Conservation Genomics and Adaptive Management of Translocated Greater Stick-Nest Rats (*Leporillus conditor*) Under Climate Change

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Contents

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Thesis Abstract

In the last two centuries, many species in Australia and around the world have experienced rapid population declines. Further biodiversity loss is predicted under the projected rising temperatures and weather extremes associated with anthropogenic climate change. Informed, adaptive management practises are therefore required to safeguard Earth's flora and fauna from further extinction risk. However, given the speed at which many species have declined, conservation managers often operate in a knowledge void, particularly when making decisions about cryptic or understudied taxa with limited biological information available. In addition, there is often little available data on species' range, diversity and population size prior to human-driven declines, making goal-setting for restoration projects difficult. Recent advances in genomic technologies and wildlife monitoring technology may offer novel solutions to this problem. Informed, multi-disciplinary, effective conservation management strategies and decision-making is of increasing importance under climate change. This thesis therefore aims to use a variety of tools, including genomics, field ecology, morphology and population viability analysis, to investigate the past and present biology of a threatened endemic species, the greater stick-nest rat (*Leporillus conditor*). The knowledge gained from these studies will then be used to provide guidelines and suggestions for future management of the species, such as optimal translocation harvesting strategies and critical refuge requirements during periods of climatic extremes. The greater stick-nest rat shares many characteristics with other Australian small mammals, as it is a highly fragmented species that is frequently translocated, has suffered a significant range contraction, is vulnerable to predation and climate change, and is relatively data-deficient. The management strategies developed from this comprehensive research will therefore be broadly applicable to many species of conservation concern under the pressures of projected climate change.

Introduction

In recent years, wildlife across the globe have experienced rapid declines (WWF 2020), leaving many threatened species data deficient in areas critical to conservation management (IUCN 2013; Bland et al. 2015). As a result, conservation programs often operate in a knowledge void, with decision-making for interventions such as translocation, genetic rescue and captive breeding programs based on structured protocols, rather than species-specific biological information. This lack of natural history data can inhibit the success of conservation efforts, particularly for species with specific habitat requirements (Michaels et al. 2014; Berger-Tal et al. 2020). However, by combining modern and historic data on species' genomics, morphology, climate and microclimate niches, social structure, and other critical aspects of biology, ecologists can begin to fill the void for threatened species in order to improve conservation outcomes. My thesis seeks to piece together the biological puzzle of one such understudied species, the greater stick-nest rat (*Leporillus conditor*), with the aim of informing future management decisions and, in a broader sense, highlighting the importance of natural history data for conservation management under climate change.

The diversity of Australia's terrestrial mammal fauna

Australia is one of the most biodiverse countries in the world and is home to a high number of endemic taxa (Chapman 2009). Of the frog species found in Australia, 94% are found nowhere else in the world, along with 93% of reptiles, 45% of birds, and 87% of mammals (Chapman 2009). Australia's mammalian fauna is also the most distinctive in the world and, along with New Guinea, the only place where all three orders of mammals occur naturally (Holt *et al.*, 2013; Woinarski, Burbidge and Harrison, 2015). Monotremes are an ancient order (~110 mya) consisting of four species (Keast 1968; Archer et al. 1999), while marsupials are far more numerous (~250 species) and can be traced in the Australian fossil record back to 55 mya (Godthelp et al. 1992; Archer and Kirsch 2006; Mitchell et al. 2014). Bats, the first placental mammals to arrive in Australia, likely appeared on the continent around the same time, dispersing naturally from Asia to Australia (Godthelp et al. 1992; Cox 2000). Most native Australian terrestrial rodents did not follow until 4.5-4 mya (Whitelaw 1991; Aplin and Ford 2014; Smissen and Rowe 2018), all of whom belong to the subfamily Murinae (Johnson 2006; Breed and Ford 2007). These murines are often referred to as 'old endemics', or Old World rats and mice, and likely diversified in New Guinea before crossing

to Australia when sea levels were low in the late Miocene and early Pliocene (Aplin 2006). Murine rodents were joined in Australia by 'new endemics', a small number of *Rattus* species, in the Pleistocene (~1.8 mya) (Aplin 2006; Breed and Ford 2007). Today, there are 59 recognised modern species of native rats and mice in Australia, residing in a range of habitats from coastal to arid (Watts and Kemper 1989).

Australia's faunal extinction record

Australia's fauna has an extensive history of not only diversification, but extinction. The most recent extinctions can be classified into three main events. The first occurred during the late Pleistocene, 126-12 kya, and is characterised by the disappearance of Australia's megafauna (>44 kg) (Johnson 2006; Saltré et al. 2019). While there has been an ongoing debate as to the cause of these extinctions, recent studies attribute this rapid decline in megafaunal diversity in Australia and beyond to a combination of climate change (glacial-interglacial transition) and human impacts (Koch and Barnosky 2006; Saltré et al. 2019; David et al. 2021).

The next notable extinction period occurred during the Holocene, when two large marsupial carnivores – the Tasmanian devil (*Sarcophilis harrisii*) and the thylacine (*Thylacinus cynovephalus*) – and the native hen (*Gallinula mortierii*) disappeared from the Australian mainland, surviving only on Tasmania. These extinctions appear to be synchronous, and occurred between 3.1 and 3.2 kya (White et al. 2018). The cause of the disappearance of these two apex predators from the mainland has also been the subject of debate – potential explanations include the arrival of the dingo (*Canis lupus dingo*) (Johnson and Wroe 2003), climate variability associated with the onset of the El Niño Southern Oscillation (ENSO) (Brown 2006; Brüniche-Olsen et al. 2018; White et al. 2018), and human intensification (Johnson and Wroe 2003).

The third, and most recent, extinction event is an ongoing wave of biodiversity loss known as the "Anthropocene", of which human impacts are the driving force. The combined human-induced pressures of habitat fragmentation, unsustainable harvesting, the spread of invasive species, pollution and climate change interact to create a "perfect storm" resulting in extinction rates exceeding than those of previous mass extinctions in the fossil record (Wilson 2010; Barnosky et al. 2011; Pievani 2014). Since the year 1500, an estimated 868

species have become extinct worldwide, a level of biodiversity loss that is up to 100 times higher than background rates (Turvey and Crees 2019). With human intensification and industrialisation in the last two centuries, the threat of extinction to flora and fauna has only grown, and is expected to increase further – extinction rates could soon rise at least five-fold under current trajectories (Johnson et al. 2017). This escalation will primarily be driven by habitat reduction and modification, the spread of invasive species, pollution, overexploitation of species, and rapidly shifting environmental conditions caused by anthropogenic climate change (Diamond et al. 1989; Millennium Ecosystem Assessment 2005; Brook et al. 2008; Wilson 2010). Annual average temperatures in Australia may rise by up to 5°C by 2090 (CSIRO 2020). The flow-on effects of these rising temperatures include, but are not limited to, habitat loss and fragmentation and associated range contractions, migration of pathogens and predators, and more frequent extreme weather events (Malcolm et al. 2006; Cahill et al. 2013).

Climate change in an Australian context

Australia has experienced climate change events before. Northward drift resulting from the breakup of Gondwana has resulted in a long-term shift towards aridity (Barlow 1981; McLoughlin 2001; Hill 2004). Multiple drying events have forced the continent's fauna through a number of arid 'filters', resulting in the evolution of adaptations to aridity in many (if not most) Australian species (Dawson and Dawson 2006). In the middle Miocene (~14 mya), rapid growth of polar ice sheets in Antarctica caused sea-levels to drop (Zachos et al. 2008). Atmospheric circulation and precipitation was affected; where Australia's climate had been warm and humid in the early Miocene, the late Miocene saw rapid drying and aridification and a major reduction in rainforest cover across most of Australia (Martin 2006; Groeneveld et al. 2017). In the Pliocene, episodic cycles of aridity resulted in the formation of Australia's stony deserts between 4-2 mya (Dodson and Macphail 2004; Fujioka et al. 2005). During the Pleistocene, glaciations caused the climate to shift rapidly between warm and wet, and cold and dry climates, ultimately resulting in increased aridification; sand dune systems developed in central Australia, while southeastern Australia became increasingly dry ~1.5-1 mya (Quilty 1994; Fujioka et al. 2009; McLaren and Wallace 2010). Approximately 42 kya, a reversal of Earth's magnetic poles coincided with Grand Solar Minima to cause changes to atmospheric ozone concentration and circulation; known as the "Adams Event", this change resulted in global climate shifts, including a intensification of ultraviolet radiation

and a shift towards aridity in Australia (Cooper et al. 2021). During the Last Glacial Maximum (LGM) (~25-16 kya), when the most recent glacial cycle was at its peak, mainland lakes contracted and the cold, windy climate allowed sand dune deserts to develop further (Galloway 1965; Bowler et al. 1976; Turney et al. 2006). A period of further drying then occurred in the mid-Holocene, ~4-2 kya, coinciding with the onset of ENSO (Shulmeister and Lees 1995).

The most recent period of climate change began ~200 years ago, with the onset of industrialisation and fossil fuel-based economy (Head et al. 2014). Atmospheric carbon dioxide (CO_2) has risen from 270-275 ppm pre-1800, to ~414.5 ppm in 2020 (Steffen et al. 2007; NOAA 2021). This increased concentration of CO_2 has led to a "greenhouse effect", in which atmospheric CO_2, water vapour and other gasses absorb energy released from the Earth's surface (Anderson et al. 2016). This is a natural feedback loop that, under normal circumstances, creates a comfortable and liveable climate – but increased fossil fuel emissions have exacerbated the process and caused a global increase in temperature (Lacis et al. 2010; Anderson et al. 2016). Since national records began in 1910, Australia's average temperature has risen by 1.44°C (CSIRO and Bureau of Meteorology 2020). There have also been significant reductions in overall rainfall in several regions (Keenan and Cleugh 2011), while extreme rainfall, flooding, fire and heat events are becoming more frequent (Gallant and Karoly 2010).

Adaptive strategies in an arid climate

As a result of these repeated drying events and an evolutionary history of variable climates, Australian fauna, particularly those found in the arid zone, have evolved a number of adaptive strategies to survive in extreme environments (Dawson and Dawson 2006). These include, but are not limited to; behavioural adaptations, such as nocturnal activity patterns and burrowing for shelter (Withers et al. 2004); dietary adaptations, including a generalist feeding strategy allowing continuous exploitation of unpredictable food sources (Fisher and Dickman 1993); physiological adaptations, such as counterflow in the blood or airways or concentrated urine (Asres and Amha 2014); and morphological adaptations, wherein body and appendage surface area adapt to maximise heat loss (Roycroft et al. 2020). Organisms may also enter torpor during periods of extreme stress, an efficient way to conserve energy until conditions are more favourable (Warnecke et al. 2010). Adaptive life history traits can

also be observed at a population level, particularly in cases where a boom and bust lifecycle occurs in response to resource availability (Robin and Heinsohn 2009; Pavey et al. 2014). Species may employ any combination of these adaptations in order to persist during periods of drought and extreme heat.

Anthropogenic impacts on Australian biodiversity

Despite this suite of adaptations that have evolved over time in response to repeated selection pressures, Australia's extinction record is one of the worst in the world (Waldron et al. 2017). Since the arrival of Europeans almost two and a half centuries ago, the continent's unique wildlife has been subjected to increased predation by feral predators such as cats (*Felis catus*) and foxes (*Vulpes vulpes*), introduced pathogens, competition with introduced grazers, alterations to fire regimes and land use, habitat clearing and pollution (McKenzie et al. 2007; Woinarski et al. 2019). These combined pressures have resulted in the extinction of 38 vascular plant species, ten invertebrates, nine birds and 34 mammals, among other taxa – overall totalling at least 90 species (Woinarski et al., 2015, 2019). But Australia's chronicle of species extinctions is far from ancient history; recently, the world's first mammalian extinction attributed to anthropogenic climate change was recorded in the country's far north. The Bramble Cay melomys (*Melomys rubicola*), declared extinct in 2016, was an endemic rodent surviving on a tiny island in Torres Strait, and its disappearance has been attributed to ocean inundation of critical habitat as a result of rising sea levels induced by climate change (Gynther et al. 2016; Watson 2016; Fulton 2017; Woinarski et al. 2017).

Solutions to Australia's extinction problem

Conservation managers in Australia face a number of challenges, particularly when planning for projected climate change. Introduced predators represent a major threat to biodiversity; feral cats alone are considered to have been a major contributor to the extinction of 22 endemic Australian mammals, and threaten many more (Woinarski et al., 2015). While lethal controls such as baiting are common methods of fox and cat management, the most effective method for mitigating these threats appear to be the establishment of populations of vulnerable species on predator-free islands or within fenced enclosures on the mainland (Doherty et al. 2017). The impact of feral predators on native fauna can be exacerbated by fragmentation and land clearing (May and Norton 1996), a process that also limits dispersal,

gene flow and population connectivity. Revegetation efforts in cleared areas have proven to be effective in some cases, however; revegetated areas near remnant vegetation have been observed to increase species richness of birds and arboreal marsupials (Munro et al. 2007). These refugia can also act as a stepping stone to assist movement through the landscape, aiding dispersal whilst providing shelter from predators (Fischer and Lindenmayer 2002).

While valuable, planted vegetation is not a conservation solution for all taxa. Less mobile species requiring a highly complex understory, such as small terrestrial mammals, do not respond as well as birds and other highly mobile taxa to new stands of vegetation (Hobbs et al. 2003; McElhinny et al. 2006). In such cases, dispersal and colonisation can be aided by the process of translocation, wherein managers facilitate the movement of individuals into an area (often within the species' historical range) in order to establish new populations (IUCN 2013). This is a particularly effective solution for species that have suffered extreme range contractions; many Australian endemics only survive in extremely fragmented habitat, on offshore islands or in fenced reserves (Woinarski et al., 2015). Translocation insures the species against local extinction and can be an effective way to assist in species recovery following a bottleneck.

Translocation is not without its challenges. For species that have suffered severe range contractions – including many endemic Australian taxa – there is often little understanding of habitat requirements, climate tolerance thresholds, historical diversity and distribution, making it difficult for managers to predict survival outcomes when conducting a reintroduction (Berger-Tal et al. 2020). This uncertainty is further compounded by projected climate change; rising temperatures and increasingly unpredictable weather patterns makes it even more difficult to predict how translocated individuals will cope in their new environment. However, if a baseline understanding of the species' life history and requirements can be reached and future environmental change is taken into account, translocation has been flagged as a valuable tool to aid in the conservation of species threatened by climate change and ultimately reduce extinction risk (Hoegh-Guldberg et al. 2008; Butt et al. 2020). Species that do not have the capacity to adapt or migrate in response to a climatic shifts may be moved to more suitable areas by wildlife managers in order to ensure their ongoing persistence. This strategy represents a shift from the traditional "restoration" paradigm of conservation biology towards a more proactive approach designed

to manage and work alongside change (Thomas 2011), a necessary transition during a time of unprecedented anthropogenic disturbance.

Learning from the past

In cases where information on historical diversity and distribution is limited, studies have shown that information gained from Indigenous knowledge, the fossil record and museum collections can offer valuable insight into past population structures and community assemblages (Godoy et al. 2004; Taylor and Jamieson 2007; Willis et al. 2007; Seddon 2010; Burney and Burney 2016). This can provide goals and direction for managers seeking to return a species or ecosystem to its former state, such as the Western Australian government's Dirk Hartog Island National Park Ecological Restoration Project, 'Return to 1616' (Algar et al. 2020). Further, knowledge gained from historical sources can also inform on a species' vulnerability to temperature shifts under climate change, by providing insight into past climatic shifts, habitat niches and temperature thresholds, as well as phenotypic and genetic responses to climate change over time (Willis and Birks 2006; Jackson and Hobbs 2009; Moritz and Agudo 2013; Holmes et al. 2016; Denney and Anderson 2020). DeLeo et al. (2020) recently used herbarium records to study phenotypic changes in thale cress (*Arabidopsis thaliana*), identifying significant change over the past two centuries in all traits studied, likely in response to anthropogenic climate change. Moritz et al. (2008) used historical field notes, photographs and trapping records to resample the small mammal communities of Yosemite National Park, USA, and found that drastic elevational range shifts had occurred in half of the species in the last 100 years. Further, exon capture of alpine chipmunk (*Tamias alpinus*) museum skins from the same region showed increased genetic subdivision as a result of range contractions (Bi et al. 2013).

Contemporary adaptive management

In conjunction with historical resources, it is also important that managers and researchers continue to study relevant aspects of species' biology in order to improve conservation strategies under climate change. Advances in modern DNA sequencing techniques have made genetic analyses for conservation more affordable and accessible than ever before (Shafer et al. 2015). By using these platforms to quantify genetic diversity in threatened populations, managers can work towards reducing inbreeding depression and enhancing

heterozygosity to not only reduce the risk of extinction (Spielman et al. 2004; Charlesworth and Willis 2009), but encourage resilience to climate extremes by increasing adaptive capacity (Reusch et al. 2005; Sgrò et al. 2011). This is often achieved through the process of genetic rescue, a targeted gene flow strategy involving supplementation of genetically depauperate populations with translocated individuals from separate populations (Frankham et al. 2010; Whiteley et al. 2015). It may also involve the establishment of entirely new populations via reintroduction or assisted colonisation, often using a mixed provenancing approach by sourcing individuals from two or more existing source populations (Hoffmann et al. 2021).

There are many other elements of population ecology that must also be considered when planning conservation strategies for threatened species. Social structure and sex-biased dispersal behaviours can result in inbreeding avoidance, kin clustering and spatial genetic patterns (Hazlitt et al. 2004; Liebgold et al. 2011), and can also have implications for resource partitioning (Holekamp and Sawdy 2019). These elements can influence the viability of threatened populations, particularly those established by translocation programs. Harvesting of founder individuals and release strategies should take into account spatial genetics and species-specific dispersal patterns in order to maximise genetic diversity and the likelihood of successful population establishment (Goldenberg *et al.*, 2019; Pacioni *et al.*, 2020). Further, comprehensive knowledge of the habitat and resource requirements of species is an important factor in conservation; an organisms' niche relates not only to foraging and predator avoidance, but also its physiological tolerances (Rice 2005). For example, a species may have specific requirements for climate refugia that allow it to withstand high temperatures and other environmental extremes (Keppel et al. 2015). Managers must ensure that these requirements are well understood and provided for in conservation programs, particularly in the face of predicted climate change. Predator suppression can also improve the outcomes of wildlife management, and has been recognised as a critical contributor to increased abundance of native mammals, birds, reptiles and amphibians (Woinarski *et al.*, 2011; Hayward, Moseby and Read, 2014; Woinarski, Burbidge and Harrison, 2015; Hunter *et al.*, 2018).

Planning for future climate change

Integrating past and present ecological knowledge is a valuable step in developing and delivering informed, dynamic and adaptive conservation strategies under climate change (Beller et al. 2020), particularly in the case of reintroduction biology. Information on the past distribution and diversity of a species can guide the selection of suitable reintroduction sites (Burney and Burney 2016), as well as modelling species distributions under projected climate change (Gavin et al. 2014). The more managers know about the biology of a species, including population genetics, behavioural and social elements, and habitat requirements, the better they can plan harvesting and release strategies to maximise population establishment and ongoing viability following translocation (Goldenberg *et al.*, 2019). Reintroduction and translocation strategies can also be informed by Population Viability Analysis (PVA), a valuable risk assessment tool that can incorporate genetic data alongside life history parameters and potential environmental stressors to predict genetic diversity, inbreeding and extinction risk (Akçakaya and Sjögren-Gulve 2000; Chaudhary and Oli 2020; Seaborn and Goldberg 2020). Further, genomic insights into adaptive capacity can allow managers to select for, and encourage, resilience under a shifting climate (Aitken and Whitlock 2013). Modern genomic technologies can even identify signals of selection in response to environmental pressures such as drought (Cummins et al. 2019), allowing researchers to predict the vulnerability of populations to climate change. All of these factors combined contribute to a more specialised, informed approach to conservation, increasing the likelihood of positive outcomes under the growing pressures of climate change.

Adaptive conservation management in practice

As climate change shifts the goal posts of threatened species management, adaptive conservation practices are required (Pressey et al. 2007; Mawdsley et al. 2009; Groves et al. 2012; Rilov et al. 2019). An example of this kind of progressive, learning-based approach is at Arid Recovery Reserve, located in the arid lands of South Australia. Established in 1998, this 123 km^2 wildlife reserve includes a number of fenced, predator-proof exclosures, and has been the site of successful reintroductions of five native species, including the greater stick-nest rat (*Leporillus conditor*), the boodie (*Bettongia lesueur*) and the greater bilby (*Macrotis lagotis*) (Moseby and O'Donnell 2003; Bolton and Moseby 2004; Moseby and Bice 2004; Moseby et al. 2018). Arid Recovery operates in partnership with stakeholders, government, local community, traditional owners and collaborative scientists, allowing their research

impact to reach far beyond the reserve into the broader conservation community (Moseby et al. 2018).

Arid Recovery focusses heavily on scientific innovation and understanding climate change and drought. As such, many of their conservation efforts are experimental, seeking to fill knowledge gaps and provide solutions to seemingly insurmountable challenges such as introduced predators. Through investigative trials, ecologists at Arid Recovery have optimised predator exclusion fencing (Robley et al. 2007), pioneered one-way gates to prevent overpopulation within fenced reserves (Crisp and Moseby 2010), and used controlled predator exposure to improve anti-predator responses in reintroduced species (Moseby et al. 2012, 2016; West et al. 2018; Ross et al. 2019). Arid Recovery ecologists have recently created experimental artificial habitats for greater stick-nest rats to provide refuge during drought and heatwaves, including erecting shade cloth over exposed nests and constructing hollow rock shelters (Arid Recovery, unpubl. data, 2020). The large area and consistent monitoring (eg. routine trapping, camera traps, transects) within the reserve allows researchers to conduct long-term studies that are rarely possible in such remote environments. The knowledge gained through these innovative approaches to conservation are invaluable in a time of unprecedented biodiversity loss. Data on species' natural history traits under stress and their capacity for climate adaptation can inform future management strategies and improve conservation outcomes far beyond Arid Recovery Reserve itself.

The greater stick-nest rat – a model species for conservation in Australia

To demonstrate the value of an adaptive, holistic and informed approach to conservation management in the face of climate change, this project aims to combine an understanding of the past and present ecology, morphological diversity and genetic diversity of an endemic Australian mammal, the greater stick-nest rat, to formulate future management strategies. The greater stick-nest rat is a model species for threatened species conservation for a number of reasons. Firstly, the species suffered an extreme range contraction in the ~150 years following European arrival, eventually leading to its mainland extinction by the 1930s (Copley 1999a). The species' distribution, once encompassing the majority of the southern half of the continent, was reduced to a single population on the Franklin Islands, off the coast of Ceduna, South Australia. Many other Australian native species have shared similar fates, including the boodie (*Bettongia lesueur*) and Western Barred Bandicoot (*Perameles*

bougainville) (Short 1999; Short et al. 1999; Woinarski et al. 2015). Further, given the rapid nature of this range contraction, very little is known about the historical diversity of the species, and its habitat requirements or climate tolerance thresholds beyond the Franklin Islands.

The greater stick-nest rat has also been the subject of a number of translocations since the 1980s, both to other islands and to mainland refuges (Pedler and Copley 1993; Moseby and Bice 2004; Short et al. 2018, 2019). Although some reintroductions failed, often due to an inability to exclude predators (Copley 1999b; Short et al. 2018, 2019), many were successful. The greater stick-nest rat now has several meta-populations that can act not only as insurance populations, but as sources for future translocations. In addition, one translocation – to Arid Recovery Reserve – reintroduced individuals from a coastal habitat in the southernmost point of the species' known distribution to a desert climate. While the translocation was considered successful, greater stick-nest rats at Arid Recovery demonstrated high rates of mortality during heat waves and drought (Bolton and Moseby 2004). This represents a unique and highly valuable opportunity to use genomic methods to assess the genetic impacts of temperature shifts on bottlenecked species, as the translocation to an arid climate can be used as a proxy for climate change.

Most biological information available on the species has been gathered during expeditions to the Franklin Islands (Robinson 1975; Copley 1988) and observations of captive and translocated populations (Pedler and Copley 1993; Ryan et al. 2003; Bolton and Moseby 2004; Moseby and Bice 2004; Procter 2007; Short et al. 2018, 2019; White et al. 2020). However, due to its rapid mainland extinction, little is known about the historical population structure of the greater stick-nest rat, as well as its past climatic niches, physiological tolerance thresholds, habitat requirements and natural history (e.g. dispersal behaviours and social structure) in the wild. Further, there is currently no genetic data on the adaptive capacity of the extant and translocated populations. Improved knowledge of this species' ecology could not only assist current conservation strategies, but assist in planning for future management under climate change. The ongoing viability of the greater stick-nest rat, and many species like it both within Australia and overseas, relies upon effective and responsive adaptive management; our best defence against the exacerbation of an already poor extinction record is to seek a deeper understanding of threatened species' biology and requirements both past and present, as well as their genetics and adaptive capability. This study presents a

comprehensive analysis of multiple aspects of the life history of a threatened native species in a conservation context, with the specific aim to contribute to future management strategies under climate change (Figure 1).

