122	0.045
ForH	-0.356	0.160	0.427	-0.069	-0.049
Dongo	-0.372	0.191	0.275	-0.009	-0.130

Principal Component Scores

Sample	SCORE1	SCORE2	SCORE3	SCORE4	SCORE5
Norn	-4.104	0.311	-0.085	-1.427	2.233
Caen	0.833	2.539	0.587	0.705	0.141
Cong	0.871	-1.489	1.379	-0.077	0.226
Gura	-0.760	1.346	-0.839	-2.340	-1.900
Mang	-0.208	3.331	0.328	1.285	0.238
BaalS	2.952	-0.234	0.509	-0.478	0.089
BaalN	-2.857	-0.812	1.056	0.846	-1.420
Knight	1.934	-0.206	0.194	-0.076	0.237
Wick	-1.976	-1.246	1.464	0.268	-0.544
Yilli	-1.835	-1.426	-1.998	1.448	-0.004
Narrak	2.490	-1.112	0.934	-0.418	0.532
ForH	1.186	-0.372	-2.184	0.247	-0.065
Dongo	1.474	-0.629	-1.343	0.016	0.237

APPENDIX 8

PRINCIPLE CO-ORDINATE ANALYSIS (PCoA)

Mitochondrial (*cytb*) DNA Genetic Distance (ϕ)

DIAGONAL ELEMENTS OF TRANSFORMED MATRIX

Squared distance of each point from centroid:

1.080015	1.280056	0.279977	1.080015	0.479964
1.080015	1.280056	1.280056	1.080015	4.280091

TRACE (total variation) = 13.200260

EIGENVALUES OF TRANSFORMED DISTANCE MATRIX

4.959795	2.857864	0.999981	0.999981	0.999981
0.999981	0.999981	0.227885	0.154811	0.000000

Eigenvalue sums:

Positive = 13.20026
 Negative = 0.0000000E+00
 All = 13.20026

Specimen coordinates

1	-0.02044	-0.56825	0.04879	-0.00747	-0.00880	0.85565	0.12387	-0.05561	-0.06014
2	-0.50316	0.58891	0.17467	-0.34777	-0.62953	-0.06839	0.33797	-0.11152	0.03138
3	-0.01636	-0.36934	0.00000	0.00000	0.00000	0.00000	0.00000	0.18823	0.32842
4	-0.02044	-0.56825	-0.69084	-0.27337	0.15036	-0.29645	0.29582	-0.05561	-0.06014
5	-0.40166	0.38277	0.00000	0.00000	0.00000	0.00000	0.00000	0.37775	-0.17152
6	-0.02044	-0.56825	-0.04199	0.46646	-0.50231	-0.22156	-0.47879	-0.05561	-0.06014
7	-0.50316	0.58891	-0.03774	0.66973	0.32879	-0.03604	0.32754	-0.11152	0.03138
8	-0.50316	0.58891	-0.13693	-0.32197	0.30073	0.10443	-0.66550	-0.11152	0.03138
9	-0.02044	-0.56825	0.68403	-0.18562	0.36074	-0.33764	0.05910	-0.05561	-0.06014
10	2.00924	0.49282	0.00000	0.00000	0.00000	0.00000	0.00000	-0.00900	-0.01049

SPACER

Principal coordinates analysis

The method was first described in:

Gower, J. C. (1966)
 Some distance properties of latent
 root and vector methods used in
 multivariate analysis.
 Biometrika, volume 53, pages 325-328.

This software was described in:

Higgins, D. G. (1992)
 Sequence ordinations: a multivariate
 analysis approach to analysing large
 sequence data sets.
 CABIOS, volume 8, pages 15-22.