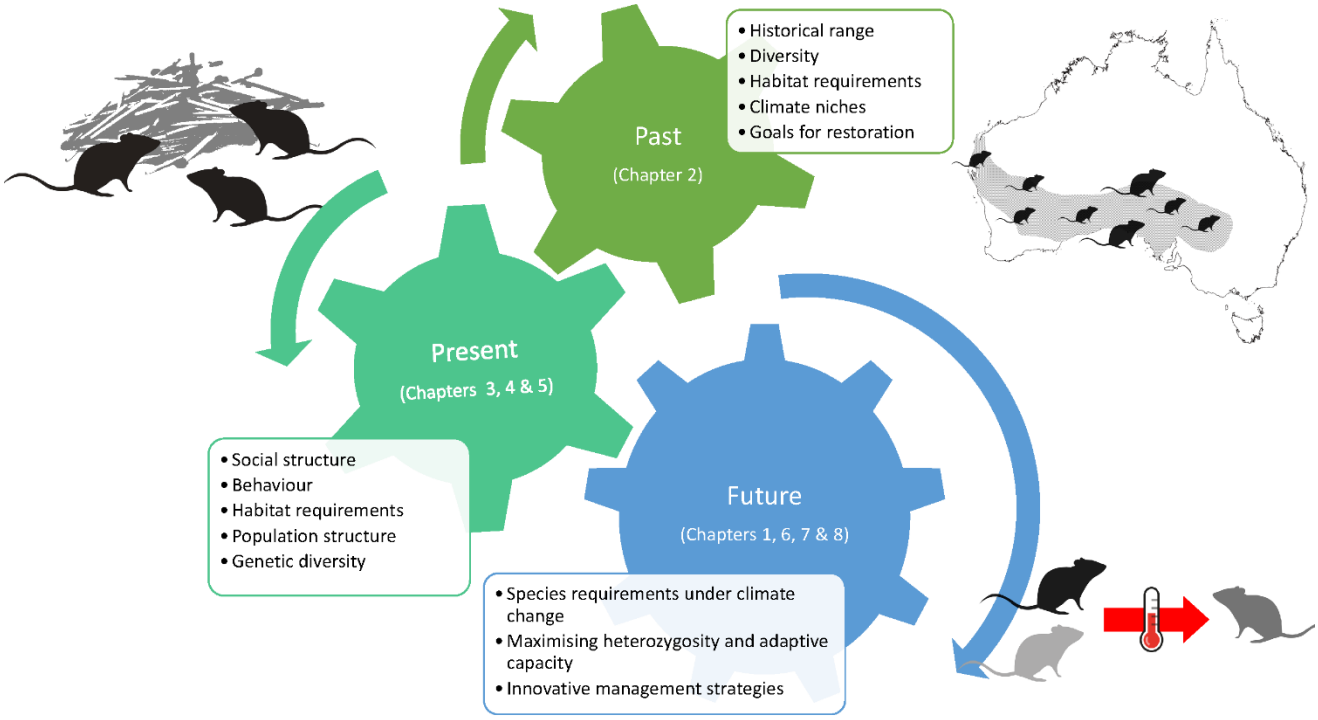

Figure 1 A summary of the thesis components and their temporal associations.

Thesis outline

Chapter One, a review of current and future genomic applications to conservation under climate change in Australia, aims to highlight potential avenues for modern DNA sequencing technology to assist in threatened species management in the face of unprecedented environmental change. I discuss the barriers to the uptake of conservation genomics in common practice, and provide examples of the ways that these obstacles are being overcome in the wildlife management community.

Chapter Two uses morphological data gathered from museum collections to determine the level of historical variation and diversity once present in the greater stick-nest rat prior to its mainland extinction. In particular, I assess whether intraspecific morphological variation was present in the species that may limit the capacity of individuals to persist following

reintroduction into parts of their historical range (i.e. specialised skull morphology in response to the local environment, island gigantism).

Chapter Three demonstrates the application of a read-doseage pipeline designed for shotgun data to a single nucleotide polymorphism (SNP) dataset generated by next-generation sequencing platform, Diversity Arrays (DArT-seq), in order to successfully determine the sex of field-collected greater stick-nest rat DNA samples in the absence of a reference genome. Given the increased uptake of next-generation sequencing in conservation biology, the paucity of reference genomes available for threatened species, and the difficulties associated with sexing in the field, this pipeline is a valuable tool for wildlife managers and researchers.

Chapter Four combines high-throughput DNA sequencing and field data to study the social structure and dispersal behaviour of the species in the early stages of translocation to Arid Recovery, a desert environment with climatic extremes similar to those predicted to occur with increasing frequency under climate change. I provide the first empirical evidence of female philopatry in the species, and use spatial genetic patterns to make recommendations for appropriate harvesting and release strategies of future translocations.

Chapter Five is an analysis of long-term temperature data gathered inside greater stick-nest rat nests at two study sites, a coastal habitat (Reevesby Island) and the arid conditions at Arid Recovery. I compare multiple nest substrates to determine the most effective climate refugia under extreme temperatures.

Chapter Six aims to determine whether signals of adaptation in response to heat stress are present in the genome of greater stick-nest rats following translocation to Arid Recovery, as the desert environment may have selected for individuals with greater physiological, morphological or behavioural traits to survive heatwaves and drought. If so, this has implications for the future of threatened species management under climate change.

Chapter Seven incorporates genetic data and life history parameters to construct a Population Viability Analysis for a planned translocation of greater stick-nest rats to Dirk Hartog Island, Western Australia. I model a variety of translocation scenarios to determine the optimal harvesting strategy that will maximise genetic diversity and potential adaptive capacity in the founding population, ultimately resulting in increased resilience to future climate change.

Chapter Eight is a general discussion of the outcomes of each chapter, consolidating the results and their combined implications for the future conservation and adaptive management of the greater stick-nest rat and other threatened small mammal species under climate change.

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Chapter 1

Genomic Approaches for Conservation Management in Australia under Climate Change

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Contribution to the Paper	Isabelle drafted the manuscript and acted as corresponding author .
Overall percentage (%)	80%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

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Review

Genomic Approaches for Conservation Management in Australia under Climate Change

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Abstract: Conservation genetics has informed threatened species management for several decades. With the advent of advanced DNA sequencing technologies in recent years, it is now possible to monitor and manage threatened populations with even greater precision. Climate change presents a number of threats and challenges, but new genomics data and analytical approaches provide opportunities to identify critical evolutionary processes of relevance to genetic management under climate change. Here, we discuss the applications of such approaches for threatened species management in Australia in the context of climate change, identifying methods of facilitating viability and resilience in the face of extreme environmental stress. Using genomic approaches, conservation management practices such as translocation, targeted gene flow, and gene-editing can now be performed with the express intention of facilitating adaptation to current and projected climate change scenarios in vulnerable species, thus reducing extinction risk and ensuring the protection of our unique biodiversity for future generations. We discuss the current barriers to implementing conservation genomic projects and the efforts being made to overcome them, including communication between researchers and managers to improve the relevance and applicability of genomic studies. We present novel approaches for facilitating adaptive capacity and accelerating natural selection in species to encourage resilience in the face of climate change.

Keywords: conservation genomics; climate change; assisted migration; genetic rescue

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1. Introduction

In the time since European arrival in Australia, native plants and animals have suffered major population decline and extinction. Ten percent of endemic mammal species known to be present in the 18th century are now extinct, and many others survive only on offshore islands and fragmented habitat [1]. Further, some 38 species of vascular plants, 10 invertebrates, 4 frogs, 3 reptiles, 1 fish, and 9 bird species have been confirmed extinct since European arrival in 1788 [2]. These impacts have been attributed to a number of factors, most notably land management changes (including land clearing for cropping and grazing), alterations to fire regimes, and the introduction of feral predators, including cats (*Felis catus*) and red foxes (*Vulpes vulpes*), and feral herbivores such as European rabbits (*Oryctolagus cuniculus*) [2–4]. However, extinction risk is being further exacerbated by human-induced climate change [5], with rapidly warming temperatures and increased frequency and magnitude of extreme weather events such as drought and fire resulting in phenological shifts, range contractions, and population declines in many taxa [5–7].

Since the late 20th century, the importance of genetic factors in the science of conservation biology has been well recognised; inbreeding depression and loss of genetic variation have been identified as potential drivers of extinction [8,9]. For example, an isolated population of mountain pygmy possums (*Burramys parvus*) at Mount Buller in Victoria suffered a considerable loss of fitness following a rapid population decline and subsequent

inbreeding [10]. Processes such as inbreeding and genetic drift, particularly in threatened species with small, isolated populations, can result in a high frequency of deleterious alleles, exacerbating extinction risk [11]. With this knowledge, genetic analyses are now a vital part of conservation biology in Australia [12–14], with several approaches currently being considered as potential strategies for maintaining, and in some cases increasing, the genetic diversity and resilience of threatened species [14,15].

With the advent of advanced DNA sequencing technologies, it is now possible to approach management of threatened species under a changing climate at the genomic level, taking into account not only genetic diversity and inbreeding effects, but fitness, gene expression, and adaptation [16,17]. The relevance and application of genetic tools to conservation have been discussed extensively in the literature [11,18–20], as have the various genomic approaches for DNA sequencing and analysis [21–23]. Here, we focus specifically on genomic approaches to conservation management under climate change in Australia—a continent with a range of climate change challenges, large latitudinal and environmental gradients, and a biota that has already suffered disproportionate rates of extinction, population fragmentation, and decline. However, the challenges presented by climate change to conservationists and the potential solutions discussed herein are applicable on a global scale. This review aims to discuss the current and expected conservation challenges associated with anthropogenic climate change, followed by the progress of conservation genomics to address these challenges. We explore some of the issues surrounding the application of such technologies to conservation and management strategies and highlight emerging opportunities to apply genomics to conservation in Australia.

2. Climate Change and Conservation Challenges

Anthropogenic climate change has caused Australia's average temperature to increase by 1 °C in the last century, and further warming is expected [24]. By the year 2090, annual average temperatures may rise by 5 °C [25]. Climate change has also been linked to an increase in extreme weather conditions [26], including more frequent and intense bushfires [27], cyclones [28], and floods [29]. These rapidly changing conditions compound the existing threats from habitat loss, fragmentation, feral predators, and competitors and are exacerbating extinction risk, all of which present new and pressing challenges for conservationists [5–7,30]. Two of the most critical issues relate to species' ability to shift their range or adapt in situ. While species may once have undergone range shifts in response to changing climates during the Late Pleistocene and Holocene [31], habitat loss and fragmentation are likely to hamper this response in the majority of species, particularly those with short dispersal distance. In the face of rapidly changing climate many species may not be able to adapt in situ due to low standing genetic variation, reduced gene flow, and/or limited phenotypic plasticity [32,33].

The initial consequences of climate change for Australian flora and fauna have been well documented in recent years and include range shifts, population declines, altered migration rates, and altered selection pressure [34–38]. Changes to the physical environment have resulted in catastrophic cascading ecosystem effects and negative feedback loops [39,40]. The impacts of climate change are evident across a range of habitats and environments, from the oceans [41] to the tropics [42] and even into the arid and alpine zones [43]. Montane species are being forced into higher altitudes as temperatures increase and will inevitably be forced "off the mountain top" [44]. Species with specific habitat and climatic tolerance ranges are predicted to be vulnerable to rising temperatures [45]; mechanistic models of future climate conditions predict a reduction in reproductive output of green sea turtles (*Chelonia mydas*) associated with marine heatwaves [46]. Conradie et al. [47] predict that by 2100, zebra finches (*Taeniopygia guttata*) will be exposed to acute lethal dehydration risk for several weeks of the year in over 50% of the species' current range. Climatic extremes have already resulted in massive diebacks of mangroves [48] and seagrass [49]. Furthermore, less resilient species with specific habitat requirements are becoming increasingly vulnerable due to shifts in their climatic niche. For example, only

30% of the current distribution of *Banksia marginata*, a highly fragmented but ecologically significant plant species, overlaps with the projected distribution under climate change by 2080 [50].

Unfortunately, despite these threats, the vast majority of management plans for threatened species do not currently include actions to improve adaptability to climate change [51].

3. Conservation Genetics in the Genomics Era

Conservation genetics is a discipline that incorporates genetic information into the planning and management of threatened species to minimise extinction risk. Genomic measures of relatedness, connectivity, and differentiation can be applied in a broad context to identify and clarify taxonomic issues and to identify evolutionarily divergent lineages within species [52]. At a local level, conservation managers can use genetic information to monitor gene flow and landscape genetics, as well as population parameters such as heterozygosity, genetic drift, and levels of inbreeding [14,53]. Genetics has also been used to inform pedigrees and breeding programs for endangered species in captivity by determining factors such as individual fitness and kinship [18,54,55].

Recent developments in high throughput DNA sequencing and its application to genomics have made genetic analysis more advanced and affordable for researchers [56]. Since 2005, DNA sequencing costs have reduced 5-fold, and the number of genetic markers available has increased by at least 2–3 orders of magnitude [57]. These genomic methods utilise high throughput sequencing technologies to sequence millions of DNA fragments in parallel, allowing thousands of genetic markers to be sequenced from hundreds of individuals in a single assay [58,59]. Previously, sequencing of mtDNA or nuclear genes or analysis of microsatellite loci limited genetic analyses to one to tens of loci and focused almost exclusively on neutral (or nearly neutral) loci [60]. While traditional methods were effective for taxonomy, phylogeography, and population genetic studies, genomic sequencing allows conservation geneticists to generate and analyse large data sets that include neutral and functional loci. The ability to assay functional variation extends the focus of conservation genetics to include processes such as natural selection and adaptation and to examine the fitness consequences of inbreeding [61,62]. Geneticists can now sequence the entire genome, use exome capture to target specific regions, or target single nucleotide polymorphisms (hereafter, SNPs) [63]. Although the massive amounts of data produced by genomic sequencing platforms necessitate advanced and diverse bioinformatics tools [58,64], such programs are constantly being improved and developed to allow genomic sequencing to reach its full potential. While there are still some uncertainties surrounding interpretation and uptake of genomic data in a management context [56], population genomics studies are increasingly being applied to conservation problems and management decision-making [65]. Genomic data have already been used to extensively study and characterise the genetic diversity of Australian wildlife, including quantifying the genetic effects of translocations in small mammal populations and identifying candidate genes associated with breeding success in marsupials [14,66–70].

The advances in genomic sequencing methods have made it an invaluable tool for conservation biologists, particularly when studying selection, adaptation, and functional diversity in threatened and economically valuable species [71,72]. For example, genomic studies of the Tasmanian devil (*Sarcophilus harrisii*) by Epstein et al. [73] revealed signals of selection in genes associated with immune function or cancer risk in three populations decimated by facial tumour disease, likely the result of an evolutionary response to the illness. This discovery has the potential to inform future selective breeding in the species, enhancing the prevalence of these resistant genotypes in insurance populations for the ongoing persistence of Tasmanian devils. SNP analysis of commercially important abalone (*Haliotis rubra*) identified genotype associations with several variable aspects of marine habitat, including sea surface temperature and ocean current, providing important insight into species resilience under fluctuating marine climates [74]. Genomic sequencing has also been used to identify local adaptation in gimlet trees (*Eucalyptus salubris*) [75] and potential

selection in response to sea surface temperatures in seaweed (*Phyllospora comosa*) [76]. SNP genotyping performed on degraded samples seized from the wildlife trade has even been used to identify population structure and differentiation of threatened species [77].

4. Application of Conservation Genomics to Climate Change Challenges

Genomics can provide critical new data to inform conservation management of threatened species under climate change in two key ways. Neutral variants—changes to the DNA sequence that have no effect on the viability of the individual—can be analysed to understand population processes such as gene flow, changes in population size, and population structure. Meanwhile, functional variants—DNA sequence changes that have fitness consequences—can be analysed to identify genetic diversity and patterns of local adaptation across potential source populations. Such knowledge may contribute to facilitating assisted range shifts, identifying suitable source populations for translocations and restoration carrying genotypes adapted to conditions at the recipient site [15], and enhancing local adaptation to climate change stress. An important application of conservation genomics is to inform species translocations, the facilitated movement of a species to an area within its historical range or to a new location with a suitable current or projected climate and habitat [78]. Traditionally, conservation managers conduct translocations to establish insurance populations, increase population size, and encourage heterogeneity [79,80]. However, Sgro et al. [81] argue that translocation should be considered not only as a method of increasing population sizes in threatened species but also as a means of creating "evolutionary resilience" to climate change. Assisted migration and genetic rescue are types of species translocation that may have the potential to offset the effects of climate change [82,83]. Furthermore, evolutionary rescue via processes such as targeted gene flow, another type of translocation, and gene editing, the process of altering DNA coding sequences to remove deleterious/insert advantageous alleles, may provide conservation solutions in the face of anthropogenic environmental shifts by quickly and efficiently improving the resilience of a population to external stressors [84–86]. These techniques are summarised in Figure 1. It is important to note that many of these technologies and approaches are still in the early stages of development, and while their potential uses are promising, limitations remain that are discussed further in subsequent sections of this review.

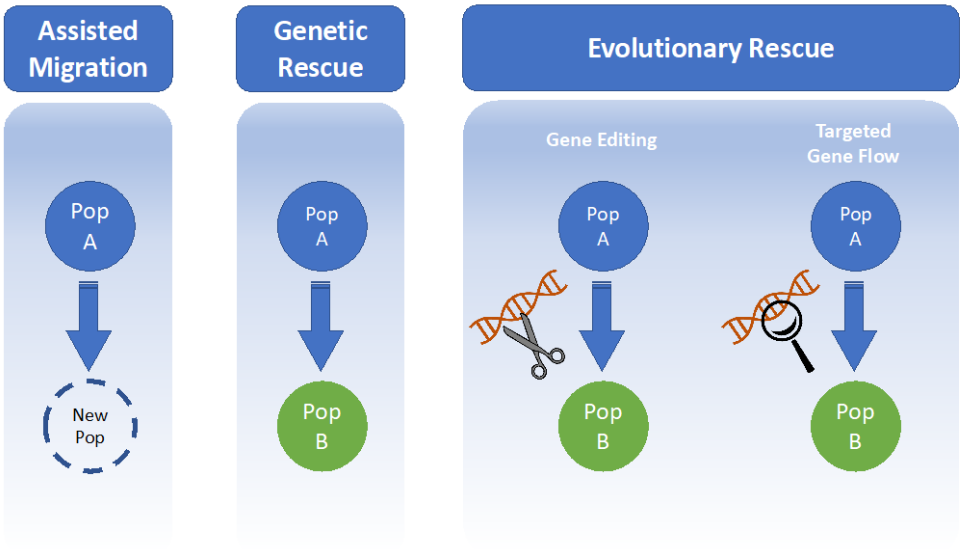

Figure 1. A summary of the conservation approaches discussed in this review that may be informed by genomics.

4.1. Assisted Migration

Assisted migration (or assisted colonisation) is the intentional movement of species to areas where habitat is predicted to become suitable as the climate changes (Figure 1) [87]. This usually refers to translocation of individuals outside their historical range but may include reintroductions to climatically suitable locations within the former range for species that have suffered large historical range contractions. Due to habitat fragmentation, many species that once encompassed large ranges no longer exist along an environmental gradient or have the capacity to disperse in response to climate change threats and stressors. In such scenarios, assisted migration may prove effective, particularly for sessile species or those with low dispersal ability [88].

Gallagher et al. [82] summarised the traits associated with species most likely to be affected by climate change and in need of assisted migration. Of most relevance to genomic applications to conservation are species with reduced adaptive capacity (poor ability to evolve in situ or disperse), small effective population size, and reduced genetic diversity. These features may be a result of recent population declines, long term effects of narrow ranges (narrow endemics) or niche specialisation, meta-population structure (new or existing barriers to gene flow), and distribution (for example, species in the tropics may have less adaptive capacity for temperature stress due to limited thermal seasonality). Examples of assisted migration outside a species' historical range are rare; however, Supple et al. [89] examined genomic variation in remnant populations of critically endangered yellow box (*Eucalyptus melliodora*) to inform restoration plantings in this species that has been reduced to less than 5% of its original range. By combining genomic data with environmental variables and climate predictions, they were able to identify sites for assisted migration and suitable source populations containing genetic variation adapted to future climate predictions.

4.2. Genetic Rescue

Translocation may also be used as a method of genetic rescue, whereby new individuals (and subsequently new genetic material) are introduced into an existing population with the aim of increasing population fitness and adaptive potential by increasing heterozygosity and adaptive capacity, masking deleterious alleles, countering the effects of inbreeding depression, and reducing genetic load (Figure 1) [15,83,86,90–93]. A well-known example of genetic rescue involves the mountain pygmy possum (*Burramys parvus*); an isolated population at Mount Buller was supplemented twice with genetically divergent males from larger populations, resulting in increased fitness and fecundity in the subsequent hybrids [10]. Genetic rescue can be applied to any taxa; experimental crosses between populations of a rare perennial daisy (*Rutidosis leptorrhynchoides*) resulted in similar or increased levels of heterosis across three generations [94]. Advances in genomics have given managers the ability to refine the science of genetic rescue further by testing for the presence of inbreeding depression in target populations, to predict the likelihood of gene flow causing outbreeding depression, to identify adaptive variation, and to closely monitor the results of population admixture for genetic rescue [95,96]. Emerging genomic technologies may even be used to predict the fitness consequences of alleles in a population, although some uncertainty remains around this method [93]. Genetic rescue is likely to become increasingly important under climate change, particularly given the tendency for environmental stress to increase inbreeding depression [97,98].

4.3. Evolutionary Rescue

A more specific variation of genetic rescue is evolutionary rescue, wherein adaptive evolutionary change is introduced to a population rather than overall genetic diversity [84]. One method of evolutionary rescue is targeted gene flow, a form of translocation that involves the introduction of new individuals with particular traits into an existing population with the aim of increasing a population's evolutionary resilience (Figure 1). In terms of climate change threats, individuals from a population with favourable alleles, e.g.,

resilience to high temperatures, could be translocated to another population of the same species that is not adapted to the threat, thereby increasing the resilience of the overall population within a few generations [99]. An example of how targeted gene flow can enhance evolutionary resilience was presented in a pioneering study by Kelly and Phillips (2019) [100], who suggested that the introduction of northern quolls (*Dasyurus hallucatus*) that avoided eating poisonous and invasive cane toads (*Rhinella marina*) to a quoll group naïve to the risks of eating the toads could result in a rapid adaptive response and, ultimately, a more resilient population. Hybrid offspring of toad-exposed and toad-naïve parents showed similar phenotypic responses to offspring of toad-exposed parents only, suggesting the presence of a dominant heritable trait for "toad-smart" behaviour. Although yet to be tested on a real-world population, the results of this study indicate that it is possible to introduce an adaptive response to a threat in a population through targeted gene flow. For targeted gene flow to be successful, however, knowledge of trait variation, heritability, and the underlying genetic variants linked to the trait are needed in order to identify suitable individuals to translocate.

Within a single species, certain populations may be better adapted to environmental stressors than others. For example, genomic sequencing has revealed within-species variation in heat stress response in both animals and plants [101–103]. This has important implications for species management under climate change. Recently, Cummins et al. [104] used the commercial genomic sequencing platform Diversity Arrays to conduct a genome-wide analysis of the Australian crawling frog (*Pseudophryne guentheri*), which revealed signals of local adaptation and limited gene flow between populations. While individuals living in the hotter, drier regions of the species' range were better adapted to predicted conditions in Australia under climate change, the more mesic individuals were not. Similarly, a study on greenlip abalone (*Haliotis laevigata*) revealed adaptive divergence across ~800 km of coastline that was strongly linked to minimum sea surface temperature and oxygen concentration [105]. In both cases, targeted gene flow between populations may encourage viability in the face of rising temperatures and other environmental shifts associated with climate change. Varied resilience to high temperatures has also been observed in coral reefs across natural temperature mosaics, with corals from warmer locations exhibiting mild selection in response to heat stress events [106,107]. A recent study by Quigley et al. [108] modelled the spread of temperature tolerant loci in corals in the Great Barrier Reef under natural and assisted scenarios. They concluded that adaptive variants are unlikely to spread fast enough to combat current rates of warming without human intervention. Targeted gene flow has therefore been flagged as a potential strategy to combat coral bleaching under climate change [109]. Further, Jordan et al. [110] identified 81 adaptive SNPs in the genome of mottlecah trees (*Eucalyptus acrocarpa)*, many of which were associated with variables of aridity, temperature, and rainfall, while Steane et al. [111] studied the genomes of a forest tree species, *Eucalyptus tricarpa*, across an area encompassing significant variation in aridity. Genomic divergence was found to be strongly correlated with temperature and moisture availability, evidence of local adaptation to environmental stressors associated with climate change predictions. The authors suggest that such information on the adaptive capacity of the species could be used to inform assisted migration in order to fix beneficial alleles and safeguard vulnerable populations against climate change.

Another underexplored genetic approach to addressing climate change impacts through evolutionary rescue is gene-editing. Already used extensively in agriculture, gene-editing involves the use of functional proteins to target a location in the genome and alter the gene's coding sequence or activity (Figure 1) [112]. Commonly, the RNA-guided Cas9 enzyme (isolated from CRISPR acquired immune systems in bacteria) is used to target and cut the DNA sequence, enabling insertion, deletion, and replacement [113]. Once considered impractical for wild populations, gene-editing technology has recently become much more accessible to conservation biologists [114]. Although research to date has focussed predominantly on the application of gene-editing to disease prevention and the suppression of invasive species, with the new capacity of genomic sequencing technology

to identify adaptive alleles associated with environmental stressors [115], it follows that the isolation, introduction, and fixation of these in a population would be possible via gene-editing [116,117].

In particular, CRISPR technology has the potential to be used for gene drives, wherein a beneficial trait is introduced and fixed in a population far more rapidly than natural selection allows [118]. For example, populations of American chestnut trees (*Castanea dentata*) have been decimated by the invasive pathogen chestnut blight fungus (*Cryphonectria parasitica*) since the early 20th century [119]. Researchers recently succeeded in developing transgenic American chestnut trees that demonstrate tolerance to the fungus by inserting a gene from wheat into the genome [117]. Gene editing could also be used to introduce deleterious alleles to populations of invasive species in order to reduce fitness and/or fecundity [114,118]. Johnson et al. [120] champion the applications of gene-editing technology for conservation, including the possibility of removing genetic disorders from a population, increasing genetic diversity following a bottleneck, or controlling the spread of invasive species. It represents a method of introducing beneficial alleles to a population that is threatened by climate change, particularly in situations where translocations are not possible [112]. In some systems, such as coral reefs, the introduction of natural or synthetic genes may aid in increasing resilience of species vulnerable to climate change effects [121]. Zafar et al. [122] discuss the possibility of using CRISPR technologies to develop novel quantitative trait loci in plants to increase resilience to abiotic environmental stressors including drought, temperature, and salinity. Further, CRISPR microinjection performed on larvae of the reef-building coral species *Acropora millepora* resulted in a ~50% mutation rate on all three target genes [123]. All target genes were putatively responsive to environmental stressors.

5. Overcoming Barriers to the Application of Genomics for Conservation Management under Climate Change

There are some barriers to the application of conservation genomics to management practices in Australia. A detailed discussion of the technical challenges associated with population genomics is beyond the scope of this paper (but see [86,124,125])—here, we aim instead to highlight the difficulties associated with the implementation of conservation genomics in management and how they can be overcome. First, the link between research and conservation practitioners must be strengthened to allow managers to set goals, make informed decisions, and integrate the findings of conservation geneticists with on-ground management practices in real-time [56,126,127]. A recent survey of 148 conservation practitioners in New Zealand indicated that although collaboration with geneticists was desired, managers did not know how to reach them [128]. Furthermore, Cook and Sgro (2017) [129] highlight the need for increased presence and engagement of evolutionary biologists in the conservation space, while Shafer et al. [56] observe that encouraging genome researchers to communicate directly with practitioners about the decreasing costs and potential uses for genomic technology, as well as its limitations, would be a step towards resolving the disconnect between scientists and stakeholders. Kadykalo et al. [130] identify the need for an interface that allows researchers to engage and connect with conservation managers, who, in turn, may communicate what types of genomic data would be helpful and applicable in the field.