Results file is 203199.pcoord

APPENDIX 9

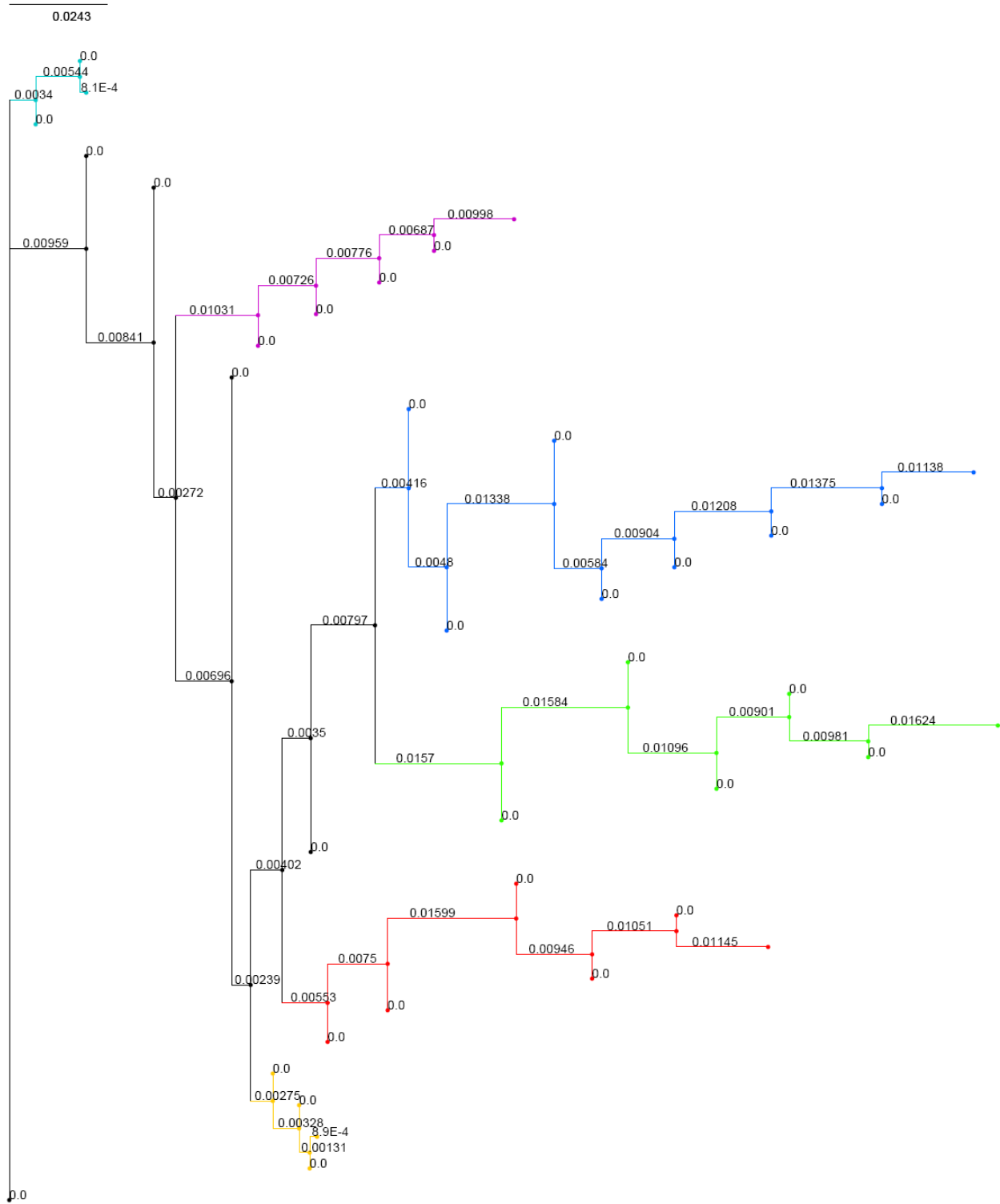
**Summary of the RTC Kinship Coefficients (Kinship Coefficient)
Across Distance Classes**

Values of computed statistics from SPAGeDi software, used to for genetic analysis at the individual level. There were 7 different loci, 60 individuals analysed and 9 distance classes calculated for Ritland's and Loiselle's Kinship Coefficients.

Dist classes	1	2	3	4	5	6	7	8	9		
Max distance	-1	5080.32	8753.32	16957.37	34080.55	37898.47	42775.12	52295.67	99058.87		
Number of pairs	0	241	267	241	267	238	258	254	250		
% partic		96.9	60.9	78.1	93.8	75	100	95.3	92.2		
CV partic		0.92	1	0.9	0.86	0.99	0.71	0.73	1.25		
Mean ln(distance)		1155.207	6437.901	12440.22	26488.29	36743.91	40621.79	46430.94	66504.21		
		7.5085	8.7523	9.4071	10.1532	10.5114	10.6116	10.744	11.0899		
Pairwise KINSHIP coefficients (Loiselle et al., 1995)											
Locus	intra-indiv	intra-group	2	3	4	5	6	7	8	9	average
ALL Loci	0.2036	0.0043	0.0054	-0.0142	0.0165	-0.0026	0.0041	-0.0228	-0.0156	-0.0029	-0.0029
Locus1	0.6705	-0.089	0.0481	-0.1088	-0.1422	0.0622	-0.0375	0.0591	-0.014	-0.021	-0.021
Locus2	-0.0712	0.0161	0.0019	0.0177	0.0079	-0.0276	0.028	0.011	-0.0492	0.0006	0.0006
Locus3	0.0752	0.0145	0.0348	-0.0503	-0.0734	0.0438	0.02	-0.0403	0.0122	-0.0007	-0.0007
Locus4	0.4462	-0.0022	0.0279	-0.0342	0.0282	0.0058	-0.0121	-0.0738	0.0138	-0.0039	-0.0039
Locus6	0.344	-0.0288	-0.001	-0.0037	0.1262	0.0028	-0.0385			-0.0086	-0.0086
locus7	0.1844	0.0217	-0.0118	-0.0057	-0.0334	-0.0154	-0.0211	0.0248	0.0246	-0.0022	-0.0022
Locus8	0.1337	-0.0122	0.0052	-0.0121	0.0863	0.0107	-0.0724	0.0029	-0.0121	-0.0027	-0.0027
Jackknifed estimators (over loci)											
Mean	0.1956	0.0034	0.0029	-0.0158	0.0447	-0.0048	0.002	-0.0208	-0.0246	-0.0015	-0.0015
SE	0.1196	0.0111	0.013	0.0194	0.0293	0.0129	0.016	0.0331	0.0231	0.0028	0.0028
Actual variance of pairwise coefficients (method of Ritland 2000)											
Estimate		0.002808	0.002632	-0.0008	0.004208	0.004145	-0.00382	0.00627	-0.00483	0.001378	0.001378
SE (jackknife over loci)		0.009738	0.010704	0.038479	0.049179	0.031037	0.021733	0.042822	0.009828	0.013525	0.013525
Pairwise KINSHIP coefficients (Ritland, 1996)											
Locus	intra-indiv	intra-group	2	3	4	5	6	7	8	9	average
ALL Loci	0.1315	0.011	-0.013	-0.0221	-0.0041	-0.023	-0.0006	-0.0116	-0.0311	-0.0117	-0.0117
Locus1	0.3734	-0.0576	-0.0218	-0.0789	-0.0917	-0.0363	-0.0596	0.0569	-0.0598	-0.0429	-0.0429
Locus2	0.017	0.0358	-0.0198	0.0034	-0.009	-0.0446	0.022	0.0095	-0.06	-0.0082	-0.0082
Locus3	0.0454	0.0042	0.0135	-0.0571	-0.0403	0.0164	0.0024	-0.0214	-0.0207	-0.0102	-0.0102
Locus4	0.1891	0.013	0.007	-0.022	-0.0072	-0.0312	-0.0202	-0.0381	0.0094	-0.0104	-0.0104
Locus6	0.3354	-0.0527	-0.0256	-0.0322	0.0693	-0.0163	-0.0415			-0.0334	-0.0334
locus7	0.0408	0.0345	-0.038	-0.0377	-0.0077	-0.0181	-0.0295	0.0165	-0.0129	-0.0124	-0.0124
Locus8	0.1345	-0.016	-0.0099	-0.0118	0.0359	-0.017	-0.0564	-0.1737	-0.1623	-0.0227	-0.0227
Jackknifed estimators (over loci)											
Mean	0.1275	0.0106	-0.0148	-0.0221	0.0101	-0.0249	0.0061	-0.0107	-0.0393	-0.0105	-0.0105
SE	0.0576	0.0105	0.0141	0.0131	0.0133	0.0144	0.0145	0.0188	0.023	0.0025	0.0025
Actual variance of pairwise coefficients (method of Ritland 2000)											
Estimate		-2.32E-05	-0.00243	-0.0005	0.000916	0.0015	0.002128	0.001992	-0.00172	0.000303	0.000303
SE (jackknife over loci)		0.012162	0.009678	0.010053	0.0566342	0.006018	0.01539	0.013842	0.008288	0.012092	0.012092