Although many practitioners have been historically averse to admixture as a conservation strategy [131], a cultural shift has recently taken place. There have been a number of cases of successful collaboration between genetic researchers and conservation practitioners in Australia, such as the "devil tools & tech" umbrella framework implemented by the Save the Tasmanian Devil Program [126] and various provenance-related research projects to facilitate ecological restoration [69,132,133]. Indeed, the inclusion of non-academic co-authors in conservation genetics and genomics studies (e.g., [134,135]) has been shown to increase the likelihood of a specific solution- or policy-orientated outcome by up to 250% [136]. Garner et al. [75] note that much of the work occurring in non-academic spaces is not prioritised for publication, but it is clear that a holistic, collaborative approach with

open communication and engagement between stakeholders is highly beneficial. Such collaboration not only facilitates the implementation of research findings but also encourages targeted studies that are directly relevant to conservation managers and policymakers and fully utilises the potential of modern genomic technology [137,138].

Second, it must be acknowledged that the application of gene flow and gene editing as management practices carries a certain level of risk. Introducing new individuals to a population may lead to outbreeding depression [139], although the risk of outbreeding depression occurring has likely been overstated, as there is little evidence of its manifestation in wild populations [15,140]. Care must be taken to ensure that deleterious alleles are not being inadvertently introduced to populations and that locally adapted alleles are maintained [88,141]. A recent genetic rescue of Trinidadian guppies (*Poecilia reticulata*) resulted in increased fitness without swamping locally adapted alleles; however, the authors note that the results are not directly transferable to other organisms and that genetic rescue should be considered and planned case-by-case [142]. Furthermore, adaptational lags to contemporary temperature increases may mean that species are not well adapted to the conditions they are currently experiencing within their home-range, necessitating thorough and careful genomic analyses to choose an effective provenancing strategy for assisted gene flow [86,143].

Gene editing is also not without its challenges; Phelps et al. [112] note that while currently used for agricultural enhancement, such an approach would be challenging in a threatened species context due to the complex nature of adaptation and selection in ecology; traits are sometimes driven by a network of genetic responses (i.e., polygenic), rather than a single genomic region [125]. Varshney et al. [144] note that the development of stress-tolerance in crops via gene-editing is difficult, as tolerance can be expressed in many ways and is often the result of many genomic mechanisms. Managers implementing evolutionary rescue in general must also consider that phenotypic expression of genotypes can be unpredictable, and as such, the introduction of a new genotype to a population or area is not guaranteed to have the desired result [86,145,146]. Incorporating phenotypic data into planning strategies may assist in predicting the persistence of species introduced to new environments. Although a significant body of work on risk assessment has emerged in recent times [19,140], there remains a need for more resources surrounding decision-making tools and guidelines for conservation managers hoping to implement conservation genetics in the planning of threatened species management strategies [147]. Careful planning and risk assessment prior to intervention using tools such as those from Rossetto et al. [148] for conservation genomics management workflow are vital if genomic data are to be routinely included in threatened species management. This not only will help prevent undesirable outcomes but also will optimise resource usage and "bridge the gap" between researchers and conservation practitioners [149].

Finally, trust and support from the general public and conservation institutions for the expansion of conservation genomics must be gained in order to provide a solid foundation for future trials and innovation. Some conservation organisations such as zoos have policies against selective breeding that were put in place to safeguard species from becoming oddities or public curiosities [54]. These policies need to be updated to allow their participation in breeding trials and genetic interventions that are conservation focussed. Such institutions also need to play a stronger role in public education regarding genetic interventions. While there are a number of inherent issues associated with captive breeding programs, including genetic risks such as inbreeding depression [150], and behavioural challenges, such as predator naivety [151], breeding establishments such as zoos, herbariums, and seed banks have been identified as potentially vital resources in conservation genomics were their policies to become more flexible, not only as sources of genetic variation and insurance populations but also through providing a controlled environment for hybridisation trials [152–154].

6. Future Opportunities and Tools to Harness Conservation Genomics in the Fight against Climate Change

Advanced genomic sequencing technology can now be incorporated into conservation management strategies through genomic analyses, targeted gene flow, assisted migration, and gene editing. These methods can all be used in breeding programs, reintroductions, revegetation programs, and translocations to encourage viability in threatened species in the face of rising temperatures and extreme climate events. We see additional opportunities for genomics methods to involve experimental studies and targeted solutions to enable better planning and management for species conservation in the face of climate change. For example, genomic data could be used to determine how phenotypic plasticity and adaptive evolution act within species across environmental gradients in order to predict species' response and vulnerability to climate change [155]. Climate change experiments, either in the field or laboratory settings, using manipulated climatic conditions and genomic data could be used to identify evolutionary responses to changes in temperature and water availability [156]. This information could then be used to guide translocations and to revise species range loss projections under different climate change scenarios [157].

Accelerating natural selection in response to current and future environmental stressors may be particularly important for the survival of species that have suffered severe range reductions, a common occurrence amongst Australian endemics. Whilst reintroduction programs are becoming common, few take into account future adaptability or, indeed, adaptive capacity of source populations [140,158,159]. Conservation practitioners now need to think seriously about the long-term viability of the populations they are managing under climate change projections. Actions could include maximising evolutionary potential by working towards increased population size, genetic variation, and gene flow in managed populations [86,153] or targeted provenancing strategies involving the selection of source individuals for translocations and reintroductions with an adaptive bias towards predicted climate change conditions [160]. Climate resilience may even be encouraged by exposing individuals to climate stressors, as per Kelly and Phillips (2019) [100]. The greater stick-nest rat (*Leporillus conditor*), for example, is a murid rodent that became extinct on the Australian mainland in the early 1900s, surviving only on a single offshore island [161]. The species became the focus of a number of translocation efforts beginning in the 1980s, including a reintroduction to Arid Recovery Reserve, a 12,300 hectare predator-free enclosure in South Australia's arid zone [162,163]. Although the translocation was initially considered a success, having retained a viable population for two decades, it was observed that the stick-nest rats demonstrated spikes in mortality during extreme summer heat events [164], a selection pressure that may lead to natural selection for animals with improved physiological adaptations to heat. Comprehensive genomic analyses of the stick-nest rat population at Arid Recovery by White et al. [14] twenty years after the species' reintroduction identified six loci under putative selection in the genome when compared with founding populations, but further research is required to determine whether these genomic regions are associated with heat stress. This differentiation may be an adaptive response to heat stress experienced during the hot summer months at Arid Recovery, implying that the translocation of greater stick-nest rats has led to the establishment of a population that is better adapted to withstand hotter, drier conditions.

A number of frameworks and guidelines have recently emerged to facilitate the application of conservation genomics and genomic sequencing to wildlife management strategies (e.g., [165]). Hoffmann et al. [153] present a decision-making framework for managers that incorporates the potential and limitations of genomic approaches, as well as guidelines for inferring adaptive capacity and the significance of gene flow in a threatened species population. They note the importance of a robust reference genome (see also [166]) but also acknowledge that this resource is not always essential for detailed analysis of population structure and signals of selection associated with environmental variables, as evidenced by Grabowski et al. [167] and Wood et al. [76].

7. Conclusions

With the advent of genomic sequencing, conservation biologists now have the capacity to assess genomic data at a higher resolution than ever before. Not only can overall genetic diversity be analysed but also signals of adaptive evolution, mutations, and inbreeding can now be identified quickly and at relatively low cost. Under a rapidly changing climate, such technology has the potential to revolutionise conservation management; assisted migration, targeted gene flow, and gene-editing can now be performed from an informed perspective, encouraging adaptive capacity and selection for advantageous alleles in threatened populations to improve viability in the face of anthropogenic climate change. Conservation genomics will be of particular value in the management of threatened species with fragmented habitats that are unable to migrate or those with low genetic diversity and limited adaptive capacity. We recommend the application of novel conservation approaches discussed in this review to such taxa in the face of projected climate change. Although such strategies diverge from the traditional in situ conservation paradigm, preservationist methods alone are no longer feasible in the face of widespread climatic shifts. The humbling realisation that, in a comparatively short period of time, humans have induced irreversible changes to the global environment that will be observable in the fossil record for millennia calls for a shift in our attitude toward the world around us [168,169]. As Thomas (2011) [170] notes, "conservation under current circumstances is about managing change; retaining or restoring past community composition is no longer feasible".

While some limitations remain—species suitability, additional conservation requirements, the risk of outbreeding depression [19,171], and communication barriers between conservation practitioners and geneticists—the potential for conservation genetics utilising genomic sequencing technology must be realised if we are to actively and successfully conserve our remaining biodiversity under the threat of anthropogenic climate change. There are many examples of successful collaborations between researchers, stakeholders, and managers in Australia, such as the Pilbara northern quoll research program, a collaborative monitoring effort between multiple universities, researchers, and Indigenous groups, as well as the Western Australian state government [172] and the Genetic Rescue Project, a network of scientists and stakeholders working towards the recovery of five threatened species (e.g., [135]). Based on the success of these cooperative approaches, we reiterate previous calls [56,126,127,129–131] for practitioners and researchers to consider the ongoing genomic viability of species in the face of climate change when planning future conservation actions, to collaborate and communicate, and to harness the wealth of information that genomic sequencing provides for more informed and targeted management strategies moving forward.

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Chapter 2

Morphological variation in skull shape and size across extinct and extant populations of the greater stick-nest rat (*Leporillus conditor*): implications for translocation

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Overall percentage (%)	75%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

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Morphological variation in skull shape and size across extinct and extant populations of the greater stick-nest rat (*Leporillus conditor*): implications for translocation

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ABSTRACT

Within-species morphological variation is often observed across spatial and climatic gradients. Understanding this variation is important to conservation planning, as specialised adaptations may influence a population's persistence following translocation. However, knowing whether local adaptations are prevalent within a species can be challenging when the species has undergone range contractions. Here, we used museum specimens to study size and shape variation of the greater stick-nest rat (*Leporillus conditor*). We aimed to determine whether intraspecific size and shape variation previously existed within the species across its historical range, and inform on possible implications for translocations of the remaining extant population. We found significantly larger skull size in the Franklin Islands and arid populations, possibly indicating a historically continuous population experiencing similar selection pressures such as high predation pressure, competition with other large arid zone rodents or climatic extremes. Conversely, skull shape variation within the species adheres to an allometric trajectory, indicating no specific local adaptations of skull shape. This absence of local skull shape adaptation suggests that the Franklin Islands population is likely suitable for mainland translocations. However, further research into the historical phylogeography of the species is recommended to identify whether large size resulted from shared ancestry or convergent evolution.

Keywords: conserved cranial allometry, intraspecific variation, local adaptation, morphology, muridae, reintroduction biology, rodent, translocation.

Introduction

Intraspecific morphological variation can vary spatially due to phenotypic plasticity, natural selection and adaptation, or genetic drift (Price *et al.* 2003; de Abreu *et al.* 2018). This variation may be a response to spatial or temporal variation in climate, competition, predation pressure, habitat or diet (Alexander *et al.* 2006; Campbell-Tennant *et al.* 2015; Foth *et al.* 2015; Lostrom *et al.* 2015; Onley *et al.* 2020). Many Australian taxa exhibit morphological variation across their range in response to various ecological and environmental changes (Keast 1968; Lostrom *et al.* 2015); the Lakeland Downs mouse (*Leggadina lakedownensis*), for example, presents considerable morphological variation across its range, including island gigantism (Cooper *et al.* 2003). However, anthropogenic range contractions, extirpations and habitat fragmentation, are known to reduce intraspecific morphological diversity and population structure (e.g. Des Roches *et al.* 2021).

Understanding intraspecific variation in morphology is relevant to threatened species conservation for several reasons. Firstly, much of conservation biology is species-orientated and descriptions of geographic variation in morphology are important for delineating biological species and resolving taxonomic issues (Dubois 2003; Godfray *et al.* 2004). For example, morphological studies of intraspecific variation in Australian bandicoots (genus *Perameles* and *Chaeropus*) has recently resulted in the identification of

a number of new species from within what was traditionally thought to be a single species (Travouillon and Phillips 2018; Travouillon *et al.* 2019). At a finer scale, knowledge of intraspecific morphological variation can complement population genetic data to identify geographic population structure and intraspecific units for conservation (Arnoux *et al.* 2014; Hounkpèvi *et al.* 2020). Further, knowledge of morphological variation is critical when planning translocations that involve two or more source populations. Mixing phenotypically different populations may prevent or reduce interbreeding if pre-zygotic isolation exists (Alexandrino *et al.* 2005; Latch *et al.* 2006), which can produce offspring that are maladapted to the local environment, or can lead to non-random mating between source populations (Charlesworth and Willis 2009; Thavornkanlapachai *et al.* 2019). Finally, morphological studies can be used to quantify how within species diversity has changed following a bottleneck (Lovatt 2007).

Identifying the extent of morphological variation within species is a necessary, but often overlooked component in planning reintroductions and translocations. Local adaptations or plasticity in fragmented populations may be a key element for survival and persistence. Although difficult to determine from morphology alone, knowledge of whether physical variation is due to natural selection or phenotypic plasticity is critical to identify whether a population could adapt to a new environment or selective pressure *in situ* or following translocation (Lema and Nevitt 2006; Ficetola *et al.* 2016). Variation due to phenotypic plasticity may produce favourable results and improve the rate of population establishment (Haddaway *et al.* 2012); for example, a mainland translocation of an island population of golden bandicoot (*Isoodon auratus*) resulted in an increase in fecundity, skeletal size and body mass within four generations, which researchers suggested was a result of a reduction in competitive pressures (Dunlop and Morris 2018). Local adaptations, however, may result in reduced fitness following translocation if they not suited to the translocation site (Hereford 2009; Taylor *et al.* 2021). For example, Taylor *et al.* (2021) suggested that Shark Bay bandicoots (*Perameles bougainville*) translocated to the arid zone of Australia may not possess the necessary auditory adaptations for predator avoidance in a desert environment. Further, sock-eye salmon (*Oncorhynchus nerka*) adapted to a beach environment demonstrated reduced reproductive success when colonising a stream environment (Peterson *et al.* 2014). This reduction in fecundity was attributed to limitations resulting from the beach-adapted salmon's larger body size, which made them more susceptible to predation and stranding, and limited their access to mates and spawning sites in shallower areas.

Rodents are exemplary for exhibiting morphological variation across wide geographical ranges and a variety of environmental conditions (Maestri *et al.* 2016; Assis *et al.* 2017). For example, species in arid habitats have larger bullae in order to detect low frequency sounds and longer nasal passages to aid respiratory water retention (Lay 1972; Alhajeri and Steppan 2018; Basso *et al.* 2020). These adaptations can result from factors such as changes in food availability, rainfall, primary productivity, or thermoregulatory requirements under varying climates, and can lead to functional differences between populations (Walsh *et al.* 2016). Therefore, when developing translocation strategies, conservationists should not assume that all populations will respond homogeneously to different environments across the species' distribution, particularly if the reintroduction site is markedly different from the source (Zaidaneen and Hasaseen 2008). However, despite being universally recognised as critical to survival (Schlichting 1986; Agrawal 2001), local morphological adaptation is rarely considered during translocation planning and assessment. This is of particular concern for species that historically had wide geographical ranges and many potential ecotypes (Mee *et al.* 2015) but have declined to a single habitat type or restricted areas. One such species is the greater stick-nest rat (*Leporillus conditor*), an endemic Australian rodent that has been the subject of multiple translocations since the 1980s. Although *L. conditor* has suffered a considerable range contraction in the past two centuries (Copley 1999), the species once inhabited a large geographical range encompassing many habitat types and bioregions, from mesic coastal environments to the arid zone. However, its rapid population decline has resulted in limited knowledge of the species' historic morphological variation, including potential adaptations to environmental variation such as maximum/minimum temperature, shelter sites and food and water availability. Increased mortality has also been noted in reintroduced *L. conditor* at an arid site during periods of extreme heat stress (Bolton and Moseby 2004), despite the site being encompassed by the species' historical range. This raises concerns for the heat tolerance thresholds of this population, having been sourced from the southernmost, and most mesic, point of the species' range and translocated to the arid zone.

In this study, we use morphometric analyses of museum specimens to identify patterns of morphological variation in skull shape and size across the species' former range. We aim to determine whether intraspecific variation existed across the historic distribution of *L. conditor* as a result of adaptations to environmental niches, and inform on possible implications for the conservation management of the species. Given that populations isolated on islands often display divergent phenotypes in comparison to their mainland counterparts (e.g. island gigantism/dwarfism) (Case 1978), it is expected that the single extant population of *L. conditor* will differ in size (and associated allometric shape variation) compared with the extinct mainland populations. Further, given the variety of habitat types encompassed (e.g. desert, plains), some morphological diversity is expected among the mainland populations in response to environmental gradients such as climate and vegetation.

Methods

Study species

Following European arrival and the introduction of feral predators and herbivores, as well as land use changes, *L. conditor* was extirpated from its entire mainland Australian range, with just a single population surviving on the Franklin Islands, off the coast of Ceduna, South Australia by the early 1900s (Copley 1999). This population was briefly classified as a separate species, *L. jonesi*, but has since been synonymised with *L. conditor* (Thomas 1921; Copley 1999). What little is known about the historical range of this murid rodent has been gathered from subfossils, nest remains, sightings by early naturalists, and voucher specimens in natural history collections (Copley 1999). In the mid-1980's, after an extensive ecological study of the Franklin Island populations, a captive breeding program began and was shortly followed by multiple translocation efforts to Reevesby and St Peters Islands, as well as several fenced reserves (Van Dyck *et al.* 2008; Short *et al.* 2019). While some reintroduction efforts have been successful, such as those at Salutation Island and the Arid Recovery Reserve, others, including translocations to reserves at Venus Bay and Faure Island, failed due to predation by species such as feral cats and raptors (Woinarski and Burbidge 2016; Short *et al.* 2019).

Samples

A total of 199 partial and whole skulls (preserved as skeletal material) of *Leporillus conditor* from 34 locations across the species' historic range were sourced from the Mammal and Palaeontology collections at the South Australian Museum, Adelaide (SAM), the Western Australian Museum, Perth (WAM) and Museum Victoria, Melbourne (MV) (Table 1, Supplementary Table S1). In addition, morphometric data recorded in Tate (1951) of the type specimen of *L. jonesi* and of a *L. conditor* specimen collected at Ooldea, South Australia by E. Troughton were included. To assess environmental variation across the geographic range of *L. conditor*, individuals were grouped according to the Interim Biogeographic Regionalisation for Australia (IBRA) classification system (Table 1, Fig. 1). IBRA regions separate Australia's landscapes into 89 geographically distinct bioregions characterised by common vegetation, habitat, geology and climate (Thackway and Cresswell 1995; Environment Australia 2000).

Cranial and dental measurements

Fifteen linear measurements of the cranium and mandible (Fig. 2, Table 2) were taken using iGaging Absolute Origin digital calipers developed from common linear morphometrics used in past studies of rodents, including features associated with climatic variation such as rostra length and width (Musser and Piik 1982; Voss 1988; Mortelliti *et al.* 2012; Fabre *et al.* 2013; Alhajeri and Steppan 2018). Although bullae were measured during data collection as a point of interest of adaptation to aridity, these features were not available for the majority (86%) of the samples, and were therefore excluded from the final analysis. Cranial material was chosen for this study over skins, as shrinkage of skins can distort physical features and may confound morphological studies (Horie 1990; Shu *et al.* 2017). Where one side of the mandible was available, measurements were taken from that side; where both were available, a side was chosen at random. Where only part of the skull was available, measurements were only recorded for features that were not broken or damaged. Specimen age was determined by examining the tooth wear of the individual, as well as the ossification of the cranial plates and of the suture between the basioccipital and basisphenoid bones (Gustafson and Malmö 1950; Morris 1972; Pankakoski 1980). In cases where a specimen was identified as juvenile, no cranial measurements were taken. With the exception of the Tate and Troughton specimens, all measurements were taken by one researcher (I.R.O) to minimise observer error. As a measure of repeatability, a subset of measurements was used to determine the intraclass correlation coefficient (ICC) using the R package 'ICC' (version 2.3.0).

Table 1. Sample sizes of *Leporillus conditor* skulls collected in each IBRA region.

IBRA Region	*n*
Carnarvon	13
Yalgoo	44
Murchison	1
Coolgardie	11
Hampton	12
Nullarbor	70
Eyre Yorke Block	30
Stony Plains	1
Simpson Strzelecki Dunefields	5
Flinders Lofty Block	10
Riverina	1
Darling Riverine Plains	3

Data analysis

All analyses were completed using the R Statistical Environment (version 4.0.2) (R Core Team 2021, R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. Available from: https://www.R-project.org/). Due to the poor condition of some of the cranial material 53% of the measurements were missing from the full dataset. In order

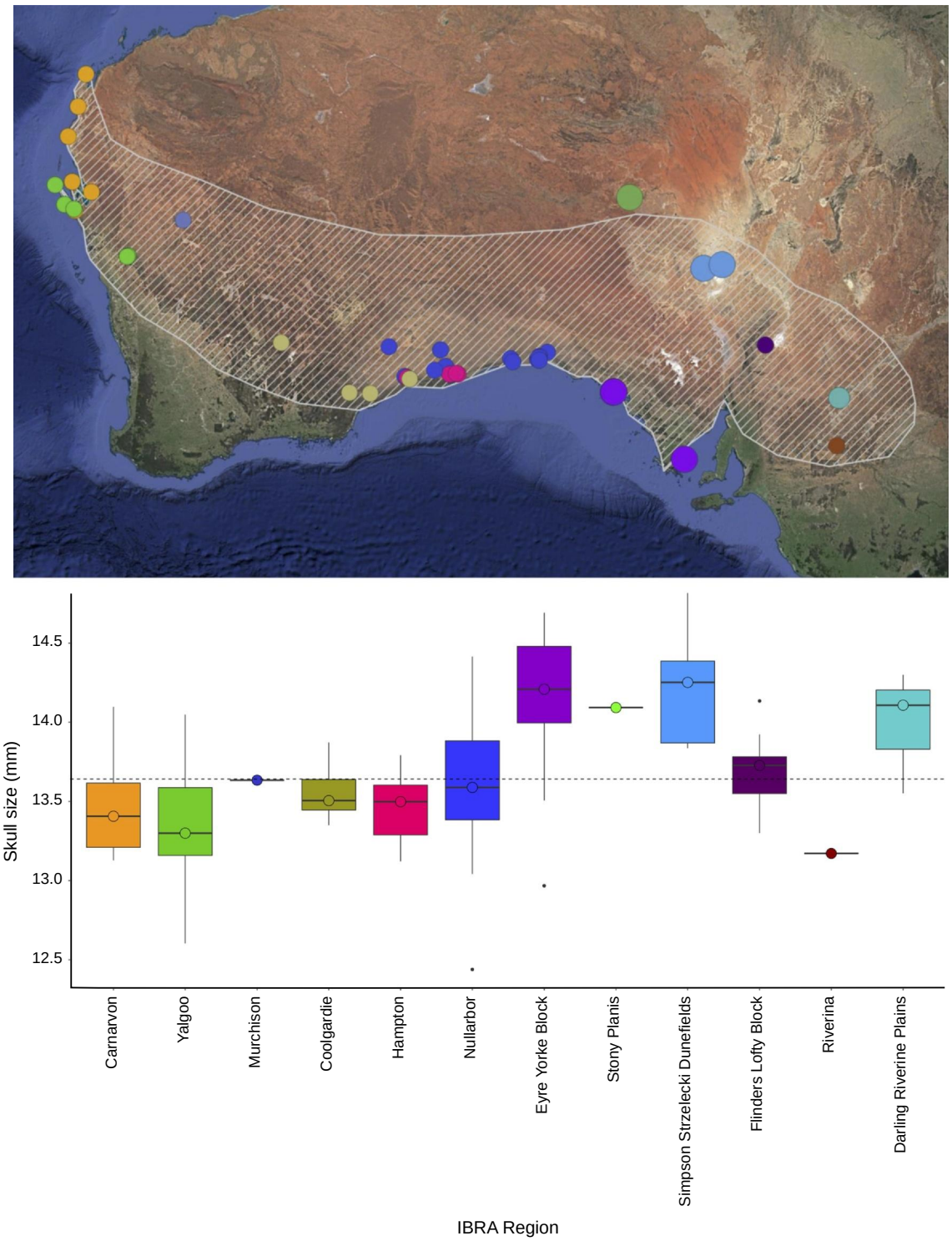

Fig. 1. Skull size (geometric mean of linear variables) of *Leporillus conditor* per IBRA region, corresponding to a map of collection locations across the historic range of the species (represented by grey hashed area). Size of points on the map reflect the size of individuals from that location. Dotted horizontal line indicates overall mean skull size. See also Table 3a.

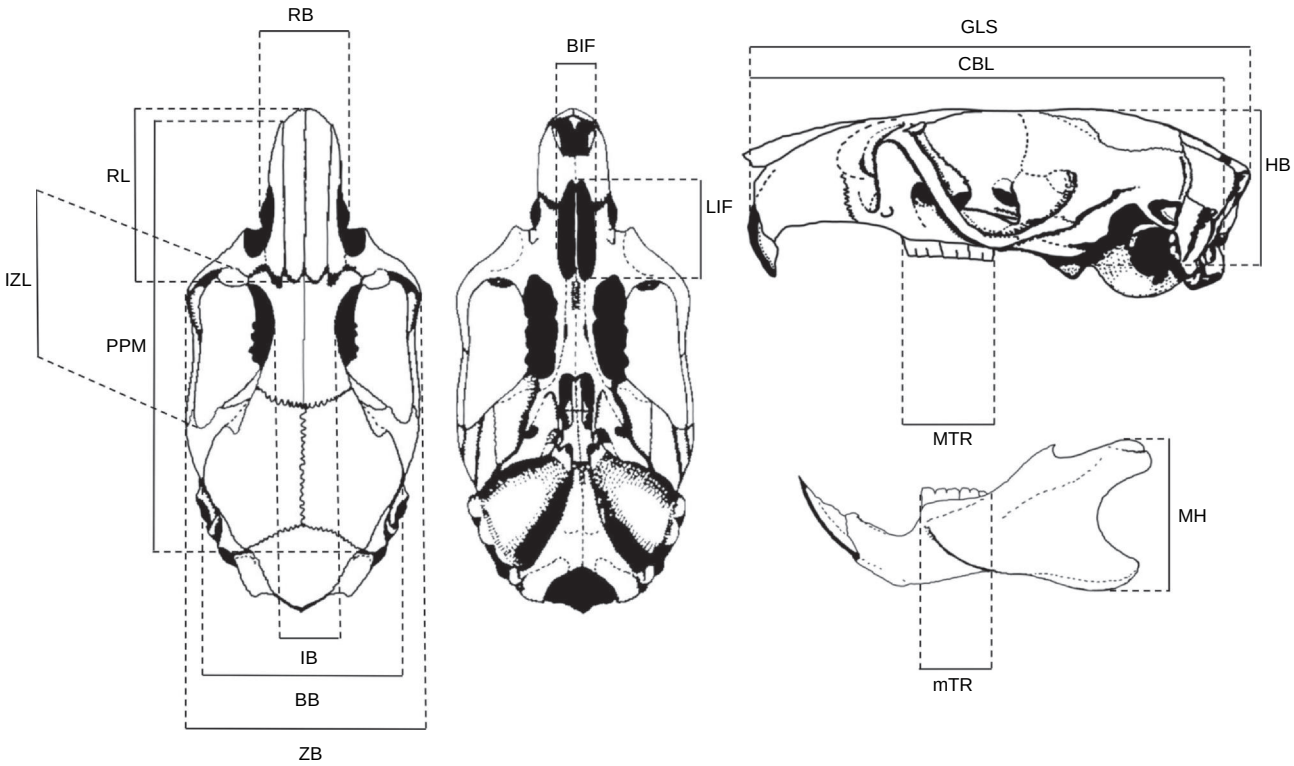

Fig. 2. Morphological measurements of *Leporillus conditor* cranial material (Image redrawn from Watts and Aslin 1981). Abbreviations are shown in Table 2.

Table 2. Definitions of abbreviations of the measurements depicted in Fig 2.

Abbreviation	Measurement
GLS	Greatest length of skull
CBL	Condylo-basal length
PPM	Parietal to pre-maxillary length
ZB	Zygomatic breadth
IZL	Internal zygomatic length
BB	Breadth of braincase
HB	Height of braincase
IB	Interorbital breadth
RB	Breadth of rostrum
RL	Length of rostrum (nasal bone)
LIF	Length of incisive foramina
BIF	Breadth of incisive foramina
MTR	Maxillary tooth row length
mTR	Mandibular tooth row length
MH	Mandibular height

to maximise the sample size among localities, missing values were imputed using the *mice* function in R package 'mice' (version 3.12.0), that creates multiple imputations for missing data based on fully conditional specification (Buuren and Groothuis-Oudshoorn 2011; Clavel *et al.* 2014). This method was chosen over single imputation procedures, as it takes into account the uncertainty of missing value estimation (Zhang 2016). The model was trained on existing measurements in the dataset, that then informed the imputation of the missing data over 100 iterations.

Skull size and shape were treated separately for analysis, but the relationship between the two (allometry) was also examined (Mosimann 1970). Skull size was calculated as the geometric mean of all variables in the imputed dataset, and taken to be a proxy for body size (Mosimann 1970; Meachen-Samuels and Van Valkenburgh 2009). This allowed for a conservative estimate of size without confounding by shape variation in individual measurements, but was supported by tests using three other common indicators of body size, greatest length of the skull (GLS) and upper and lower molar tooth row length (MTR/mTR) (Millien and Bovy 2010; Freudenthal and Martín-Suárez 2013; Bertrand *et al.* 2015). Skull shape was calculated using the log-shape ratio approach to standardise for isometric scaling differences, where the imputed linear variables were divided by the skull size of all variables and log-transformed (Mosimann and James 1979).

To determine if there were differences in skull size between rats sampled from different IBRA regions, the skull sizes of individuals in each region were compared

using a non-parametric one-way analysis of variance (ANOVA; Kruskal–Wallis test), followed by a pairwise Wilcoxon rank sum test to identify which groups were significantly different, implemented in the R package 'stats' (version 4.1.0). This approach was used as the data were not normally distributed, even when a log transformation was applied. Box plots were used to visualise cranial size variation within and among regions.

To determine if there were differences in skull shape among IBRA regions, a non-parametric ANOVA for multivariate data was implemented using the *procD.lm* function in the R package 'geomorph' (version 3.3.2). Here the model included log-transformed skull size as a covariate to calculate the proportion of variance in the dataset that was due to allometry (the size term), while the proportion due to regional differences was provided by the size:region interaction term. To ensure that the imputation method was consistent and reliable, a loop was created that completed 100 iterations of the above process, and the mean and standard deviations of the coefficient of determination (R^2) and *P*-values were inspected. For graphical representation of the results, a multivariate regression analysis was applied to visualise the allometric shape variation, using the regression score approach (Drake and Klingenberg 2008), and a principal components analysis of the regression residuals was performed to visualise the non-allometric shape variation among IBRA regions.

Finally, to test whether morphological variation was correlated with environmental variables, we ran linear regressions between morphological measurements and two key climate variables (mean annual temperature and mean annual precipitation), as well as latitude and longitude. Climate data were extracted from the Atlas of Living Australia's Spatial Portal (https://spatial.ala.org.au/) using the following layers: CSIRO Ecosystem Sciences mean annual temperature (°C) and mean annual rainfall (mm).

Table 3. Analysis of variance model results for *Leporillus conditor* skull size (log-transformed geometric mean) against IBRA region, and skull shape (log-shape ratios) against size and region.

(a) Size vs IBRA region			
	F	***R*²**	***P*-value**
Mean (±s.d.)	11.40 (±1.3289)	0.3976 (±0.0283)	0.001 (±0)
Min	8.321	0.3263	0.001
Median	11.360	0.3980	0.001
Max	14.580	0.4590	0.001
(b) Shape vs size			
	F	***R*²**	***P*-value**
Mean (±s.d.)	37.20 (±3.9172)	0.1411 (±0.0129)	0.001 (±0)
Min	27.15	0.1108	0.001
Median	37.46	0.1409	0.001
Max	46.08	0.1723	0.001
(c) Shape vs IBRA region			
	F	***R*²**	***P*-value**
Mean (±s.d.)	4.592 (±0.2907)	0.2107 (±0.0105)	0.001 (±0)
Min	3.963	0.1874	0.001
Median	4.559	0.2097	0.001
Max	5.483	0.2419	0.001
(d) Shape vs size: IBRA region			
	F	***R*²**	***P*-value**
Mean (±s.d.)	1.3495 (±0.2282)	0.0410 (±0.0066)	0.1333 (±0.1286)
Min	0.8037	0.0251	0.0030
Median	1.3273	0.0401	0.0975
Max	2.4139	0.0722	0.8120

Test statistics (*F*), coefficients of determination (R^2) and *P*-values are provided with standard deviations from the 100 iterations of 'mice' missing data imputation.

Results

Of the 201 individuals in the dataset, 13 had no missing data, 26 had 1–25% missing data, 64 had 26–50% missing data, and 98 had more than 50% missing data. Across all samples there was a total of 53% missing data. Multiple imputation has been found to remain unbiased to ~50% missingness, and so this proportion of missing data was considered acceptable (Marshall *et al.* 2010; Lee and Carlin 2012; Haji-Maghsoudi *et al.* 2013). Following ICC analysis of a subset of measurements to determine repeatability, the ICC value was determined to be >0.9, indicating excellent reliability of measurements (Wolak 2015; Koo and Li 2016).

Skull size and shape variation

IBRA regions accounted for 40% (mean $R^2 = 0.3976$) of size variation (Table 3a) among all individuals (*P*-value < 0.001). Pairwise comparisons using the Wilcoxon rank sum test revealed that the individuals that differ most from all others were those from the Eyre Yorke Block and Simpson Strzelecki Dunefields (although they were not significantly different from each other) (Supplementary Table S2). Skulls from individuals from these two regions were the largest in the dataset (Fig. 1). Tests using the standard size-proxy linear variables GLS, MTR and mTR corroborated this pattern (Supplementary Fig. S1).

For skull shape, size accounted for 14% (mean $R^2 = 0.1411$; Table 3b) and IBRA regions accounted for 21% (mean $R^2 = 0.2107$; Table 3c) of the variation among individuals (both *P*-value < 0.001). Samples followed a global allometric trajectory (Fig. 3*a*), and while some regional groups were separated along this trajectory there was clear overlap of groups spanning the size distribution. Only 4% (mean $R^2 = 0.041$) of shape variation was due to

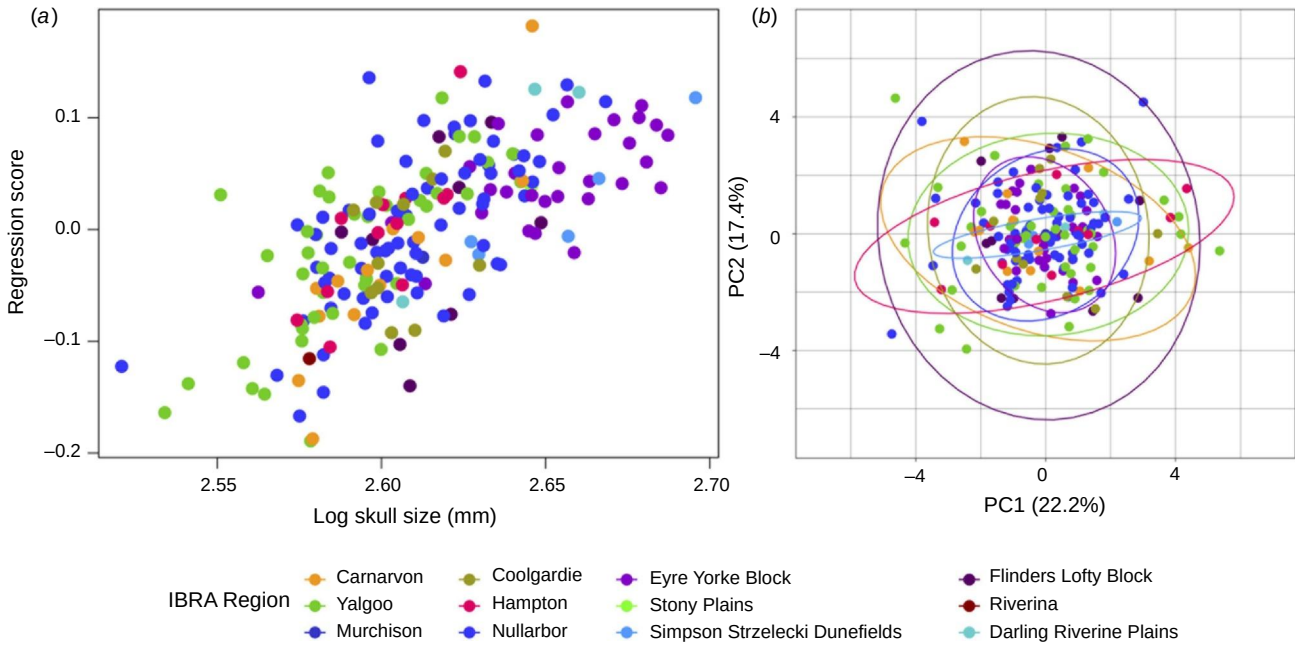

Fig. 3. (*a*) Multivariate regression analysis of *Leporillus conditor* skull size (log-transformed geometric mean) against skull shape and (*b*) the first two axes of a principal components analysis of the regression residuals. Size accounts for 14% (mean R^2 = 0.1411) of the shape variation (see Table 3a). Points represent individuals, coloured by IBRA region, and 95% confidence ellipses for each region are drawn in *b*.

regional size differences, and these differences were not statistically significant (mean *P*-value 0.1333) (Table 3d). No differences among groups were found in the skull shape regression residuals (Fig. 3*b*). This indicates that there is skull shape variation between regional groups, but this is mostly due to allometric differences corresponding to the observed size variation (Fig. 1) and not specific local adaptation acting on skull shape. No individual areas of the skull emerged as having noticeable shape variation across the IBRA regions, and so further study into individual linear variables was not deemed necessary. Individuals from the Eyre Yorke Block and Simpson Strzelecki Dunefields clustered at the larger end of the spectrum, indicating a larger skull size and inferred body size.

Spatial and climatic correlations

Given that skull size emerged as the dominant morphological trait varying among IBRA regions, we tested for spatial and climatic correlations in skull size variables only. Significant positive correlations were apparent between skull size and annual mean precipitation (*P*-value = 0.0042), latitude (degrees south) (*P*-value < 0.001) and longitude (*P*-value < 0.001). There was a significant negative correlation between skull size and annual mean temperature (*P*-value < 0.001). However, all but one model had considerable outliers, as evidenced by their low R^2 values (Fig. 4). Longitude produced the best fit, with an R^2 value of 0.25. *L. conditor* individuals increased in size as longitude increased (i.e. from west to east).

Discussion

Morphometric analysis of *L. conditor* skull size and shape revealed considerable size differences between sampled locations and predictable shape variation across its historical distribution. Allometric shape (the component proportional to size) dominated the variation among individuals of *L. conditor*, indicating that apparent skull diversity is due to body size differences and does not suggest local adaptation acting on skull shape. This is a common observation in Australian rodents; a study by Marcy *et al.* (2020) of 38 Australian rodent species found low variation in skull shape across all taxa, with size explaining the majority of the variation. The authors suggested that this universal skull shape is an evolutionary adaptation dating back over ten million years and is the secret to rodents' success in a variety of habitats. It is therefore unsurprising that little shape variation is present in historical populations of *L. conditor*, despite the variety of environmental conditions the species encompassed.

Skull size, a proxy for body size, varied significantly across the historical range. Our analyses indicate that individuals from the Eyre Yorke Block IBRA region (containing the Franklin Islands and a population translocated to

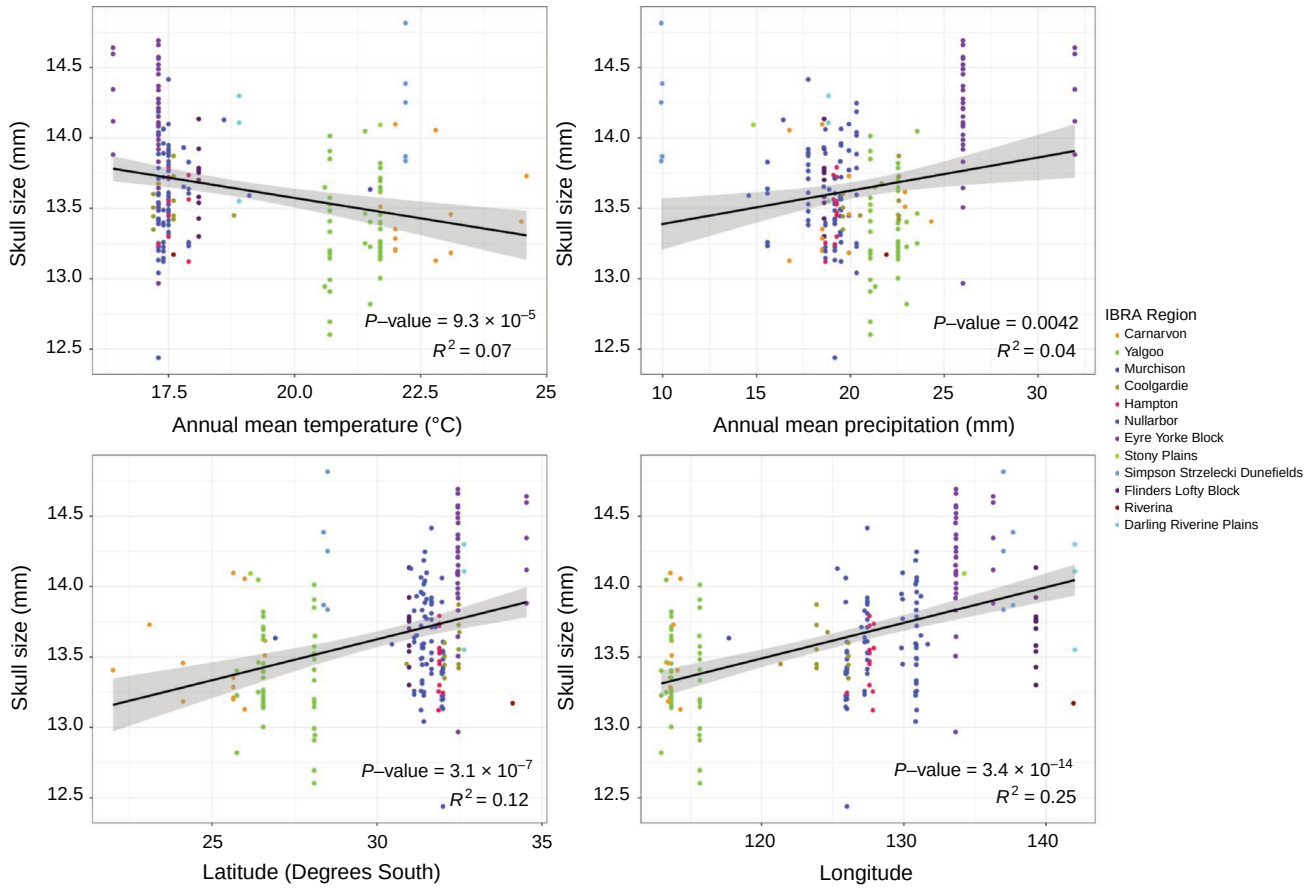

Fig. 4. Linear regression analysis of *Leporillus conditor* skull size (log-transformed geometric mean) against climate and spatial variables. Points represent individuals and are coloured by IBRA region. Note that latitude is displayed as degrees south rather than negative values.

Reevesby Island from the Franklins) and individuals from the Simpson Strzelecki Dunefields are significantly larger than all other sampled locations. While our models using climate variables did not reveal a clear correlation with skull size, there are several possible ecological explanations for these observations. As no other major herbivores inhabit the Franklin Islands (Copley 1999), the observed size increase in individuals belonging to the Franklin Island populations may be due to predation pressure from black tiger snakes (*Notechis ater niger*) and barn owls (*Tyto alba*) that regularly prey on juvenile *L. conditor* (and likely smaller adults) (Robinson 1975; Read 1984; Copley 1988, 1999). The equally large size of individuals from central arid Australia (Simpson-Strzelecki Dunefields) (all of which were collected in close proximity to the Lake Eyre Basin but were not collected following a flood year) may be due to similar predation pressures from desert reptiles such as snakes and goannas (Bolton and Moseby 2004). Indeed, the similarity in size between these populations of *L. conditor* and their geographical proximity suggest that these larger individuals may once have belonged to a continuous population that became separated by rising sea levels ~8000 years ago (Robinson *et al.* 1996). Genetic analysis of historical specimens would further inform on this possibility.

An alternative explanation for the large body size of the arid *L. conditor* may be character displacement, or ecological release, intensified by limited resources in a desert environment (Brown and Wilson 1956; Grant 1972; Strong *et al.* 1979; Herrmann *et al.* 2021). Species that are closely related and of similar size often compete more intensely than those of disparate size (Larsen 1986; Violle *et al.* 2011). Increased competition with other rodents such as the long-haired rat (*Rattus villosissimus*) in the arid zone may therefore have resulted in the evolution of larger body size in the northern population of *L. conditor*, in order to expand its niche and access alternative resources in a competitive environment (Bowers and Brown 1982; Bolnick *et al.* 2010). Another alternative selection pressure that should be considered is that smaller animals can be more sensitive to extreme temperatures as they have a larger surface area to volume ratio and a narrower thermal neutral zone, meaning that thermoregulatory costs are lower for larger animals when temperatures are highly variable (Grodzinski and Weiner 1984; Degen *et al.* 1997). As daily

temperature ranges of 15–20°C are typical in the Australian desert (Trewin 2006), climate extremes may have acted as a selection pressure for larger body size in *L. conditor*. Support for this comes from a study of fat sand rats (*Psammomys obesus*), where under extreme ambient temperatures body mass of adults correlated positively with time spent foraging, suggesting that larger size allows for better thermoregulation in a desert environment (Haim *et al.* 2006).

Individuals from the easternmost region, the Darling Riverine Plains, straddled the margin between the two apparent size morphotypes in the dataset. Although not significantly larger than the other mainland populations, individuals in this region were not significantly smaller than the larger morphotypes, either. This pattern may be consistent with a west-east size gradient. Indeed, of our climate and spatial correlation analyses, longitude was found to be the variable of best fit to skull size. There are several examples of east-west variation in other Australian taxa, such as the Hooded Plover, (Weston *et al.* 2020); however, in many cases genetic studies have determined this variation to represent multiple species, with the Nullarbor Plain acting as a driver of speciation (Rix *et al.* 2015). Evidence of east-west vicariance has been observed in many taxa, including phascogales (Spencer *et al.* 2001), pygmy perch (Buckley *et al.* 2018), aquatic beetles (Hawlitschek *et al.* 2011), and eucalypts (Ladiges *et al.* 2010). The individual from the Riverina, however, did not adhere to this pattern, but with a sample size of one we cannot make sound inferences for this region. Indeed, our small sample size and sparse spatial distribution overall prevents any robust conclusions here, but molecular phylogeographic studies would provide further insight.

Limitations

Due to the incomplete preservation of many of the skulls used in this study, our dataset had a high degree of missing values (53%). Although imputations using the 'mice' R package produced consistent results, the uncertainty associated with this amount of missing data must be acknowledged as a caveat. Another limitation that must be considered is the small sample size and patchy representation across *L. conditor*'s former range. As the species became extinct on the mainland almost a century ago, very little material is available that characterises its historic distribution. Here we have attempted to obtain a representative sample of the variety of habitat types and environmental conditions experienced by the species, but acknowledge that the sample sizes are not equal between regions, and there remains much that we do not know about *L. conditor*'s former life history.

Implications for translocation

Leporillus conditor has been used in several translocation programs in recent decades, with the Franklin Islands population acting as the primary source (Pedler and Copley 1993; White *et al.* 2018; Short *et al.* 2018, 2019). Our analyses show that these individuals are likely larger than their extinct counterparts in most mainland locations, with the exception of central Australia. Whether this morphological variation has an impact on fitness when translocating Franklin Island individuals to other areas of Australia is difficult to determine, as the relationship between form and function is highly complex and context-dependent (Koehl 1996). Small morphological changes may have considerable consequences for some species, such as Darwin's finches (Grant and Grant 2002; Herrel *et al.* 2005), while in other cases phenotypic variation has no influence on performance (Warner and Shine 2006).

Encouragingly, however, the lack of non-allometric shape variation in *L. conditor* among regions indicates that the species likely conforms to the universally well-adapted cranial form observed in many Australian rodent species, and may be capable of simply scaling its body size when necessary to adapt to an ecological niche (Marcy *et al.* 2020). Further studies on body size changes over time in relation to community composition in translocated *L. conditor* populations would provide more clarity here. In addition, genetic analysis of historic populations of *L. conditor* would provide insight as to genetic spatial variation and phylogeography within the species prior to its mainland extinction, as well as determining whether the large size of some *L. conditor* populations is the result of phenotypic plasticity or variation in genetic structure. Morphological studies of species that have undergone significant declines and range contractions are encouraged prior to conducting reintroductions, as this information may assist with population establishment.

Supplementary material

Supplementary material is available online.

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Data availability. Data used to generate these results are contained in the supplementary material and are available at the University of Adelaide FigShare (https://doi.org/10.25909/18319349).

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Supplementary Information 1

Metadata of individual *Leporillus conditor* specimens measured. SAM = South Australian Museum, MV = Museums Victoria, WAM = Western Australian Museum, AM = Australian Museum, BMNH = British Museum of Natural History. All measurements are in millimetres (mm).