APPENDIX 10

Ritland's Kinship Phylogram with Relatedness values



APPENDIX 11

Species Catch List For The Dryandra Woodlands (2003-2007)

Species Common Name	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
Shell Duck			x					
Bee-eater		x	x					
Black-faced Cuckoo Shrike	x		x			x		
Blue-breasted Fairy-wren	x		x		x	x		x
Bronze-winged Pigeon				x				
Brown Honeyeater								x
Dusky Wood Swallow	x		x		x			
Elegant Parrot	x			x	x	x		x
Grey Currawong			x					
Grey Shrike Thrush	x	x	x					
Horsefield Bronze Cuckoo	x		x					
Kookabarra				x				
Little Red Wattle Bird					x			
Magpie			x	x				
New Holland Honey-eater	x							
Pallid Cuckoo		x						
Purple-crowned Lorikeet			x					
Red-capped Robin			x					
Regent Parrot								x
Ringed necked Parrot		x	x	x				x
Raven			x	x				
Restless Flycatcher		x		x		x		x
Rosella Parrot		x			x	x		
Rufous Treecreeper	x	x	x	x	x	x	x	x
Rufous Whistler		x		x				x
Sacred Kingfisher		x	x		x	x	x	x
Scarlet Robin		x						
Scrub Wren	x							
Smoker Parrot				x				x
Striated Pardalote		x			x	x	x	
Southern Boobook Owl	x							
Tawny-crowned Honeyeater								x
Tree Martin			x	x				x
Wilie Wagtail	x	x	x	x	x	x		
Wee Bill	x						x	
Western Warbler							x	
Western Yellow Robin		x		x			x	x
White-browed Babbler				x	x			
White-cheeked Honeyeater								x
White-naped Honeyeater								x
Yellow-rumped Thornbill		x						
Yellow-plumed Honeyeater	x	x	x	x	x	x	x	x
Wedge-tailed Eagle			x					
Total Species	13	15	19	15	11	9	7	16

APPENDIX 12

Percentage Foliage Cover, Rainfall Data and Location Co-ordinates

Annual Rainfall Data

Wandering (010917)

1999	505.4
2000	485.2
2001	441.6
2002	440
2003	604.8
2004	516.2
2005	626.6
2006	392.8
2007	537
2008	515.6
2009	524.6
2010	277.4

Location Co-ordinates

Site	Easting	Northing
Dryandra		
Norn	492310.4	6369955
Mangart	489648.3	6369158
Bradford	492901.9	6364631
Gura	495605.5	6375195
Skelton	487756.7	6372207
BaalucNth	493646.7	6370470
BaalucSth	493213.1	6369380
Marri	492799.3	6373432
Borranning	501718.4	6367029
Wandering	484207.3	6366644
Candy	508200.8	6372172
Penny	503539.9	6378408
Region		
Boyagin	487564.6	6403141
Wickpin	533585.8	6390072
NYilliminning	535414.8	6366292
Commondine	529568.8	6372975
Birdwhistle	545862.7	6360736
Warren	513255.7	6338767
Narrakine	516804.1	6337741
Dongolocking	565238.5	6341680
Norseman	979989.4	6439000