Catalogue Number	Collection	Locality	Year	Latitude	Longitude	IBRA 7 Regions	GLS	CBL	PPM	ZB	IZL	BB	HB	IB	RB	RL	LIF	BIF	MTR	mTR	MH
M14051	SAM	Tieyon Homestead	1986	-26.1667	134.25	Stony Plains	46.62	44.32	40.88		15.68	18.92	13.48	6.04	7.29		9.78	3.25	9.12		
M4377	SAM	Lake Eyre	1907	-28.5	137	Simpson Strzelecki Dunefields	45.16	42.19	39.16	21.07		17.35	13.49	5.68		16.55	7.82	3.45	10.12	9.03	13.2
M4372	SAM	Lake Eyre	1907	-28.5	137	Simpson Strzelecki Dunefields	47.15	45.2	41.63	22.24	15.85	18.44	13.81	5.01	7.8	17.7	9.98	2.9	10.09	9.19	13.75
M4371	SAM	Lake Eyre	1907	-28.5	137	Simpson Strzelecki Dunefields	47.32	43.87	42.91	22.85	16.01	19.05	14.33	5.44		18.6	10.61		9.56	9.1	14.17
CHG613.2.3	SAM	Chambers Gorge	1976	-30.97	139.28	Flinders Lofty Block			38.11	20.45	14.91	17.11	13.07	5.53	7.64		8.68	3.04	8.7		
CHG613.2.1	SAM	Chambers Gorge	1978	-30.97	139.28	Flinders Lofty Block			38.65	21.21	15.51	17.74	11.96	5.49	7.37		9.89	3.41	9.57		
CHG613.3.5	SAM	Chambers Gorge	1978	-30.97	139.28	Flinders Lofty Block			38.97	20.82	14.39			5.96			8.99	3.51	9.09		
CHG613.2.2	SAM	Chambers Gorge	1978	-30.97	139.28	Flinders Lofty Block			39.2		15.53	16.55	12.41	5.21			8.55	3.68	9.58		
CHG613.3.3	SAM	Chambers Gorge	1978	-30.97	139.28	Flinders Lofty Block											9.51	3.32	9.73		
CHG613.3.2	SAM	Chambers Gorge	1978	-30.97	139.28	Flinders Lofty Block				19.88				5.76			8.57	3.27	9.15		
CHG613.2.5	SAM	Chambers Gorge	1978	-30.97	139.28	Flinders Lofty Block													8.9		
CHG613.3.1	SAM	Chambers Gorge	1978	-30.97	139.28	Flinders Lofty Block				20.6	15.73			5.72			9.02	3.1	9.19		
CHG613.3.4	SAM	Chambers Gorge	1978	-30.97	139.28	Flinders Lofty Block													9.26		
CHG613.2.4	SAM	Chambers Gorge	1978	-30.97	139.28	Flinders Lofty Block				20.05	14.97	18.72		6.36			8.24	3.23	8.96		
NSB1.1	SAM	Number 6 Bore	1989	-31.2	131.2	Nullarbor			38.03	21.18	15.55	17.3	12.8	5.69			9.04	3.41	8.88		
NSB1.2	SAM	Number 6 Bore	1989	-31.2	131.2	Nullarbor			39.25	21.6	15.58	17.33	13.11	5.92	7.77		9.29	3.41	9.1		
IVC.2.1	SAM	Ivy Cave	2011	-31.417	130.827	Nullarbor			37.72	21.36	15.81	18.11	13.01	5.98	6.8		8.26	3.45	8.45		
IVC.2.2	SAM	Ivy Cave	2011	-31.417	130.827	Nullarbor			38.22	22.19	16.12	17.27	13.15	6.06	7.91		9.19	3.63	9.96		
IVC.2.3	SAM	Ivy Cave	2011	-31.417	130.827	Nullarbor	45.51	42.23	39.58	20.08	15.53	17.58	12.27	5.71	6.68		8.47	3.22	8.46		

IVC.2.4	SAM	Ivy Cave	2011	-31.417	130.827	Nullarbor			38.41		14.92	17.2	12.77	5.8	6.59		8	3.11	8.15		
IVC.2.5	SAM	Ivy Cave	2011	-31.417	130.827	Nullarbor	45.45	43.65	39.77	22.8	15.96	18.54	13.3	6.1	7.4	17.07	8.67	3.56	9.13		
IVC.2.6	SAM	Ivy Cave	2011	-31.417	130.827	Nullarbor			37.39	19.48	15.33		12.47	5.93	6.5		8.1	3.28	8.84		
M6308.006	SAM	New Cave	1960	-31.3333	130.9	Nullarbor			36.37	20.71	14.66	17.3	12.8	5.93	6.45		8.55	3.02	9.03		
M6308.004	SAM	New Cave	1960	-31.3333	130.9	Nullarbor			37.49	20.02	15.71	17.82	12.08	5.75	7.15		8.58	3.19	8.57		
M6308.001	SAM	New Cave	1960	-31.3333	130.9	Nullarbor			38.84	19.73	15.93	17.84	13.31	5.91	6.73		8.9	3.03	9.16		
M6308.003	SAM	New Cave	1960	-31.3333	130.9	Nullarbor			39.26	19.98	16.01	18.9	13.6	6.88			9.73	3.21	9.9		
M6308.005	SAM	New Cave	1960	-31.3333	130.9	Nullarbor			39.94	22.24	15.59	18.47	13.6	5.82			9.34	3.4	9.44		
M6308.007	SAM	New Cave	1960	-31.3333	130.9	Nullarbor								5.95		16.91	8.99	2.99	9.01		
MUC.1.2.1	SAM	Murrawijinie Cave	1989	-31.365	130.875	Nullarbor	44.75	42.84	39.08	21.58	15.05	17.4	12.6	6.08	7.11	16.65	10.19	3.49	8.73		
MUC.1.2.2	SAM	Murrawijinie Cave	1989	-31.365	130.875	Nullarbor			38	20.55	14.93	17.32	13.19	5.78	6.24		8.68	3.21	8.67		
MUC.1.2.3	SAM	Murrawijinie Cave	1989	-31.365	130.875	Nullarbor			38.45	21.43	15	17.44	12.77	5.28	6.9		8.83	3.16	9.01		
MUC.1.1.1	SAM	Murrawijinie Cave	1989	-31.365	130.875	Nullarbor			38.15	20.73	15.61	17.99	12.61	6.03	6.65		9.04	3.3	9.43		
MUC.1.1.2	SAM	Murrawijinie Cave	1989	-31.365	130.875	Nullarbor	44.57	43.21	39.03	20.61	15.8	17.25	13.08	5.41	6.91		8.9	3.22	8.48		
MUC.1.1.3	SAM	Murrawijinie Cave	1989	-31.365	130.875	Nullarbor			37.07		15.13			5.76	6.99		8.72	3.32	8.73		
MUC.1.1.4	SAM	Murrawijinie Cave	1989	-31.365	130.875	Nullarbor								6.06	7.12		8.79	3.18	9.05		
M6307.002	SAM	Koonalda Cave	1960	-31.4	129.8333	Nullarbor			38.03		16.16	18.48	13.68	6.39			9		9.55		
M6307.001	SAM	Koonalda Cave	1960	-31.4	129.8333	Nullarbor			38.36	21.18	14.98			6.24	7.5		9.39	3.23	8.89		
M6306.005	SAM	Weekes Cave	1960	-31.5	129.9167	Nullarbor			37.49	22.23	15.59	17.47	13.41	5.71	6.98		9.2	3.45	9.13		
M6306.004	SAM	Weekes Cave	1960	-31.5	129.9167	Nullarbor			38.1	21.71	15.95	17.21	12.44	5.75	6.98		8.83	3.35	9		
M6306.002	SAM	Weekes Cave	1960	-31.5	129.9167	Nullarbor			39.26	22.62	15.37	18.18	12.23	6.17					8.99		
M6306.006	SAM	Weekes Cave	1960	-31.5	129.9167	Nullarbor			40.48			18.35	13.19	5.85	7.43		9.23	3.56	8.77		
M11959	SAM	West Franklin Island	1982	-32.4333	133.65	Eyre Yorke Block	45.52	43.47	39.68	22.32		17.85	13.83	5.44		17.19	8.76	3.47	9.61	9.45	13.8

M7860	SAM	East Franklin Island	1969	-32.4431	133.6694	Eyre Yorke Block	45.6	44.02	38.96	21.89	15.94	16.66	12.49	5.49	7.91	16.26	8.88	3.6	9.59	9.05	13.28
M7859	SAM	East Franklin Island	1969	-32.4431	133.6694	Eyre Yorke Block	45.84	43.59	39.74	21.8	15.89	17.12	13.29	5.06	8.48	16.4	9.18	3.25	9.58	9.25	13.69
M8607	SAM	Franklin Islands	1970	-32.45	133.6667	Eyre Yorke Block	45.78	43.2	38.71	21.83		16.91	12.9	5.89	7.66	17.16	8.51	3.44	9.5	9.4	13.25
M8182	SAM	Franklin Islands	1970	-32.45	133.6667	Eyre Yorke Block	45.04	42.18	38.93	20.59			12.95	5.86		15.7	7.96	3.33	9.82	9.22	12.79
M9509	SAM	Franklin Islands	1970	-32.45	133.6667	Eyre Yorke Block	45.76	43.68	39.39	21.74		17.6	12.98	5.64	8.16	17.34	8.81	3.56	9.88	9.77	13.45
M7862	SAM	Franklin Islands	1969	-32.45	133.6667	Eyre Yorke Block	45.66	43.16	39.91	22.14		17.45	13.74	5.72	8.22	17.29	8.45	3.43	9.69	9.52	13.24
M7858	SAM	Franklin Islands	1969	-32.45	133.6667	Eyre Yorke Block	46.35	44.36	39.93	22.26		17.9	14.1	5.3	7.57	17.18	8.72	3.24	9.73	9.23	13.41
M7863	SAM	Franklin Islands	1969	-32.45	133.6667	Eyre Yorke Block	46.62	44.12	40.08	21.99		17.84	14.7	5.42	7.71	17.24	8.97	3.3	9.69	9.61	14.15
M21372	SAM	Franklin Islands	1985	-32.45	133.67	Eyre Yorke Block	46.94	44.76	40.18	22.47	15.66	17.48	13.75	5.55	8.3	17.25	8.39	3.11	9.74	9.08	13.36
M7861	SAM	Franklin Islands	1969	-32.45	133.6667	Eyre Yorke Block	47.22	44.64	40.41	22.75		17.16	13.67	5.57	8.42	17.71	8.89	3.76	10.19	9.54	13.87
M9508	SAM	Franklin Islands	1969	-32.45	133.6667	Eyre Yorke Block	47.09	45.25	40.48	22.89	15.32	17.72	13.7	5.74	8.42	17.07	9.31	3.65	9.83	9.51	13.98
M15747	SAM	Franklin Islands	1971	-32.45	133.67	Eyre Yorke Block	46.76	44.25	40.53	22.27	16.78	17.19	13	5.58	7.87	16.63	9.34	3.68	10.19	9.31	13.77
M7865	SAM	Franklin Islands	1969	-32.45	133.6667	Eyre Yorke Block	48.03	45.04	40.83	22.62	16.97	18.01	13.76	5.4	8.59	17.16	9.04	3.92	9.81	9.82	14.05
M8183	SAM	Franklin Islands	1970	-32.45	133.6667	Eyre Yorke Block	47.36	44.35	40.87	22.9		17.74	13.78	5.76		17.75	9.24	3.67	10.18	9.61	12.81
M21396	SAM	Franklin Islands	1985	-32.45	133.67	Eyre Yorke Block	47.68	44.9	41.29	22.81	16.96	18.01	13.76	5.15	8.06	17.37	8.74	3.56	10.19	9.2	14.6
M7864	SAM	Franklin Islands	1969	-32.45	133.6667	Eyre Yorke Block	48.08	45.68	41.4	22.25		17.83	14.13	5.94	8.42	17.44	9.31	3.62	9.4	9.03	14.77
M7850	SAM	West Franklin Island	1969	-32.4569	133.6375	Eyre Yorke Block	44.39	42.25	38.79	19.56	15.02	17.09	13.09	5.26	6.95		8.61	3.3	9.42	9.44	11.98
M8617	SAM	West Franklin Island	1970	-32.4569	133.6375	Eyre Yorke Block	45.67	43.16	39.1	22.03		17.46	13.28	5.48		16.25	8.46	3.42	9.42	9.1	12.94
M7851	SAM	West Franklin Island	1969	-32.4569	133.6375	Eyre Yorke Block	45.49		39.29	20.94	16.03	17.35	13.87	5.68					9.6	9.65	13.2
M8616	SAM	West Franklin Island	1970	-32.4569	133.6375	Eyre Yorke Block	45.75	43.75	39.54	21.87		17.36	12.6	5.05	7.44	16.87	8.51	3.47	9.56	9.2	13.04
M8619	SAM	West Franklin Island	1970	-32.4569	133.6375	Eyre Yorke Block	46.1	43.12	40.04	22.3		18.08	13.62	5.81	8.31	16.89	8.83	3.99	9.88	9.15	13.93
M8618	SAM	West Franklin Island	1970	-32.4569	133.6375	Eyre Yorke Block													8.82	8.63	
M16410	SAM	Reevesby Island	1990	-34.53	136.28	Eyre Yorke Block			38.75	21.38	15.86	17.42	13.96	5.3	7.74		9.04	3.63	9.6	9.3	12.96

M21156	SAM	Reevesby Island	1999	-34.53	136.28	Eyre Yorke Block	46.16	43.51	39.74	20.66	16.05	17.56	12.98	5.4	7.38	17.01	8.93	3.38	9.31	9.02	13.21
M16590	SAM	Reevesby Island	1990	-34.53	136.28	Eyre Yorke Block	45.89	44.01	39.95	21.32	15.86	17.93	14.38	5.56	8.17	17.61	8.92	3.58	9.84	9.48	13.37
M16402	SAM	Reevesby Island	1991	-34.53	136.28	Eyre Yorke Block	47.78	45.57	40.79	22.87	15.69	17.62	14.05	5.2	8.46	17.45	9.67	3.67	10.05	9.7	14.53
M16591	SAM	Reevesby Island	1991	-34.53	136.28	Eyre Yorke Block	47.72	45.04	41.51	23.33	16.4	17.97	14.22	5.94	8.63	17.25	8.87	3.53	9.94	9.43	12.85
NMV:C.101	MV	Murray and Darling Rivers Junction	1857	-34.112	141.922	Riverina			38.02	19.83	15.2			5.89	6.53	15.52	8.09	2.85	8.99	8.72	
NMV:C.33830	MV	Cooper Creek		-28.38	137.68	Simpson Strzelecki Dunefields	47.19	44.14	40.95	21.84		17.85	13.71	5.95	8.57	17.53	8.98	3.48	9.62	9.22	13.32
NMV:C.33831	MV	Cooper Creek		-28.38	137.68	Simpson Strzelecki Dunefields	45.78	43.22	39.55	20.79		17.71	12.96	5.72	7.7	16.78	8.72	3.25	9.4	9.31	13.54
M829	WAM	Franklin Islands	1926	-32.45	133.6667	Eyre Yorke Block	46.98	45.31	41.26	22.62	16.82	17.64	13.3	5.3	7.73	17.89	8.8	3.4	9.78	9.35	14.18
M63287.01	WAM	Mundrabilla Station	2018	-31.866	127.821	Hampton			36.9	19.32	15.64	17.79	12.92	6.3	6.1		8.32	3.05	9.08		
78.1.61.1	WAM	Dirk Hartog Island	1979	-25.75	112.95	Yalgoo	41.85	40.3	37.21	19.56	14.44	16.65	12.17	4.83	6.85		8.84	3.3	8.32		
78.1.61.2	WAM	Dirk Hartog Island	1979	-25.75	112.95	Yalgoo													8.9		
78.1.61.3	WAM	Dirk Hartog Island	1979	-25.75	112.95	Yalgoo														8.02	13.34
71.7.59.1	WAM	Edel Land	1970	-26.4	113.3	Yalgoo														8.46	12.92
71.7.59.2	WAM	Edel Land	1970	-26.4	113.3	Yalgoo														7.99	13.47
71.7.59.3	WAM	Edel Land	1970	-26.4	113.3	Yalgoo														9.09	
71.7.24.1	WAM	Edel Land	1970	-26.6	113.68	Carnarvon														8.49	
71.7.24.2	WAM	Edel Land	1970	-26.6	113.68	Carnarvon														8.58	
73.1.218	WAM	Weld Range	1963	-26.917	117.7	Murchison														8.33	
67.4.42	WAM	Coolgardie	1967	-30.896	121.331	Coolgardie														8.41	14.2
68.11.99	WAM	Menindee	1967	-32.636	142.015	Darling Riverine Plains														8.52	
68.11.100	WAM	Menindee	1967	-32.636	142.015	Darling Riverine Plains														8.36	
68.11.101	WAM	Menindee	1967	-32.636	142.015	Darling Riverine Plains														9.12	
GJGS84.1	WAM	Tallering Gorge	2003	-28.094	115.659	Yalgoo														7.54	

GJGS84.2	WAM	Tallering Gorge	2003	-28.094	115.659	Yalgoo														8.7	
GJGS84.3	WAM	Tallering Gorge	2003	-28.094	115.659	Yalgoo														8.6	
WGS84.1	WAM	Tallering Gorge	2003	-28.094	115.659	Yalgoo			37.97	19.62	15.48	17.42	13.22	5.11	5.91	15.02	8.61	3.32	8.99		
WGS84.3	WAM	Tallering Gorge	2003	-28.094	115.659	Yalgoo											8.52	3.47	7.91		
WGS84.4	WAM	Tallering Gorge	2003	-28.094	115.659	Yalgoo			37.25	19.71	15.24	17.41	12.69	5.46	6.72	15.54	8.41	3.37	8.7		
WGS84.5	WAM	Tallering Gorge	2003	-28.094	115.659	Yalgoo				18.27	13.93			5.1					8.4		
WGS84.6	WAM	Tallering Gorge	2003	-28.094	115.659	Yalgoo														7.87	12.66
WGS84.7	WAM	Tallering Gorge	2003	-28.094	115.659	Yalgoo														7.74	12.91
WGS84.8	WAM	Tallering Gorge	2003	-28.094	115.659	Yalgoo														7.63	12.79
WGS84.9	WAM	Tallering Gorge	2003	-28.094	115.659	Yalgoo														8.54	12.58
WGS84.10	WAM	Tallering Gorge	2003	-28.094	115.659	Yalgoo														7.83	
WGS84.11	WAM	Tallering Gorge	2003	-28.094	115.659	Yalgoo			35.93	18.35	14.75			5.44	6.33		7.54	3.03	8.04		
WGS84.12	WAM	Tallering Gorge	2003	-28.094	115.659	Yalgoo				19.55	14.06			5.36					8.69		
WGS84.13	WAM	Tallering Gorge	2003	-28.094	115.659	Yalgoo				18.66	13.49			5.67	6.14	13.83	7.95	2.81	8.33		
ABRS115/2.1	WAM	Tallering Hill	1985	-28.117	115.62	Yalgoo														8.3	12.27
ABRS115/2.2	WAM	Tallering Hill	1985	-28.117	115.62	Yalgoo														7.85	12.31
ABRS124/3.1	WAM	Yaringa Station	1985	-25.987	114.297	Carnarvon													8.49		
ABRS124/3.2	WAM	Yaringa Station	1985	-25.987	114.297	Carnarvon														8.13	
ABRS125/2.1	WAM	Peron Peninsula	1985	-25.643	113.585	Carnarvon														8.05	12.68
ABRS125/2.2	WAM	Peron Peninsula	1985	-25.643	113.585	Carnarvon														8.15	
ABRS125/2.3	WAM	Peron Peninsula	1985	-25.643	113.585	Carnarvon														7.86	11.66
ABRS125/2.4	WAM	Peron Peninsula	1985	-25.643	113.585	Carnarvon														7.89	
ABRS125/2.5	WAM	Peron Peninsula	1985	-25.643	113.585	Carnarvon													8.55		
MEE.1	WAM	Cocklebiddy	1985	-32	126	Nullarbor			35.45	18.54	14.86	16.85	11.72	5.29			7.98	2.84	7.21		

MEE.2	WAM	Cocklebiddy	1985	-32	126	Nullarbor			37.24	20.74	15.05	16.97	12.66	5.9	6.22		8.19	3.15	8.9		
MEE.3	WAM	Cocklebiddy	1985	-32	126	Nullarbor			38.14	20.63	15.7	17.81	12.61	5.97	6.6		8.59	3.41	9.11		
MEE.4	WAM	Cocklebiddy	1985	-32	126	Nullarbor													9.07		
MEE.5	WAM	Cocklebiddy	1985	-32	126	Nullarbor														8.06	
MEE.6	WAM	Cocklebiddy	1985	-32	126	Nullarbor				19.65	14.59			5.46	6.49	14.37	8.62	2.99	8.95		
MEE.7	WAM	Cocklebiddy	1985	-32	126	Nullarbor														8.54	12.94
MEE.8	WAM	Cocklebiddy	1985	-32	126	Nullarbor														8.1	13.07
MEE.9	WAM	Cocklebiddy	1985	-32	126	Nullarbor														8.89	
MEE.10	WAM	Cocklebiddy	1985	-32	126	Nullarbor														6.97	10.69
MEE.11	WAM	Cocklebiddy	1985	-32	126	Nullarbor			37.88	20.45	15.29	17.14	12.52	5.61	6.83		8.72	3.42	8.89		
68.3.81.1	WAM	Eucla Basin	1966	-31.892	127.583	Hampton				20.44	14.61	18.43	12.32	5.67					9.46		
68.3.81.2	WAM	Eucla Basin	1966	-31.892	127.583	Hampton														8.04	13.31
68.3.81.3	WAM	Eucla Basin	1966	-31.892	127.583	Hampton														8.35	13.48
68.7.50	WAM	Eucla Basin	1966	-31.892	127.583	Hampton			37.98		15.19	18.02	12.69	5.73	6.83		9.71	3.72	9.51		
68.7.51	WAM	Eucla Basin	1966	-31.892	127.583	Hampton			38.09	21.75	15.69	18.59	12.68	6.32	6.58		9.21	3.02	8.95		
68.2.301	WAM	Eucla Basin		-31.967	125.918	Nullarbor			37.9	21.02	16.08	18.65	13.12	6.08	7.11		9.41	3.51	9.14		
68.2.302	WAM	Eucla Basin		-31.967	125.918	Nullarbor			39.3	21.87	16.36	17.06	13.2	5.88	7.06		9.35	3.54	9.2		
68.2.303	WAM	Eucla Basin		-31.967	125.918	Nullarbor				20.29				5.63	6.86	15.86	8.51	3.24	9.02		
68.2.304	WAM	Eucla Basin		-31.967	125.918	Nullarbor				18.94				5.57			8.41	3.05	8.87		
68.2.305	WAM	Eucla Basin		-31.967	125.918	Nullarbor			37.65	20.05	15.46	16.23	11.68	5.58	6.23		9.02	3.11	8.78		
68.2.306	WAM	Eucla Basin		-31.967	125.918	Nullarbor														8.23	12.08
68.2.307	WAM	Eucla Basin		-31.967	125.918	Nullarbor														8.36	12.35
69.7.753	WAM	Eucla Basin		-31.889039	127.889036	Hampton			39.45	20.65	14.69	17.65	13.13	5.53	6.91	16.15	9.5	3.51	9.09		

73.1.100	WAM	Eucla Basin	1967	-31.889039	127.889036	Hampton			37.86	21.91	15.29	17.74		5.81	6.59		9.38	3.16	8.77		
67.4.178	WAM	Eucla Basin	1966	-31.125	127.243	Nullarbor					15.64			5.8					8.93		
67.4.179	WAM	Eucla Basin	1966	-31.125	127.243	Nullarbor					15.33			6.07					8.66		
67.4.180	WAM	Eucla Basin	1966	-31.125	127.243	Nullarbor								6.08							
67.4.181	WAM	Eucla Basin	1966	-31.125	127.243	Nullarbor			39.4	20.28	16.54	17.73	13.29	5.86	6.97		8.37	3.29	9.73		
67.4.189	WAM	Eucla Basin	1966	-31.125	127.243	Nullarbor				19.79				5.6			8.56	3.23	8.77		
67.4.295	WAM	Nullarbor Plain	1966	-31.65	127.43	Nullarbor				21.71	15.54	18.26	13.19	5.94					9.55		
67.4.296	WAM	Nullarbor Plain	1966	-31.65	127.43	Nullarbor								6.32	7.05		9.19	3.36	9.2		
67.4.297	WAM	Nullarbor Plain	1966	-31.65	127.43	Nullarbor				22.24	15.43	17.02	12.57	5.88					9.23		
67.4.298	WAM	Nullarbor Plain	1966	-31.65	127.43	Nullarbor			38.31	20.15	15.28	16.89	12.17	5.79	6.69		9	3.34	9.41		
67.4.299	WAM	Nullarbor Plain	1966	-31.65	127.43	Nullarbor			39.25	21.81	15.12		13.22	6.2			9.61	3.17	8.93		
67.4.300	WAM	Nullarbor Plain	1966	-31.65	127.43	Nullarbor														8.19	
67.4.301	WAM	Nullarbor Plain	1966	-31.65	127.43	Nullarbor														8.45	12.89
67.4.302	WAM	Nullarbor Plain	1966	-31.65	127.43	Nullarbor														7.93	
67.4.303	WAM	Nullarbor Plain	1966	-31.65	127.43	Nullarbor														8.36	13.88
72.1.882	WAM	Nullarbor Plain		-31.65	127.43	Nullarbor													8.52		
72.1.697	WAM	Nullarbor Plain		-31.65	127.43	Nullarbor													9.52		
72.1.696	WAM	Nullarbor Plain		-31.65	127.43	Nullarbor													8.38		
72.1.1114	WAM	Nullarbor Plain		-31.65	127.43	Nullarbor			37.82	21.5	15.78	17.26	12.59	5.72			8.47	3.14	8.89		
WAM1	WAM	3 Bays Island	2013	-26.55	113.65	Yalgoo			37.14	19.43	15.13	17.65	11.74	5.15			9.2	3.39	9.15		
WAM2	WAM	3 Bays Island	2013	-26.55	113.65	Yalgoo			39.18	21.48	16.13	18.54	12.92	5.44	7.63		9.63	3.57	8.5		
WAM3	WAM	3 Bays Island	2013	-26.55	113.65	Yalgoo				20.81	15.55			5.21	6.81		9.52	3.36	7.94		
WAM4	WAM	3 Bays Island	2013	-26.55	113.65	Yalgoo			36.94	19.31	14.52	17.31	11.81	5.02	7.51		8.79	3.65	8.42		

WAM5	WAM	3 Bays Island	2013	-26.55	113.65	Yalgoo			40.44	20.92	16.15	18.02	12.7	5.16	7.16	15.82	9.16	3.53	9.52		
WAM6	WAM	3 Bays Island	2013	-26.55	113.65	Yalgoo				20.1	15.15			5.21	6.77		8.52	3.09	8.78		
WAM7	WAM	3 Bays Island	2013	-26.55	113.65	Yalgoo			37.79	19.69	14.64	17.96	12.88	5.51	6.72		8.34	3.26	8.36		
WAM8	WAM	3 Bays Island	2013	-26.55	113.65	Yalgoo				19.85	15.18	17.74		5.23	6.16		8.75	3.43	8.56		
WAM9	WAM	3 Bays Island	2013	-26.55	113.65	Yalgoo			37.39	20.56	14.98	18.13	12.03	5.45			9.1	3.25	8.49		
WAM10	WAM	3 Bays Island	2013	-26.55	113.65	Yalgoo				19.8				5.22	6.73		8.71	3.04	8.48		
WAM11	WAM	3 Bays Island	2013	-26.55	113.65	Yalgoo														7.66	13.28
WAM12	WAM	3 Bays Island	2013	-26.55	113.65	Yalgoo														8.07	13.21
WAM13	WAM	3 Bays Island	2013	-26.55	113.65	Yalgoo														8.47	12.75
WAM14	WAM	3 Bays Island	2013	-26.55	113.65	Yalgoo														8.02	13.98
WAM15	WAM	3 Bays Island	2013	-26.55	113.65	Yalgoo														7.87	12.33
WAM16	WAM	3 Bays Island	2013	-26.55	113.65	Yalgoo														8.01	13.3
WAM17	WAM	3 Bays Island	2013	-26.55	113.65	Yalgoo														8.02	12.47
WAM18	WAM	3 Bays Island	2013	-26.55	113.65	Yalgoo														8.16	13.23
WAM19	WAM	3 Bays Island	2013	-26.55	113.65	Yalgoo														8.07	12.54
WAM20	WAM	3 Bays Island	2013	-26.55	113.65	Yalgoo														8.32	12.69
WAM23	WAM	3 Bays Island	2013	-26.55	113.65	Yalgoo				20.75				5		15.99	9.36	3.62	9.11		
ABRS121/2.1	WAM	Quobba Station	1985	-24.119	113.435	Carnarvon														8.28	
ABRS121/2.2	WAM	Quobba Station	1985	-24.119	113.435	Carnarvon													8.62		
CARD1	WAM	Cardabia Homestead	1987	-23.104	113.804	Carnarvon													9.18		
QC1	WAM	Cape Range		-21.997	114.096	Carnarvon													8.91		
68.7.50	WAM	Eucla Basin	1966	-31.892	127.583	Hampton			37.97		15.26	17.81	12.45	5.83	6.55		9.12	3.54	9.67		
68.7.51	WAM	Eucla Basin	1966	-31.892	127.583	Hampton			37.89	21.66	15.48	18.7	12.58	6.25	6.35		8.9	2.96	9.08		
70.4.241	WAM	Mundrabilla Station	1966	-31.866	127.821	Hampton				19.37	15.3	17.48	12.5	5.82	6.45		8.3	3.23	8.97		

BAL1	WAM	Eucla Basin	1971	-32.472	123.862	Coolgardie	45.75	43.34	39.42	20.78	15.33	18.45	13.37	5.96	7.08	16.27	9.31	3.27	9.1		
BAL2	WAM	Eucla Basin	1971	-32.472	123.862	Coolgardie			38.9		15.68	18.14	12.28	5.43	7.01		9.38	3.45	9.37		
BAL3	WAM	Eucla Basin	1971	-32.472	123.862	Coolgardie								5.79	6.77		8.21	3.32	8.9		
BAL4	WAM	Eucla Basin	1971	-32.472	123.862	Coolgardie														8.34	
BAL5	WAM	Eucla Basin	1971	-32.472	123.862	Coolgardie														8.57	
NR1	WAM	Nullarbor Plain	1989	-31.45	130.896	Nullarbor				21.61	15.9	17.45		5.67	6.85		9.25	3.19	9.85		
NR2	WAM	Nullarbor Plain	1989	-31.45	130.896	Nullarbor			39.72	21.47	16.08	18.01	12.46	5.44	7.19		8.79	3.21	9.64		
NR3	WAM	Nullarbor Plain	1989	-31.45	130.896	Nullarbor			39.02	21.53	15.83	17.9	13.33	6.19	7.05		9.3	3.51	9.55		
NR4	WAM	Nullarbor Plain	1989	-31.45	130.896	Nullarbor		45.05	39.68	22.75	16.52	17.22	13.34	5.51	7.71		9.39	3.7	9.29		
73.1.85	WAM	Cocklebiddy		-32	126	Hampton				20.55	14.58	17.68	12.7	6.11					8.9		
68.5.58	WAM	Rawlinna		-31.024	125.33	Nullarbor			40.04	21.9	16.31	17.55	12.61	5.64			8.72	3.55	9.93		
69.7.583	WAM	Eucla Basin	1962	-32.043	126.096	Coolgardie			37.38	20.33	16.01		12.66	6.3	6.5		9.2	3.16	9.18		
69.7.584	WAM	Eucla Basin	1962	-32.043	126.096	Coolgardie			36.6	20.39	15.44	17.29	12.46	5.33	6.98		8.89	3.2	8.84		
69.7.591	WAM	Eucla Basin	1962	-32.043	126.096	Coolgardie														8.39	13.07
69.7.593	WAM	Eucla Basin	1962	-32.043	126.096	Coolgardie														8.53	13.97
ABRS32B	WAM	Eucla Basin	1984	-32.497	124.635	Coolgardie				20.22	15.42	17.79	13.34	5.65	7.5		9.36	3.47	8.59		
67.10.94	WAM	Eucla Basin	1967	-31.769	127.0198	Nullarbor	45.35	42.76	39.35	22.51	15.75	17.41	12.96	5.53	7.42	16.83	8.15	3.36	9.12		
L jonesi Type (B.M.21.7.3.2)	BMNH	Franklin Islands	1920	-32.45	133.6667	Eyre Yorke Block	45.9			23.1				5.6			8.5		9.5		
M.3062 (measured by Troughton)	AM	Ooldea	1921	-30.45	131.68	Nullarbor	45.4			20.4							9.2		9.3		

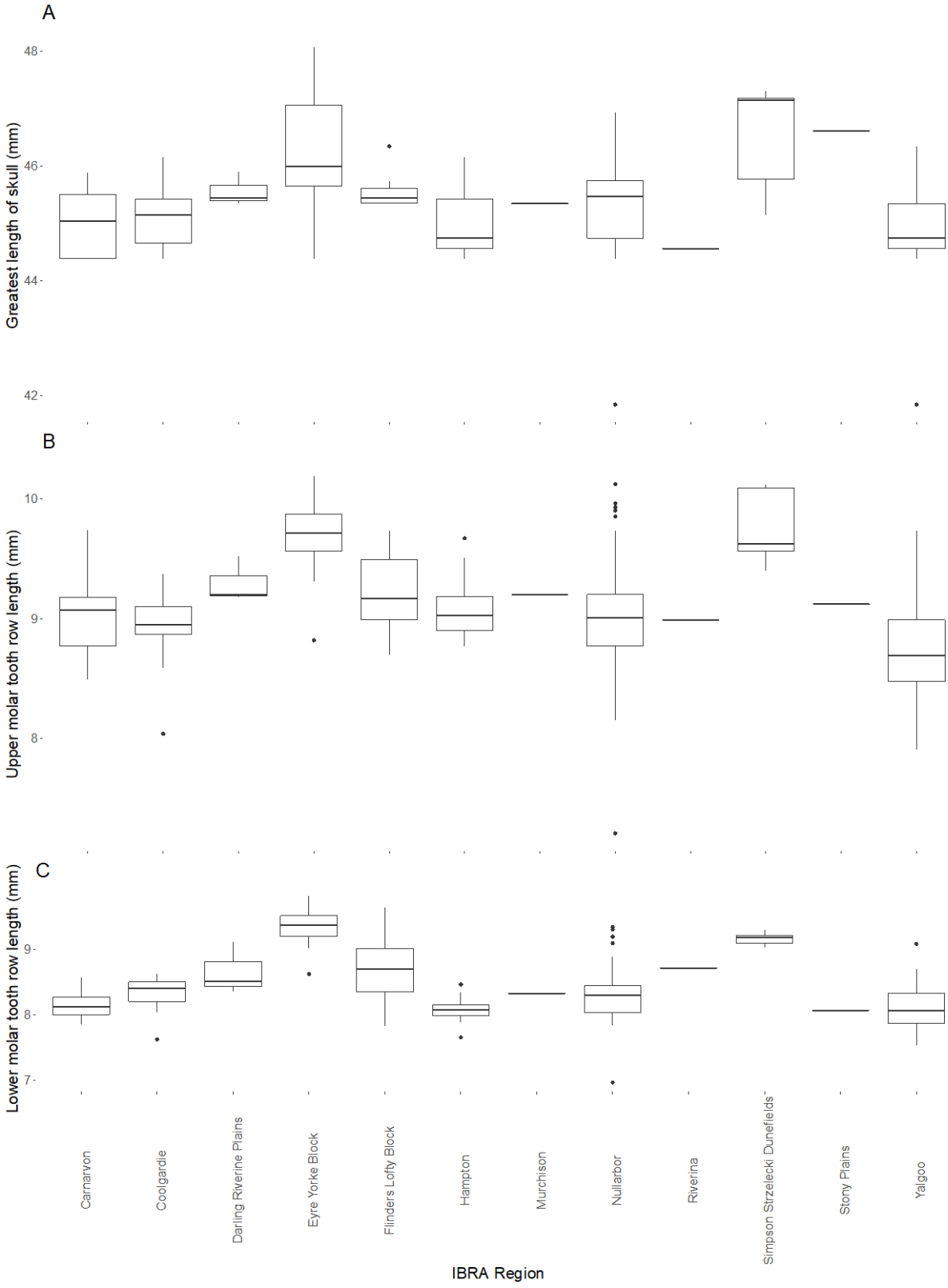
Supplementary Information 2

Wilcoxon rank sum test pairwise comparison of skull size (geometric mean) per IBRA Region. Significant p-values are indicated in bold.

	Carnarvon	Coolgardie	Darling Riverine Plains	Eyre Yorke Block	Flinders Lofty Block	Hampton	Murchison	Nullarbor	Riverina	Simpson Strzelecki Dunefields	Stony Plains
Coolgardie	0.49530957	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Darling Riverine Plains	0.15252101	0.25226947	NA	NA	NA	NA	NA	NA	NA	NA	NA
Eyre Yorke Block	**4.82E-05**	**2.64E-05**	0.59747546	NA	NA	NA	NA	NA	NA	NA	NA
Flinders Lofty Block	0.15816205	0.34375	0.4852071	**0.00157834**	NA	NA	NA	NA	NA	NA	NA
Hampton	0.7806597	0.7806597	0.15816205	**1.32E-05**	0.15816205	NA	NA	NA	NA	NA	NA
Murchison	0.75428571	0.78571429	1	0.37010709	1	0.77484277	NA	NA	NA	NA	NA
Nullarbor	0.34375	0.77075623	0.25226947	**4.97E-08**	0.59747546	0.37010709	0.94015885	NA	NA	NA	NA
Riverina	0.4852071	0.34375	0.6875	0.34375	0.36363636	0.49530957	1	0.34375	NA	NA	NA
Simpson Strzelecki Dunefields	**0.01540616**	**0.01208791**	0.75428571	1	**0.03767661**	**0.00237018**	0.51162791	**0.02172944**	0.51162791	NA	NA
Stony Plains	0.4852071	0.34375	1	0.82173175	0.54545455	0.34375	1	0.34375	1	1	NA
Yalgoo	0.4852071	0.11537346	0.05535615	**1.90E-11**	**0.01455582**	0.34375	0.68652482	0.00204808	0.77484277	**0.00048456**	0.15816205

Supplementary Information 3

Comparisons of specimens from each IBRA Region using greatest length of the skull (GLS) (A), upper (MTR) (B) and lower (mTR) (C) tooth rows as proxies for body size.



Chapter 3

Sex assignment in a non-model organism in the absence of field records using Diversity Arrays Technology (DArT) data

Statement of Authorship

Title of Paper	Sex assignment in a non-model organism in the absence of field records using Diversity Arrays Technology (DArT) data
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Principal Author

Name of Principal Author (Candidate)	Isabelle Onley		
Contribution to the Paper	Isabelle coordinated submission of samples to DArT, analysed data, drafted the abstract, introduction, results and discussion, and acted as corresponding author.		
Overall percentage (%)	70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	13/09/2021

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Kieren Mitchell		
Contribution to the Paper	Kieren analysed the data, drafted the materials, methods and figures and provided feedback on the final manuscript.		
Signature		Date	14/09/2021

Name of Co-Author	Jeremy Austin		
Contribution to the Paper	Jeremy coordinated the submission of samples to DArT, and provided feedback and editing on the final manuscript.		
Signature		Date	18/10/21

Please cut and paste additional co-author panels here as required.

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METHODS AND RESOURCES ARTICLE

Sex assignment in a non-model organism in the absence of field records using Diversity Arrays Technology (DArT) data

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Abstract

Conservation genomics research often relies on accurate sex information to make inferences about species demography, dispersal, and population structure. However, field determined sex data are not always available and can be subject to human error, while laboratory sex assignment methods such as PCR assays can often be costly and challenging for non-model species. Conservation genomics programs increasingly use reduced-representation genome sequencing to assess neutral and functional genetic diversity, population structure, gene flow and pedigrees in threatened species. Here we demonstrate that sex can be determined from reduced-representation sequencing data produced by the increasingly popular Diversity Arrays Technology sequencing workflow (DArT-seq) using a program originally designed for application to shotgun data. This program—*sexassign*—compares the "dosage" of sequencing reads mapping to autosomes versus the X chromosome. In the present study, *sexassign* was used to identify the sex of 60 field-collected Greater Stick-Nest Rat (*Leporillus conditor*) samples, despite the absence of an annotated reference genome for the species. This "read-dosage" approach is not only more accurate and affordable than traditional sex assignment methods, but can be applied to any diploid organism with a heterogametic sex determination system—including non-model and understudied species of conservation importance—by using FASTQs generated by DArT.

Keywords Conservation genomics · Sex assignment · Bioinformatics · DArT-seq

Introduction

Accurate sex assignment is an integral aspect of conservation genomics research, particularly when studying parameters such as relatedness, dispersal, and philopatry. Sexing of individuals used in conservation genomics studies typically takes place in the field at the time of collection. However, sex assignments recorded in the field are not always reliable and there is a wide margin for human error, particularly for species that do not demonstrate sexual dimorphism or when researchers are working in difficult conditions. Further, field records can easily be lost or incorrectly transcribed during trapping and monitoring. Genetic sex assignment is a favourable alternative or complement to field identification, as it is an objective, highly standardised, and accurate approach that eliminates the possibility of upstream sex misidentification confounding genomic studies (Hrovatin and Kunej 2017).

While PCR-based sex identification methods have been used for several decades to identify and amplify sex chromosomes in individual samples (Akane et al. 1992; Clapcote and Roder 2005; McFarlane et al. 2013), such processes can be time consuming and expensive. In addition, they require taxon-specific primers that are not always available or applicable to the target species. With the advent of high-throughput sequencing (HTS) technology it is now possible to produce high-resolution genomic data that may allow researchers to determine the sex of sequenced individuals bioinformatically. For example, single nucleotide polymorphisms (SNPs) in the genome can often be linked to the sex chromosomes in model organisms, allowing sex to be determined on chromosomal presence-absence basis (Fowler and Buonaccorsi 2016; Lambert et al. 2016). For non-model organisms where a well-assembled and well-annotated

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reference genome is unavailable, the overall "dosage" of sequencing reads mapping to the sex chromosomes can be assessed to determine whether the individual is heterogametic or homogametic and thus to identify the sex (Bover et al. 2018; Gamble 2016; Gower et al. 2019; Pečnerová et al. 2017).

Read-dosage-based approaches to sex assignment have only been applied using shotgun sequencing data, where molecules are randomly sampled and sequenced (Flamingh et al. 2020; Motahari et al. 2013; Skoglund et al. 2013). However, many conservation programs employ reduced-representation sequencing approaches (e.g. RADseq), where sequenced molecules belong to a subset of genomic loci. One commercial provider of reduced-representation sequencing that is growing in popularity in the conservation genomics field is Diversity Arrays Technology (DArT) (Cummins et al. 2019; Ewart et al. 2019; Pazmiño et al. 2018; Sansaloni et al. 2011; Schultz et al. 2018; van Deventer et al. 2020). The DArT workflow uses restriction enzymes to reduce genomic complexity, allowing identification of informative markers that are subsequently sequenced for all submitted samples (Kilian et al. 2012). However, despite the growing popularity of DArT for conservation genomics projects, no simple and widely applicable sex-assignment framework has emerged that can be applied to DArT data. In the present study we apply a read-dosage sex-determination approach to DArT data from an Australian rodent, the Greater Stick-Nest Rat (*Leporillus conditor*), and demonstrate that—despite being originally designed for application to shotgun data—this method remains robust when applied to FASTQ files generated as part of the DArT workflow.

Materials and methods

DNA submitted to DArT was extracted from 60 *L. conditor* tissue samples collected by staff during routine trapping events at Arid Recovery Reserve, South Australia, between 1999 and 2003. DNA extraction was completed following the methods described by Barclay et al. (2006) and samples were subsequently stored at − 20 °C prior to sequencing by DArT. Following library preparation and sequencing by DArT using their proprietary workflow, we obtained the raw Illumina data in FASTQ format. We used the Paleomix v1.2.14 pipeline to process these data: AdapterRemoval2 v2.3.1 was used to trim residual adapter sequences (using default parameters) and filter reads shorter than 30 bp, after which all remaining reads were mapped against the repeat-masked house mouse genome assembly (GRCm38) using BWA v0.7.17 *mem* algorithm. We then used the *idxstats* command in SAMtools v1.10 to extract the number of reads mapping to each scaffold of the reference assembly.

To determine the sex of the Greater Stick-Nest Rat samples we used Gower et al.'s (2019) python script *sexassign* (https://github.com/grahamgower/sexassign), which uses a likelihood ratio test to assign samples to either male or female on the basis of the observed ratio of reads mapping to the X chromosome versus the autosomes. Following Gower et al. (2019), X chromosome read-dosage is used in preference to the Y chromosome because references for the latter are either unavailable or poorly assembled for most species (Janečka et al. 2018). However, *sexassign* assumes that the X chromosome in homogametes (females, in this case) should receive the same read-dosage as an autosome of the same length (i.e. read dosage of ~ 1X versus ~ 0.5X in heterogametic males), so we first checked that our data conformed to this assumption by visualising read-dosage (proportion of total reads mapped versus scaffold length) for each sample using RStudio v1.3.1073 (Fig. 1). We observed that the mean proportion of reads mapping to the X chromosome (length = 171,031,299 bp) for the putatively female samples (0.0308) was substantially lower than the expectation (0.0656) based on the relationship between the proportion of reads mapped and scaffold length inferred from the autosomes, perhaps due to the DArT marker-selection and filtering process or a depletion of the restriction motif on the X chromosome. Consequently, before proceeding with analysis using *sexassign* we first multiplied the number of reads mapping to the X chromosome for all samples (regardless of putative sex) by a factor of 2.12 (the expected read-dosage for the X chromosome in a female, 0.0656, divided by the observed mean read-dosage for the X chromosome in the putatively female samples, 0.0308).

Results

The proportion of reads mapping to each of the autosomes was highly consistent between samples (Fig. 1). Further, autosomal read-dosage appeared to be positively correlated with scaffold length, as expected if restriction motifs are randomly distributed. We tested this correlation by performing a linear regression in RStudio (proportion of reads ~ scaffold length), which resulted in a slope coefficient of $3.833e^{-10}$ (adjusted $R^2 = 0.7$, $p < 2e^{-16}$). Unlike the autosomes, values for the proportion of reads mapping to the X chromosome formed two clusters, putatively representing females (with higher read-dosage values) and males (with lower read-dosage values).

The read-dosage sex-assignment program (*sexassign*) allowed us to successfully assign all individuals in the dataset as either male (heterogametic, XY; X read-dosage = ~ 0.5X) or female (homogametic, XX; X read-dosage = ~ 1X, Fig. 2, Table 1). Of the 60 individuals sequenced, 33 were determined to be female and 27 to be

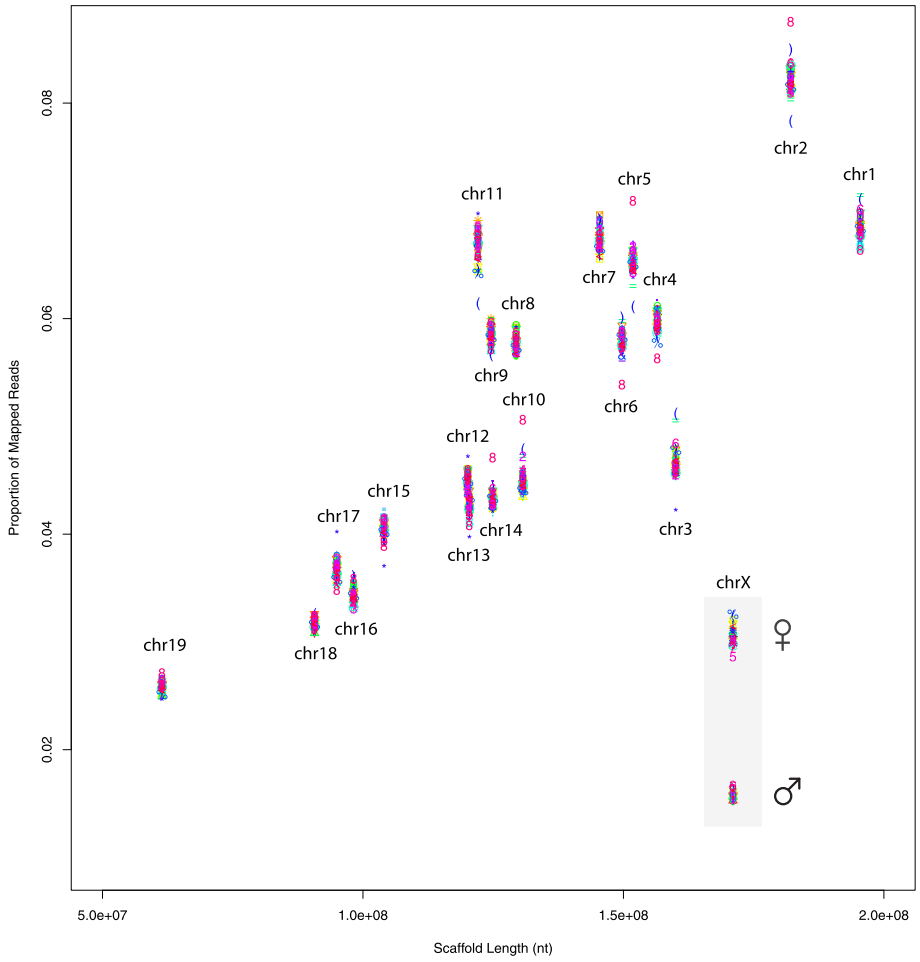

Fig. 1 Proportion of reads mapped to autosomes and the X chromosome in the *L. conditor* DArT dataset. Colour/symbol combinations represent different individuals. Read dosage of autosomes was positively correlated with scaffold length, while reads for the X chromosome form two distinct "dosage" clusters indicative of homogametic (XX) and heterogametic (XY) individuals

male, consistent with the typical sex ratio in rodent populations under normal conditions (Labov et al. 1986; Rosenfeld et al. 2003). Genetic sex assignment had a ~ 94% concurrence rate with field determined sex, a typical human error margin considering the lack of obvious sexual dimorphism within the species and the difficulty of accurately sexing rodents in the field, particularly during non-reproductive periods (Hoffmann et al. 2010; Jacques et al. 2015).

Discussion

Our results demonstrate that the FASTQ-formatted data routinely generated by Diversity Arrays Technology (DArT) as an intermediate step in their workflow can reliably be used to determine the sex of samples from non-model organisms, confirming or replacing field-based sex identification and eliminating the need for additional costly laboratory sexing analyses. Importantly, a reference genome from the species of interest does not appear to be necessary, as we obtained robust results by mapping our data to the reference assembly for the house mouse (*Mus musculus*), which shared a common ancestor with *L. conditor* 10 million years ago (Steppan and Schenk 2017). While the house mouse genome is assembled to the chromosome-level, making identification of reads mapping to the X chromosome straightforward, this approach should also work with scaffold-level reference assemblies.

Gower et al. (2019) identified X-linked scaffolds in the polar bear genome (UrsMar1.0) by first mapping all scaffolds against the chromosome-level dog reference assembly (CanFam3.1), then applied *sexassign* to shotgun sequencing data from a third species—brown bears (*Ursus arctos*)—that they mapped to the putative polar bear X-linked scaffolds. Given that scaffold-level assemblies are increasingly available for a wide range of taxa, our results suggest that most DArT end-users working on mammals should be able use their FASTQ data to determine the sex of their samples. Indeed, the read-dosage approach to sex assignment should be applicable to any diploid organism with a heterogametic sex-determination system, such as birds, lizards, and many invertebrates, regardless of which sex is homogametic.

Fig. 2 Plot of X chromosome read dosages for all sequenced *L. conditor* individuals, with confidence intervals for male heterogametes (red) and female homogametes (blue)

Table 1 Results of DNA-based sex assignment using *sexassign* compared to sex determined in the field for 60 greater stick-nest rats

ID[†]	Field sex	M_X[‡]	Sex	N_X[§]	N_A[¶]
ET002	nd	0.474	M	26,392	802,947
ET101	nd	0.962	F	23,290	349,580
ET102	nd	0.480	M	12,663	380,070
ET103	nd	0.953	F	25,446	385,924
ET106	M	0.515	M	12,856	359,385
ET119	F	0.930	F	60,581	941,980
ET133	F	0.963	F	26,462	396,880
ET146	M	0.517	M	8416	234,263
ET147	F	0.979	F	26,407	388,782
ET147B	nd	0.976	F	12,858	190,022
ET148	M	0.507	M	14,526	412,334
ET149	F	1.020	F	29,618	417,327
ET151	F	0.975	F	24,507	362,393
ET152	F	1.002	F	28,024	402,617
ET153	nd	0.970	F	26,335	391,810
ET154	nd	0.946	F	25,894	395,471
ET155	nd	0.482	M	14,054	420,275
ET157	M	1.026	F	28,484	399,170
ET158	nd	0.950	F	26,525	403,485
ET162	nd	0.503	M	13,867	397,200
ET163	nd	0.946	F	24,215	370,137
ET163B	nd	0.473	M	14,299	436,150
ET17	F	0.942	F	58,158	892,183
ET173	F	0.956	F	27,789	419,868
ET176	F	0.938	F	22,451	346,121
ET177	nd	0.952	F	26,275	398,926
ET18	F	0.905	F	39,676	635,045
ET183	nd	0.495	M	13,220	384,279
ET184	nd	0.487	M	32,640	966,813
ET185	F	1.010	F	26,952	384,146
ET186	nd	0.473	M	28,294	863,292
ET187	nd	0.996	F	25,964	375,434
ET188	nd	0.503	M	12,563	359,345
ET189	nd	0.972	F	22,913	339,929
ET192	M	0.500	M	14,444	416,297
ET193	M	0.489	M	13,108	386,485
ET195	nd	0.960	F	25,194	378,761
ET196	F	0.977	F	27,030	398,915
ET198	M	0.512	M	28,970	813,496
ET198B	nd	0.484	M	12,733	378,965
ET203	M	0.475	M	11,469	348,138
ET209	F	0.971	F	25,533	379,373
ET217	M	0.480	M	13,460	404,344
ET231	nd	0.493	M	12,745	372,720
ET233	nd	0.480	M	12,353	370,852
ET255	F	0.952	F	24,282	368,459
ET259	M	0.478	M	11,357	342,534
ET261	F	0.958	F	26,557	400,394
ET277	M	0.488	M	29,029	857,827
ET29	F	0.939	F	30,250	465,742
ET29B	nd	0.959	F	23,511	354,200
ET3	F	0.991	F	27,316	397,077
ET32	M	0.509	M	13,059	369,309
ET37	M	0.467	M	26,816	828,029
ET5	M	0.491	M	27,564	809,456
ET50	F	0.485	M	11,802	350,729
ET50.2	nd	0.981	F	23,708	348,582
ET5967	nd	0.958	F	26,131	393,719
ET61	M	0.491	M	7566	222,226
ET62	F	0.987	F	36,015	526,076

The length of the X chromosome was 171,031,299 bp and the total length of the autosomes was 2,462,745,373 bp (Gower 2019)

[†]ID = ear tag number for *L. conditor* individual, *nd* not determined

[‡]M_X = read dosage on X chromosome

[§]N_X = count of reads mapped to the X chromosome (after multiplying by 2.12)

[¶]N_A = count of reads mapped to the autosome

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Author contributions IRO and JJA coordinated submission of samples to DArT. IRO and KJM analysed the data. IRO drafted the abstract, introduction, results, and discussion. KJM drafted the materials and methods and figures. All authors contributed to the interpretation of results and provided feedback on the final manuscript.

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Data availability The reads generated for this study have been deposited at the Sequence Read Archive (NCBI) with BioProject ID PRJNA702840 (http://www.ncbi.nlm.nih.gov/bioproject/702840).

Code availability The original code can be found on Dr Graham Gower's GitHub repository https://github.com/grahamgower/sexassign.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval Live animal trapping and sampling at Arid Recovery was conducted under South Australian Wildlife Ethics Committee permit numbers 27/98, 4/99, 22/99, 2/2000, 19/2000, and 18/2000.

Consent to publish The authors give consent for the publication of this manuscript.

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Chapter 4

Understanding dispersal patterns can inform future translocation strategies: a case study of the threatened greater stick-nest rat (*Leporillus conditor*)

Statement of Authorship

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Principal Author

Name of Principal Author (Candidate)	Isabelle Onley
Contribution to the Paper	Isabelle developed the methodology, collected and analysed the data, drafted the manuscript and acted as corresponding author.
Overall percentage (%)	80%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date: 13/09/2021

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate to include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Jeremy Austin
Contribution to the Paper	Jeremy supervised the collection and analysis of data and contributed to the development and editing of the manuscript.
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Contribution to the Paper	Katherine provided access to data, supported the analysis of the data and assisted with the editing and development of the manuscript.
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Name of Co-Author	Kieren Mitchell		
Contribution to the Paper	Kieren assisted with data analysis and development of the manuscript.		
Signature		Date	14/09/2021

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Understanding dispersal patterns can inform future translocation strategies: A case study of the threatened greater stick-nest rat (*Leporillus conditor*)

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Abstract Dispersal behaviour and sociality are significant factors influencing survival at both the individual and population levels. In translocation and breeding programmes, social structure and sex-biased philopatry and dispersal should be considered in order to maximise population viability and conservation outcomes. Here, we use the greater stick-nest rat (*Leporillus conditor*), a native Australian rodent, as a case study to understand how knowledge of social structure and dispersal can inform conservation and translocation programmes. We combine high-throughput DNA sequencing with field trapping data from a translocated population of greater stick-nest rats at Arid Recovery Reserve, South Australia, to provide the first empirical evidence of female philopatry and male-biased dispersal in this species. Males were found to disperse, on average, 1.5 km from the natal nest, while females typically did not disperse beyond 500 m. Further, recapture data showed that females demonstrated a higher degree of nest fidelity than males over time. Based on these findings, we make two key recommendations for future translocations of the species. Firstly, founders should be harvested in small groups at adjacent nest sites with groups separated by a minimum of 1.5 km allowing family group structure to be retained during translocation while simultaneously maximising genetic diversity. Secondly, translocated individuals should be released in family cohorts into patches of optimal habitat that contain adequate shelter substrates interspersed over short distances (~300–500 m, the maximum dispersal distance of females found in this study), thereby facilitating nest establishment and maintenance of family groups. The results of this study have implications for conservation and reintroduction biology as a whole; we highlight the importance of considering spatial genetic structure during all stages of translocations to improve outcomes, and the value of combining genetic and field data to better understand species' social and spatial preferences.

Key words: conservation genetics, ecology, reintroduction biology, spatial genetics.

INTRODUCTION

Sociality in mammals has many benefits at both the individual and population levels, particularly in regard to female fitness (Silk 2007). A common observation in mammalian social systems is that males will disperse from their natal territory, while females will demonstrate philopatric behaviour and remain close to their place of birth (Greenwood 1980). This pattern typically results in distinct local matrilines, with daughters inheriting territories, warrens or nests from their mothers or other female relatives (Holekamp & Sawdy 2019). Female philopatry can have a number of benefits, including sharing of knowledge about food distribution and landscape cover for predator avoidance, as well as kin-selected social behaviours such as cooperative care of young (Hamilton 1964; Clutton-Brock & Lukas 2012). Female philopatry may also be advantageous when shelter sites are limited or require considerable investment, as female offspring can inherit a shelter site from their mother. Male-biased dispersal, which is often the counterpart to female philopatry, aids in inbreeding avoidance (Dobson *et al.* 1997; Liebgold *et al.* 2011). There are genetic consequences of sex-biased dispersal (Goudet *et al.* 2002; Peakall *et al.* 2003; Matocq 2004; Banks & Peakall 2012; Shaw *et al.* 2018); for example, potential outcomes of female philopatry include mitochondrial DNA-specific population structure, wherein reduced movement of females results in genetic differentiation visible only in the mitochondrial genome (Ruppell *et al.* 2003), and increased pairwise relatedness between females within territories (Hazlitt *et al.* 2004).

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In translocation and breeding programmes, social structure and sex-biased philopatry and dispersal should be considered in order to maximise population viability and conservation outcomes (Kleiman 1989; Gouar *et al.* 2012; Garnier *et al.* 2021). For example, a study on near-threatened brush-tailed rock-wallabies (*Petrogale penicillata*) in Australia revealed evidence of female philopatry and male-biased dispersal, suggesting that females were less likely to disperse between colonies (Hazlitt *et al.* 2004). On the basis of these results, Hazlitt *et al.* (2004) cautioned that a geographically restricted collection of source animals for relocation would likely include highly related females, which could have adverse consequences for the translocated brush-tailed rock-wallaby population, such as inbreeding depression and reduced genetic diversity. However, several studies have noted that the harvesting of social groups during translocation is vital for population establishment in several species, including the black-tailed prairie dog (*Cynomys ludovicianus*; Shier 2006), as it allows individuals to continue cooperative behaviour such as nest building and allogrooming with neighbours and relatives following translocation (Shier & Swaisgood 2012; Goldenberg *et al.* 2019).

Management strategies for maximising genetic diversity and maintaining cohesive family units are likely to be species-specific, highlighting the need to understand dispersal behaviour and patterns of philopatry on a species-by-species basis for effective conservation. These factors are likely to be particularly important when selecting founding individuals, as the success of translocation programmes is often determined by the viability of the founding population (e.g. sex and age ratios, numbers, genetic diversity; Singer *et al.* 2000; Chauvenet *et al.* 2013; Pacioni *et al.* 2019).

One species that has been the focus of multiple translocations over recent decades is the greater stick-nest rat (*Leporillus conditor*), a relatively large (up to 450 g), polygynous murid rodent, which was once widespread across the semi-arid and arid zones of southern mainland Australia (Copley 1999; Pearson *et al.* 1999; Webeck & Pearson 2005). The greater stick-nest rat produces up to three litters a year, with a typical lifespan of 5 years in the wild and a generation length of approximately 2 years (Procter 2007; Pacifici *et al.* 2013; Woinarski & Burbidge 2016). With the arrival of introduced predators and grazing herbivores with European settlers in the 1800s, greater stick-nest rats became extinct on the mainland by the 1930s, with the only surviving population on the Franklin Islands of South Australia (Robinson 1975; Copley 1999). Due to this rapid contraction of population size and geographical range, little was known about its habitat preferences and life history until monitoring commenced on the Franklin Islands and, in recent decades, translocation programmes began on a number of islands and fenced mainland reserves (Robinson 1975; Pedler & Copley 1993; Copley 1999; Moseby & Bice 2004; Short *et al.* 2017, 2019). Greater stick-nest rats are nocturnal, feeding on vegetation, predominantly succulents (Ryan *et al.* 2003) and constructing large nests of sticks and stones to shelter from predators and temperature extremes during the day (Watts 1976; Copley 1999). Nests are often constructed beneath perennial shrubs, under rocky overhangs or over historical warrens dug by other species (Copley 1999; Short *et al.* 2019). While the nests are communal and believed to be shared within family groups (Copley 1988, 1999), little is known about how the nests are passed on from generation to generation.

Although the behaviour of greater stick-nest rats in the wild is still understudied, in captivity they have been observed to exhibit a matriarchal hierarchy, with the eldest female in the nest assuming the dominant role (Procter 2007) and occasionally behaving aggressively towards males in the vicinity of the female's natal nest (P. Copley, pers. comm., 2020). In addition, field observations suggest that females in wild and reintroduced populations appear to be sedentary, while males disperse readily (Robinson 1975; Pedler & Copley 1993; Copley 1999). Such behaviour suggests greater stick-nest rats may exhibit female philopatry and male-biased dispersal; however, no data have yet been published to demonstrate this. Such social patterns are common in other matrilineal rodent species, such as the black-tailed prairie dog (Hoogland 1995); females demonstrate strong philopatric behaviour, whilst males are more wide-ranging and less territorial (Christian 1970; Aguilera-Miller *et al.* 2018).

We studied a translocated population of greater stick-nest rats at the Arid Recovery Reserve, South Australia, in order to understand the social behaviours of the greater stick-nest rat and inform future translocation strategies. Arid Recovery Reserve is located in an arid environment with limited rainfall near the northern edge of the species' former range (Moseby *et al.* 2011; Short *et al.* 2019). The translocation programme began in 1998 with a trial reintroduction, shortly followed by a full-scale reintroduction the following year (Moseby & Bice 2004). The reintroduction was considered successful (Short *et al.* 2019), with population growth, limited inbreeding, and up to 98% of genetic diversity retained from their founding groups (Moseby *et al.* 2011; White *et al.* 2018); however, greater stick-nest rats demonstrated increased mortality during the summer months and the population size was adversely affected by drought and overbrowsing of vegetation by burrowing bettongs (*Bettongia lesueur*; Moseby *et al.* 2018).

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By investigating the dispersal behaviours of the greater stick-nest rat, we aimed to establish whether sex-biased dispersal and philopatry were present in the species and should therefore be considered during the planning of subsequent translocation programmes to increase their chance of success. Previously, philopatry and dispersal in the wild have been difficult to determine except through long-term observational studies. Here, we use high-throughput sequencing of DNA samples collected during the first 4 years following the reintroduction of greater stick-nest rats at Arid Recovery Reserve to determine patterns of dispersal and philopatry in this species.

METHODS

Sample collection & DNA sequencing

The Arid Recovery Reserve is located 20 km north of Roxby Downs, South Australia, and includes a 14 km^2 rabbit, cat and fox-proof exclosure of 50 mm mesh fencing (the Main Exclosure) encompassing a dune and swale landscape vegetated predominantly by chenopod and wattle (*Acacia* spp.; Moseby & Bice 2004). A 30-mm mesh foot netting runs along the bottom of the fence, although greater stick-nest rats have been observed to climb this netting and disperse through the 50-mm mesh. Following a successful trial release in 1998, 92 greater stick-nest rats were released into the Main Exclosure in 1999 at random across a number of release sites, as described by Moseby *et al.* (2011). From 1999 to 2002 (inclusive), tissue samples (tail tips, ~5 mm length) were collected from a total of 56 individuals across 18 nest sites during routine trapping and monitoring at Arid Recovery Reserve and stored at −20°C in 70% ethanol. Trapping effort was equal across all nest sites and included all known nests in the reserve. Nests were located by radiotracking rats to nest sites. Individuals were a mixture of age-classes, some were part of the translocated cohort, and some were born in the reserve. Information on the sex, trapping coordinates, age and nesting site of each individual were recorded in the field. Traps were set in close proximity to the nest, and individuals caught were presumed to inhabit that nest. Where multiple captures were recorded during the lifetime of an individual, trapping location and data from the first adult capture were used (adults were identified as animals >180 g according to Procter (2007)). DNA was then extracted from tissue by S. Barclay using the method described in Barclay *et al.* (2006). These samples were submitted to commercial sequencing company Diversity Arrays Pty Ltd (DArT) for single nucleotide polymorphism (SNP) genotyping. Diversity Arrays employs a complexity reduction method (DArTseq) to generate SNP data for each individual (Egea *et al.* 2017; Melville *et al.* 2017). DArT provided both raw FASTQ files for each individual (subsequently used for sex assignment) and a coded matrix of SNP loci by individual, which was then passed to a genlight object for kinship analysis.

Sex assignment

Although field-determined sex data were available for most of the samples, a genetic sex assignment approach was used also to ensure that sexing was accurate (Onley *et al.* 2021). Briefly, greater stick-nest rat FASTQ sequencing data were first aligned to the house mouse (*Mus musculus*) genome reference using the 'mem' algorithm in BWA v0.7.17 (Li & Durbin 2009), after which per-scaffold read counts were extracted using SAMtools v1.10 (Li *et al.* 2009). As described in Gower *et al.* (2019), we then used the Python script 'sexassign' (https://github.com/grahamgower/sexassign) to construct two binomial models (one for males and one for females) for the X chromosome 'read-dosage' *versus* that of the autosomes and conduct a likelihood ratio test between them. Sex assignment using this method resulted in ~94% concordance with field-determined sex, with the discrepancies determined to be due to misidentification of individual sex in the field (Onley *et al.* 2021). This is consistent with previously reported rates for human error when sexing rodents in the field, which are typically around 10% (particularly during non-reproductive periods; Williams *et al.* 2004; Hoffmann *et al.* 2010; Jacques *et al.* 2015).

Kinship analysis

Kinship analysis was performed on the DArTseq data to determine the degree of relatedness of individuals within and between nest sites. Data filtration was performed on the SNP matrix using the 'dartR' package in R v3.6.2 (Gruber *et al.* 2019). Monomorphic and secondary loci were removed from the dataset, and SNPs with a locus call rate <0.80 and a repeatability <0.9 were filtered out (Massault *et al.* 2021). Observed and expected heterozygosity were also calculated. We chose not to filter the dataset based on minor allele frequencies, as this has been shown to mask population structure in large data sets (Linck & Battey 2019; Wright *et al.* 2019). Following this, an identity-by-descent (IBD) analysis using the KING method of moment was conducted using the R package 'SNPRelate' (Zheng *et al.* 2012). This returned an estimated kinship coefficient for pairings within the population, which was then used to create a network graph to visualise relatedness. To confirm kinship pairings, SNP data were also run through the program COLONY v2.0.6.5 using a full likelihood analysis to produce full and half sibling dyads with associated probability values. Due to memory constraints, 500 randomly selected SNP markers were used for the COLONY run, with the following settings: polygyny for both males and females, inbreeding present, medium run length, locus error rate of 0.02, and an allelic dropout rate of 0.

To determine whether male and female greater stick-nest rats displayed a higher degree of relatedness at the cooperative group (nest site) level than within the population as a whole, a Wilcoxon rank sum test was performed on kinship coefficients of pairings within and between nest sites according to sex. A Wilcoxon rank sum test was chosen because the data were not normally distributed. If sex-biased dispersal was occurring, individuals of the dispersing sex were expected to demonstrate lower relatedness than the philopatric sex at the cooperative group level (Liu *et al.* 2015).

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Spatial autocorrelation

To further examine the spatial genetic structure (i.e. the distribution of genetic variation within the reserve space) of the Arid Recovery Reserve population in relation to nest sites, spatial autocorrelation analyses were conducted using GenAlEx v6.5 (Peakall & Smouse 2012). In order to meet GenAlEx memory requirements, we randomly selected 5000 filtered SNPs as a representative sample of the filtered data set. As the aim of this analysis was to determine how related individuals dispersed across the landscape, only individuals that appeared in kinship pairings determined by the IBD-KING analysis were used for spatial autocorrelation analysis. Data were then transformed to the appropriate format using the 'poppr' package in R (Kamvar *et al.* 2014). The SNP data were split into two separate datasets for males and females (each with the same 5000 SNPs), and pairwise genetic distance was calculated separately for each sex. Decimal latitude and longitude values of the nest locations for each individual were used to calculate a matrix of geographic distance. Using these distance matrices, a spatial structure analysis was implemented to test for spatial heterogeneity at even distance classes of 0.5-km intervals and to determine a correlation coefficient, *r*. This analysis was conducted using a permutation procedure with 999 simulations to test for deviations from zero and 1000 bootstraps to estimate the confidence intervals around *r*. Where *r* exceeded the 95% confidence intervals of the permutations and the bootstrap confidence intervals did not exceed zero, spatial genetic structuring was declared (Peakall *et al.* 2003; Hazlitt *et al.* 2004). Heterogeneity is determined by calculating an 'Omega' value and testing whether the observed value is larger than expected under the null hypothesis of homogenous genetic structure, wherein no significant spatial autocorrelation is observed ($P > 0.01$ where P = Omega-rand $\geq$ Omega-data; Smouse *et al.* 2008; Banks & Peakall 2012).

Male *versus* female nest fidelity

Finally, to corroborate any evidence of female philopatry, field trapping data were analysed to identify rates of recapture over time by sex at the same nest site. This data set included recorded captures for individuals not included in the genetic analysis, so field recorded sex was used where genetic sex determination data were not available.

RESULTS

Samples and SNP data

Fifty-six individuals (32 females and 24 males) were captured across 18 nests with 1–7 individuals sampled per nest (mean = 2.9; Appendix S1). The average male:female ratio per nest was 1:1.33. Four individuals (two males and two females) did not have nest site recorded (Appendix S1). The initial data set contained 21 792 SNPs. After filtering, 17 787 SNPs remained, with an expected heterozygosity of 0.323 and observed heterozygosity of 0.301.

Kinship analysis

Our IBD-KING analysis yielded 130 kinship pairings, with kinship coefficients ranging from 0.032 to 0.25 (Fig. 1), which corresponded to the pairings calculated by the COLONY run (Appendix S2). A kinship coefficient of 0.25 represents a parent-offspring or full sibling relationship, while 0.15 is consistent with half siblings (Lopes *et al.* 2013). Thirteen individuals showed no (or very low) genetic relatedness to any other sampled individuals, while the remaining 43 individuals formed two clusters (Fig. 1). One cluster contained 11 individuals mostly from three nests (1, 2 and 15) from the north-eastern section of the Main Exclosure, while the second cluster contained 32 individuals from 12 of the 18 nests distributed across the entire sampling area (Fig. 1).

Of the pairings determined by IBD-KING analysis, 35 were female–female and 23 were male–male. Female–female kinship coefficients were significantly lower between nests than within nests (mean = 0.11 $\pm$ 0.05 SD, cf. mean = 0.18 $\pm$ 0.04 SD), whereas male-male kinship coefficients were low and not significantly different between *versus* within nests (mean = 0.10 $\pm$ 0.06 SD, cf. mean = 0.11 $\pm$ 0.02 SD; Fig. 2).

Cohabiting females demonstrated a significantly higher degree of relatedness than cohabiting males (mean 0.18 *vs*. 0.11, *P*-value 0.02; Fig. 3).

Spatial autocorrelation

Results of our spatial autocorrelation analyses for genetic data indicated that heterogeneous spatial structuring was present for both males and females. For both sexes, the Omega value was larger than expected under the null hypothesis of homogeneous genetic structure, indicating spatial heterogeneity. Correlograms demonstrate that the correlation coefficient between genetic and geographic distance, *r*, of females is strongest in shared locations (i.e. distance class = 0), well above the upper 95% confidence intervals of no observed spatial autocorrelation (indicated by U and L in Fig. 4), and decreases as physical distance increases, while the *r* value for cohabiting males is much lower and relatively stochastic until the distance class exceeds 1.5 km (Fig. 4). This indicates that, while females did not disperse far from their family groups, males may disperse up to 1.5 km from their natal nest. However, confidence intervals overlap zero for both males and

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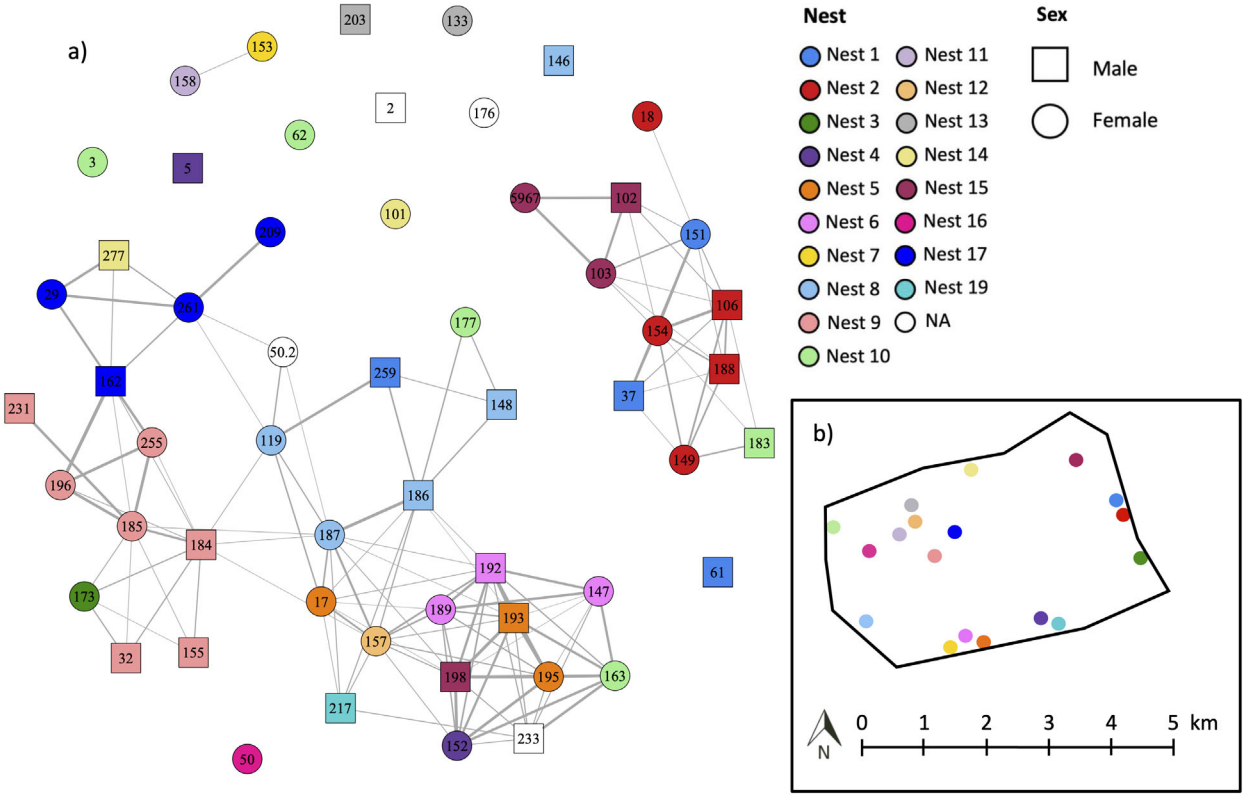

Fig. 1. (a) Relatedness network of male (squares) and female (circles) greater stick-nest rats (*Leporillus conditor*) within the Main Exclosure at Arid Recovery Reserve, coloured by nesting site. Thickness of links corresponds to degree of relatedness; (b) Location of the 18 sampled nests within the Main Exclosure.

females in the first distance class, so some level of uncertainty (likely due to small sample size) must be acknowledged. There is also a slight rise in *r* at 4 km in both sexes, possibly due to high post-release dispersal.

Male *versus* female nest fidelity

In the trapping dataset, 14 individuals were recaptured on multiple occasions over periods of 2–24 months (Table 1). Of these, 12 were females and two were males. Nine of these females were recaptured at the same nest over periods of up to 16 months. The mean period of recapture at the same nest site was nine months. The remaining three females were each recaptured at one adjacent nest site to their natal nest. The distance of these adjacent nests from the home nest did not exceed 330 m. Conversely, the two recaptured males were trapped across multiple nest sites over a period of up to 12 months, at distances that ranged from 3.38 to 1.52 km. This appears consistent with the network graph (Fig. 1), in which some individuals (e.g. ET183) were trapped at nests across the exclosure from their closely related kin. Of the two individuals that were recaptured as subadults and then again as adults – one male (ET198) and one female (ET147) – the male was recaptured at a different nest site while the female was recaptured in the same nest.

DISCUSSION

Evidence for female philopatry and male-biased dispersal

Our results demonstrate a significantly higher degree of relatedness between female and female pairings of greater stick-nest rat individuals sharing nest sites compared to those inhabiting different nests, a trend not evident in male–male pairings within the same population. Further, there was a significantly higher degree of relatedness between cohabiting female–female pairings than male–male pairings. Females were repeatedly recaptured in the same or adjacent nest sites, while recaptured males were recorded at multiple nest sites around the reserve. One female was also captured in the same nest as a subadult and as

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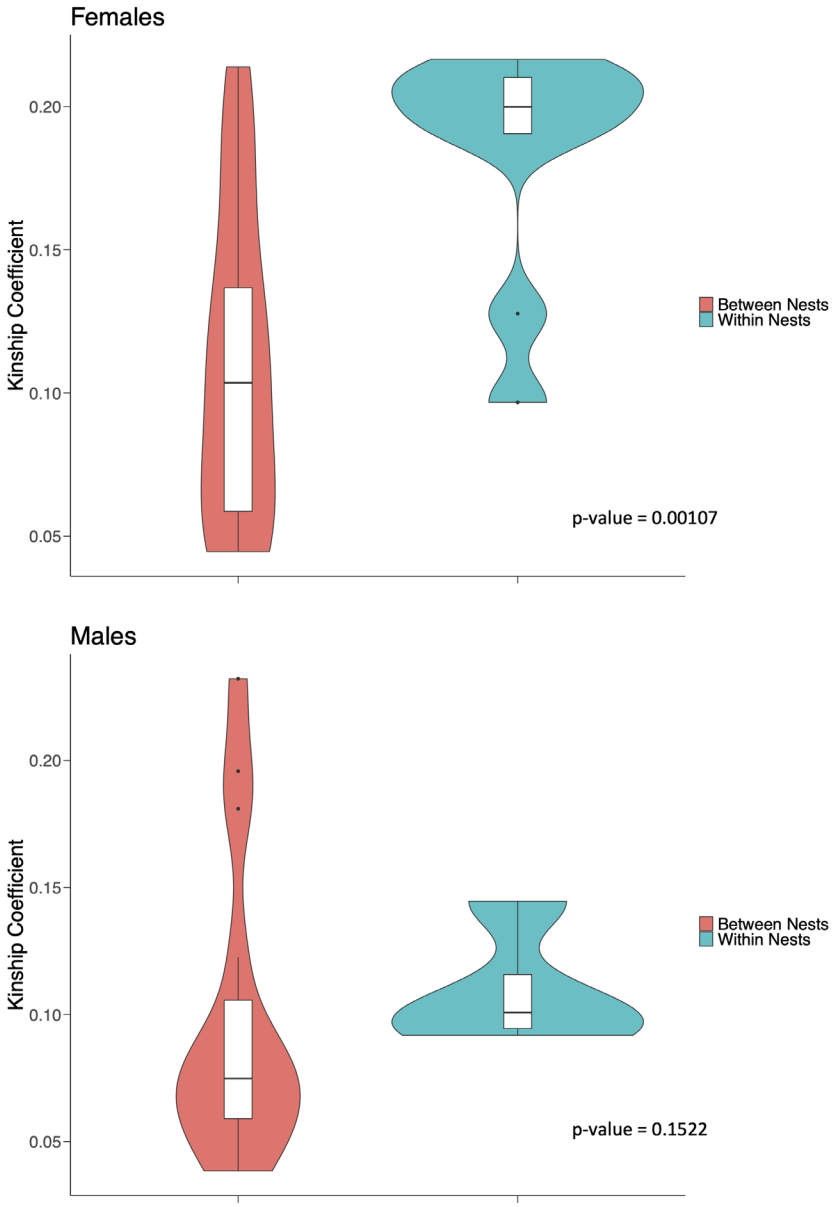

Fig. 2. Violin plots for pairwise kinship coefficients between female (top panel) and male (bottom panel) greater stick-nest rats (*Leporillus conditor*) trapped in the same or different nests at Arid Recovery Reserve.

an adult, consistent with matrilineal nest inheritance – although the small sample size makes robust conclusions based on this observation difficult. This is the first genetic evidence of female philopatry in greater stick-nest rats, wherein males disperse from the natal nest and females remain in their familial territory, a pattern that is often observed in other polygynous mammals (Greenwood 1980).

There are a number of potential advantages to male-biased dispersal strategies in polygynous species, namely that males increase their chances of breeding by gaining access to multiple females, while females maintain strong knowledge of their home range and available resources, improving the chances of survival for both themselves and their young (Moses & Millar 1994; Pärt 1995; Ruusila *et al.* 2001). Female site fidelity has been linked to increased survival and reproduction success in several taxa (Cockburn *et al.* 1985; Bose *et al.* 2017; Patrick & Weimerskirch 2017), particularly in species like the greater stick-nest rat that invest considerable energy in nest or burrow construction, such as prairie dogs and yellow-bellied marmots (Armitage 1991; Shier 2006). Over time, such systems can result in geographically restricted matrilines, with members of the resident sex in nesting sites or territories becoming closely related (Kappeler *et al.* 2002). Our field results supported the genetic data, with individual females exhibiting higher recapture rates in the same or closely spaced nests over time compared to males.

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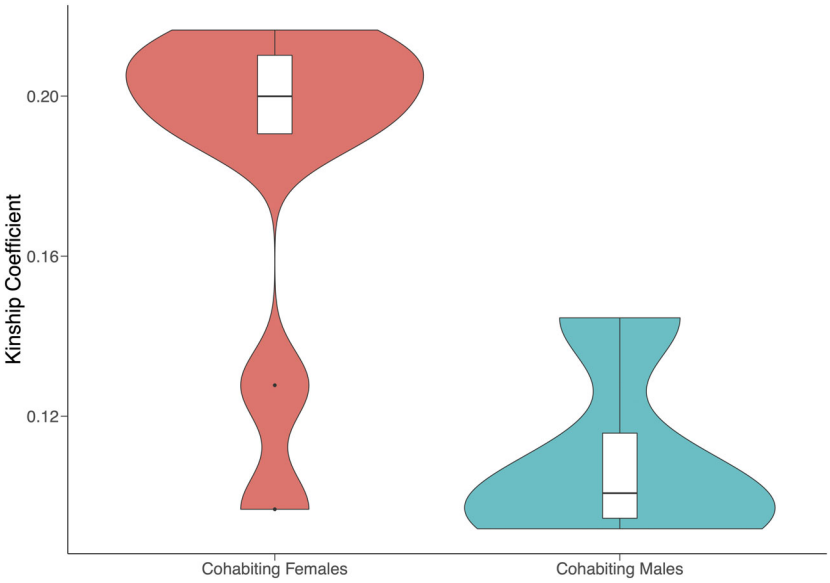

Fig. 3. Violin plots for pairwise kinship coefficients between cohabiting females and cohabiting males of greater stick-nest rats (*Leporillus conditor*) at Arid Recovery Reserve (*P*-value = 0.01958).

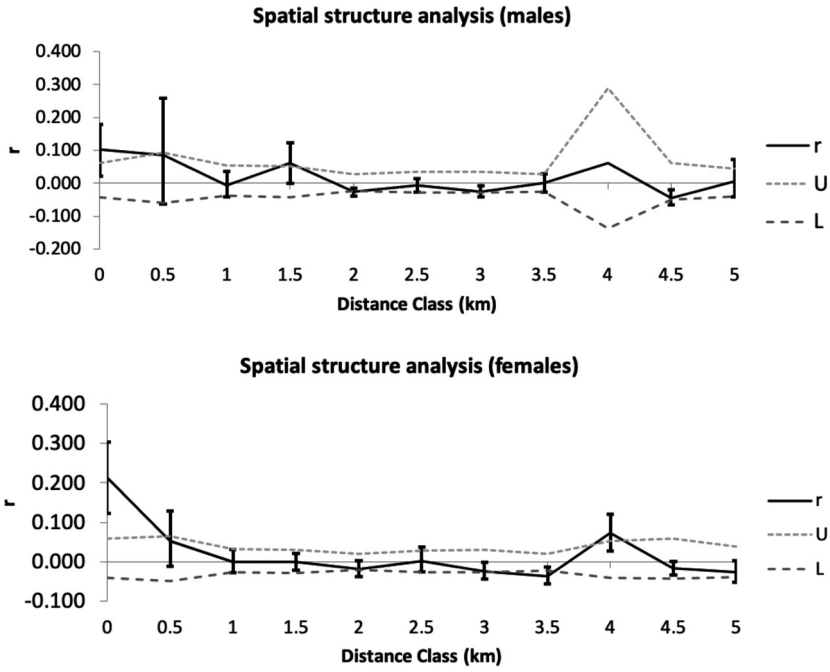

Fig. 4. Correlograms showing spatial genetic structure in male and female greater stick-nest rats (*Leporillus conditor*). Genetic correlation coefficient (*r*) is displayed with 95% confidence intervals (U = upper, L = lower) and error bars determined by bootstrapping. Upper and lower confidence intervals correspond to no observed spatial autocorrelation.

While our results provide evidence for male-biased dispersal in the greater stick-nest rat population at Arid Recovery Reserve, the applicability of our findings to other greater stick-nest rat populations is subject to some caveats. Arid Recovery Reserve is a fenced reserve, and greater stick-nest rats used in this study were confined within a 14-km^2 area. Dispersal distance was likely to have been limited by the presence of fences. Further research is needed to determine whether reserve size impacts male dispersal distance in this species. In addition, Arid Recovery Reserve is located in an arid environment, and it is unclear whether climate and resource availability impact greater stick-nest rat dispersal distance. Similar monitoring of populations in coastal or more mesic habitats would inform on this. In any case, we believe that our results have a number of implications for conservation of the greater stick-nest rat, particularly concerning the planning, execution and subsequent management of translocation programmes.

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Table 1. Nest site locations for individual greater stick-nest rats (*Leporillus conditor*) recaptured between August 1999 and October 2002 by capture month and sex

		Capture month and nest site												
	ID	08/ 1999	09/ 1999	01/ 2000	02/ 2000	03/ 2000	04/ 2000	05/ 2000	06/ 2000	11/ 2000	12/ 2000	03/ 2001	10/ 2001	10/ 2002
Female recaptures	ET29									17			17	
	ET42						2*	2*						
	ET44						1		1	1	1			
	ET55				6	7	7			7	7	7		
	ET63									6		6	5	5
	ET133											13	13	
	ET147											6*	6	
	ET149											1	2	
	ET3140	2	2			2	2	2	2	2	2			
	ET3599						12			12				
	ET5976											15	15	
	ET5997							9				9		
Male recaptures	ET198												5*	15 & 20
	ET5992			7		9				7				

Asterisks indicate individuals that were subadult at the time of trapping. Cells shaded in light grey stipple represent a capture at a different site to the individual's preferred or original nest site.

Conservation implications & recommendations for future translocations

Post-release dispersal is an important, but often overlooked, component of translocation success or failure (Gouar *et al.* 2012), so understanding dispersal patterns of greater stick-nest rats is likely to be important for the ongoing success of future translocation programmes. Selection of wild-caught individuals for translocation from a source population is often opportunistic or transect-based and heavily impacted by factors such as trapping success and accessible terrain (Coulson & Eldridge 2010). Further, guidelines around sampling regimes for translocations are limited (Ewen *et al.* 2012). However, sex-biased dispersal can result in fine-scale spatial genetic structuring, a factor that should be considered when harvesting individuals to establish a new colony (Hazlitt *et al.* 2004; Banks & Peakall 2012; Pacioni *et al.* 2020). For example, low levels of female dispersal in black-tailed deer (*Odocoileus hemionus columbianus*) have led researchers to suggest that matrilineal groups should be treated as the basic unit of genetic structuring in species demonstrating female philopatry, a major consideration for conservation management (Bose *et al.* 2017).

Selection of multiple females from the same location in a species demonstrating female philopatry will likely result in a higher degree of relatedness than desired and could increase the risk of inbreeding depression in the new population. For example, a genetic evaluation of translocated freshwater fish (*Notropis heterodon* and *Notropis heterolepis*) in Illinois, U.S.A., determined that the lack of consideration for kinship structure during harvesting had resulted in the selection of multiple full and half sibship pairings, thereby lowering the effective population size of the reintroduced stock (Ozer & Ashley 2013). Ozer and Ashley (2013) suggested that harvesting from multiple sites and across multiple trapping events may decrease the overall relatedness of the new population and improve genetic representation.

However, it must also be acknowledged that several studies on mammals demonstrating kin clustering and female philopatry have noted an increase in translocation success when entire family groups were harvested. This has been attributed to the benefits associated with resource sharing, as well as reduced aggression and stress and increased site fidelity during reintroduction (Watson *et al.* 1994; Bradley *et al.* 2005; Gusset *et al.* 2006; Shier & Swaisgood 2012; Goldenberg *et al.* 2019; but see also Franks *et al.* 2020). Consequently, when translocating a species demonstrating female philopatry, managers should consider the importance of increasing long-term genetic diversity by selecting unrelated founding individuals against the potential survival benefits of maintaining close familial associations.

Pacioni *et al.* (2020) proposed a spatially explicit approach to selection of individuals for translocation, wherein prior knowledge of a species' dispersal patterns is applied to determine the appropriate separation distance between candidates to minimise relatedness. This approach can be applied to all species with a predictable dispersal pattern. Trials using this method on woylies (*Bettongia penicillata ogilbyi*)

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have proven far more effective than conventional transect and grid trapping designs, with resulting samples exhibiting higher genetic diversity and lower relatedness, while requiring minimal increases in time and resource investment by managers (Pacioni *et al.* 2020). While some uncertainty exists around the spatial autocorrelation analysis due to the small sample size of this study, our results have shown that relatedness is significantly decreased beyond a 0.5 km radius of nest sites for females and 1.5 km for males; an appropriate harvesting strategy would therefore involve selecting small cohorts of males and females from multiple adjacent nest sites which are then separated from the next group by a minimum distance of 1.5 km. This would allow for founding females to retain family groups, while simultaneously maximising genetic diversity and reducing the risk of inbreeding. Post-release monitoring of future translocations would inform on the consistency of this spatial genetic structure when dispersal distances are not limited by fencing.

Female philopatry is an important adaptive behaviour that increases breeding success, ensuring long-term viability in a population (Stacey & Ligon 1991). In greater stick-nest rats, permanent nest structures appear to be inherited maternally and are maintained and used by subsequent generations of related females, a strategy that has been shown to improve offspring survival in other species (Armitage 1991; Moses & Millar 1994; Hatchwell & Komdeur 2000; Lutermann *et al.* 2006). As the construction of such large and complex shelter sites is energetically expensive, resource inheritance by female kin has an added survival advantage, namely that subsequent generations of females in established nests are not required to expend large amounts of energy on founding a new nest and can therefore prioritise foraging for food and caring for young (Myles 1988; Hansell 1993; Almond *et al.* 2019). Since nest sites are central to the breeding behaviour and, consequently, the population viability of the greater stick-nest rat (Aslin 1972; Copley 1999; Procter 2007), the presence of adequate nesting sites should be a consideration for future conservation of the species. An abundance of sticks and dry grass should be present for nest construction. More importantly, rock overhangs and fissures, warrens and burrows, and low, thick perennial shrubs such as *Maireana* spp. and *Rhagodia* spp. act as important substrates for nest building. The latter also supply additional protection from predators and environmental extremes, as well as providing a source of food (Copley 1988, 1999; Moseby & Bice 2004; Short *et al.* 2019). Suitable habitat for future translocations of the greater stick-nest rat should contain a variety of these structures within close proximity, providing ample shelter for both dispersing males and females remaining in their natal territory. Shelter substrates should ideally not be more than 300–500 m apart, as this was the maximum distance travelled by females in the trapping data set that visited nearby nests.

Finally, although our results suggest that maintaining related female groups with closely spaced nests should be facilitated and encouraged during translocation, female greater stick-nest rats have been observed to demonstrate aggressive territorial behaviour in captivity, thus overcrowding and reduced capacity for dispersal may increase aggression within a population (Jackson 2003; Procter 2007). During a trial reintroduction of greater stick-nest rats at Arid Recovery Reserve into an 8-ha release pen, the two largest of the three females quickly established territories that did not overlap; the youngest female roamed between the two territories, but whether this was due to her immaturity or the small size of the enclosure is unclear (Moseby & Bice 2004). Small release pens for family groups may therefore also be used to limit stress, maintain kin clusters and promote shelter establishment (Moseby *et al.* 2014, 2020), but managers should consider the long-term implications of this strategy; once the translocated population has become settled and nests established – greater stick-nest rats at Arid Recovery Reserve built nests within a few months of translocation (Moseby & Bice 2004) – larger areas should be provided to facilitate male dispersal, an important mechanism for inbreeding avoidance (Cockburn *et al.* 1985; Wolff *et al.* 1988; Szulkin & Sheldon 2008).

CONCLUSION

Here, we have presented the first empirical evidence of sex-biased dispersal behaviour in the greater stick-nest rat. Data were collected within 5 years of the start of the reintroduction program, suggesting that distinct local matrilines in the greater stick-nest rat can develop over only a few generations, and that male dispersal is likely the primary mechanism for inbreeding avoidance in the species. Based on these results, we present two key recommendations for future translocations of greater stick-nest rats using wild stock. Firstly, an adaptive design for trapping founders, such as the method proposed by Pacioni *et al.* (2020), would involve selecting small cohorts of males and females from multiple adjacent nest sites that are then separated from the next group by a minimum distance of 1.5 km. Secondly, as greater stick-nest rat matrilines rely on the generational construction and maintenance of nest sites that require a high degree of energy investment, future conservation programmes should consider releasing founder individuals in family groups into patches of optimal

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nesting habitat ideally interspersed at distances not exceeding 300–500 m, thereby encouraging shelter establishment, maintaining group structure and limiting panic dispersal.

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AUTHOR CONTRIBUTIONS

Isabelle Onley: Conceptualization (equal); Data curation (equal); Funding acquisition (lead); Investigation (lead); Methodology (lead); Writing-original draft (lead). **Jeremy Austin:** Conceptualization (equal); Data curation (equal); Investigation (supporting); Supervision (lead); Writing-review & editing (supporting). **Kieren J Mitchell:** Investigation (supporting); Methodology (supporting); Writing-review & editing (supporting). **Katherine Moseby:** Resources (lead); Supervision (supporting); Validation (lead); Writing-review & editing (supporting).

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

FUNDING

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ETHICAL APPROVAL

Live animal trapping and sampling at Arid Recovery Reserve was conducted under South Australian Wildlife Ethics Committee permit numbers 27/98, 4/99, 22/99, 2/2000, 19/2000 and 18/2000.

CODE AVAILABILITY

Code used in sex assignment can be found on Dr Graham Gower's GitHub repository https://github.com/grahamgower/sexassign. Code used in the kinship analysis can be found on Isabelle Onley's GitHub repository https://github.com/ionley/sticknestratdispersal.

DATA AVAILABILITY STATEMENT

SNP data set and trapping metadata used in the kinship analysis can be found on Isabelle Onley's GitHub repository https://github.com/ionley/sticknestratdispersal.

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SUPPORTING INFORMATION

Additional supporting information may/can be found online in the supporting information tab for this article.

Appendix S1. Nest site capture data used in kinship and nest relatedness analysis (first adult capture) of greater stick-nest rats (*Leporillus conditor*).

Appendix S2. Probability of full and half sibling dyads of greater stick-nest rats (*Leporillus conditor*) as determined by COLONY run.

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Chapter 5

The importance of alternative heat refuges for a nest-building rodent translocated to the arid zone

Statement of Authorship

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Principal Author

Name of Principal Author (Candidate)	Isabelle Onley		
Contribution to the Paper	Isabelle collected data, developed the methodology, analysed the data, drafted the manuscript and acted as corresponding author.		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Katherine Tuft		
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Name of Co-Author	Katherine Moseby
Contribution to the Paper	Katherine assisted with developing the methodology, contributed ideas and contributed to the manuscript.

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Name of Co-Author	Jeremy Austin		
Contribution to the Paper	Jeremy assisted with experimental design, development of ideas and edited the manuscript.		
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The importance of alternative heat refuges for a nest-building rodent reintroduced to Australia's arid zone

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Isabelle Onley: Funding acquisition, conceptualisation, methodology, formal analysis, investigation, data curation, visualisation, writing – original draft. **Katherine Tuft:** Conceptualisation, methodology, validation, writing – review & editing. **Jeremy Austin:** Conceptualisation, methodology, validation, writing – review & editing, supervision. **Katherine Moseby:** Conceptualisation, methodology, validation, writing – review & editing, supervision.

Highlights

- Understanding thermal properties of refugia is important under climate change
- Greater stick-nest rats build nests under shrubs but also in burrows and rocky ledges
- Rocky substrates provide better thermal buffering in extreme climates
- Alternative heat refuges may need to be provided for nesting species in the future

Abstract

Effective heat refuges are of increasing importance for nesting species under climate change, particularly in the arid zone, with heatwaves predicted to become more frequent and intense. The greater stick-nest rat shelters in nests built beneath vegetation, under rocky outcrops and in the burrows of other species. This study aimed to determine whether rocky substrates

provide a more thermally buffered environment than vegetation, and whether this is more important in the arid zone than mesic environments. We compared internal temperatures of nests beneath different substrates in two environments – arid and coastal – to quantify their thermal buffering capabilities. We found that rocky substrates typically provided a more stable microclimate than nests beneath vegetation, particularly in the arid environment during extreme temperatures and heatwaves. However, above ground nests within large shrubs provided a warmer microclimate during winter which may assist with thermoregulation during breeding. We suggest that optimum habitat for greater stick-nest rats may include areas where large shrubs and rocky warrens are both present. Future management strategies of nesting species vulnerable to climate change should ensure that rocky shelters, either natural or artificial, are available. Further, reintroducing ground nesting mammals in tandem with burrowing species will also increase the prevalence of warrens as an alternative heat refuge.

Key words

Refugia, climate change, translocation, ecology, thermoregulation, nesting rodent

Declarations

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Conflicts of interest

The authors declare no conflicts of interest.

Ethics approval

All monitoring was conducted under the University of Adelaide Animal Ethics Committee, Approval Number S-2019-074, subject to the requirements of the Animal Welfare Act (SA), the Australian Code for the care and use of animals for scientific purposes and other relevant animal welfare Codes of Practice. Scientific Research Permit Number Q26904 was granted by the Department of Environment and Water, Government of South Australia.

Introduction

Periods of extreme heat have been linked to recent mass mortality events in several species, such as flying foxes (*Pteropus* spp.), wrinkle-lipped bats (*Chaerephon plicatus*), blue mussels (*Mytilus edulis*) and Carnaby's Black Cockatoo (*Calyptorhynchus latirostris*) (Welbergen et al. 2008; Saunders et al. 2011; McKechnie et al. 2012; Pruvot et al. 2019; Ratnayake et al. 2019; Seuront et al. 2019). Heatwaves are predicted to become more frequent and intense under climate change, with potentially disastrous consequences for biodiversity on both regional and global scales (Perkins-Kirkpatrick and Gibson 2017). This is of particular concern for arid species that already experience high ambient temperatures. During times of heat stress in arid environments, thermal refugia such as nests, tree hollows, burrows and vegetation are a vital resource, and many species utilise these microhabitats to provide a stable thermal environment for themselves and their young (Pike and Mitchell 2013). Burrowing behaviour is common among arid vertebrates and invertebrates, and warrens serve a dual purpose as shelter from both climate extremes and predators. A variety of other species also utilise these underground shelters, particularly during the summer months (Kinlaw 1999; Read et al. 2008; Dawson et al. 2019).

One species known to build nests for protection against weather and predators is the greater stick-nest rat (*Leporillus conditor*), a murid rodent once found across the arid and semi-arid regions of southern Australia. Stick-nest rats are characterised by their large, resilient nest structures that they build as a central refuge (Robinson 1975). These nests are typically comprised of sticks bonded together by the rats' sticky urine, that crystallises to form amberat (Copley 1999a). The structure of the nests are fairly heterogeneous, with a series of tunnels leading to a central chamber lined with soft vegetation and occasionally feathers (Robinson 1975). The nests are communal, and are inhabited by successive matrilinial generations (Copley 1999a; Onley et al. 2021). They are typically built within shrubs or under low

hanging trees or rocky breakaways, measuring from less than 50 cm in height and 80 cm in diameter to up to 1 m high and 2 m in diameter (Robinson 1975; Moseby and Bice 2004). Early European explorers noted that stick-nest rat nests were so robust that they could not be pulled apart manually (Le Souef 1922). Despite the species' use of nests as a predator avoidance strategy, the introduction of rabbits, cats and foxes by Europeans, in addition to pastoralism, resulted in the mainland extinction of the greater stick-nest rat (Copley 1999a). Conservation efforts for the species have included a number of translocations from its remaining extant population on the Franklin Islands, including a reintroduction to Arid Recovery Reserve, near Roxby Downs, South Australia (Moseby and Bice 2004; Moseby et al. 2011). While some other reintroductions of greater stick-nest rats have failed (Short et al. 2018), the population at Arid Recovery has remained viable for twenty years; however, high mortality rates have been observed during the hot summer months (Bolton and Moseby 2004).

The thermal insulation provided by nests is of particular importance to species living in extreme environments, such as the arid zone of Australia, where diurnal temperatures can fluctuate by up to 35°C and water is scarce (National Climate Centre 2008). Extreme heat stress, or hyperthermia, can have a number of detrimental effects on rodents, including rapid water loss, hyperventilation, loss of coordination and, in extreme cases, organ damage, neurological complications, and death (Haveman et al. 2005; Leon et al. 2010; Quinn et al. 2014). It is therefore important to develop our understanding of heat refuges for the greater stick-nest rat, particularly under the projected temperature increases under climate change. The thermal properties of stick-nest rat nests have not been extensively studied, and other rodents that exhibit similar nest-building behaviours have been the subject of limited research. A laboratory study on the nest material preferences of the European ground squirrel (*Spermophilus citellus*) found that the insulation properties of nests built from fresh grass were superior to those built from dry grass, likely due to the flexibility of fresh nesting material allowing for a thicker, less permeable structure (Gedeon et al. 2010). Another study on woodwool nests built by short-tailed field voles (*Microtus agrestis*) in captivity determined that the most important factor influencing nest insulation was found to be wall thickness (Redman et al. 1999). Temperatures inside nest structures built by pack rats (*Neotoma spp*.) have been recorded up to 10°C below ambient air temperature in the warmer months, when outdoor temperatures reached up to 37°C (Whitford and Steinberger 2010).

Comparable studies have also recorded the internal nest temperatures of ground-nesting birds; the nests of bobwhites in Oklahoma were monitored during periods of extreme diurnal temperature fluctuations, and it was observed that when ambient temperatures reached ≥39°C, mortality rates were lower in nests that remained, on average, 6°C cooler than other – however, no structural variables that may be linked to the thermoregulatory properties of nests were determined in this study (Carroll et al. 2015).

Given that the thermoregulatory capacity of nesting sites in many taxa (including birds, mammals, and invertebrates) has been linked to reproductive success and mortality in extreme environments (Flaquer et al. 2014; Michielsen et al. 2019), and that heat stress has been recorded as a cause of mortality in greater stick-nest rats in the summer months (Bolton and Moseby 2004), nest insulation is likely to be an important consideration for greater stick-nest rats translocated to desert areas such as Arid Recovery. Further, greater stick-nest rats translocated to the Arid Recovery Reserve would experience a desert climate and average temperatures several degrees higher than those experienced by the founding populations located in coastal areas (Australian Bureau of Meteorology 2021). If greater stick-nest rats have developed lower heat tolerance thresholds in response to cooler, coastal environments, this may increase the importance of thermally buffered refugia when translocated to arid regions. We monitored the internal temperatures of greater stick-nest rat nests located in rocky shelters (ledges or warrens) and under vegetation at both the mesic founder site (Reevesby Island) and the arid translocation site (Arid Recovery) to inform shelter suitability during periods of heat stress. We expect that i) nests built in or under rock will be more thermally buffered than nests built under vegetation at both sites and ii) nests on Reevesby Island will be exposed to much lower temperatures than those at Arid Recovery. In good conditions, greater stick-nest rats have been observed to breed year-round, but high summer temperatures may limit breeding to annual events in cooler months in semi-arid and arid areas (Copley, 1988; Moseby and Bice 2004). Given that females demonstrate a high degree of nest fidelity (Onley et al. 2021), understanding the conditions inside nests and how they may impact breeding success will be an important outcome for the ongoing management of the species. In addition, it may assist in identifying optimum release sites for future translocation efforts and predicted persistence under the increasing temperatures and projected weather extremes of anthropogenic climate change.

Methods

Study Sites

Reevesby Island

Reevesby Island is a 344 ha island located 20 km south east of Tumby Bay in the Spencer Gulf, South Australia. It consists of low dunes and sandplain, limestone outcrops, open shrublands, chenopods and grassland, and the climate is characterised by temperate, dry summers (mean maximum temperature 26.3 ºC in January), with higher rainfall in winter (mean monthly rainfall 60.3 mm in June as opposed to 15.0 mm in January) (annual average rainfall 388.5 mm) (Australian Bureau of Meteorology 2021). It was used for grazing livestock until the mid-1970's, and since this time feral predators, including cats (*Felis catus*) have been eradicated. The introduced plant African Boxthorn (*Lycium ferocissimum*) has spread across the island (Pedler and Copley 1993). The lack of feral predators, as well as skeletal evidence that greater stick-nest rats once inhabited the island, made Reevesby Island a suitable release site for the species, and a translocation program began in 1990 (Copley 1988; Pedler and Copley 1993). A population was successfully established, with numbers varying between 600-5000, and greater stick-nest rats utilised the boxthorn for nesting, as well as the rocky outcrops along the shoreline (Pedler and Copley 1993; Copley 1999b; Short et al. 2019).

Arid Recovery

Arid Recovery is a fenced 123 km^2 reserve located 20 km north of Roxby Downs, South Australia. It has an arid climate, with hot summers (mean maximum temperature 37.1 ºC in January) and unpredictable rainfall (annual mean rainfall is 139 mm, but has been recorded as low as 35.2 mm and as high as 320.2 mm in the last twenty years) (Australian Bureau of Meteorology 2021). Habitats within the Arid Recovery Reserve include longitudinal dunes and chenopod swales (Moseby and Bice 2004). Following a trial release in 1998, greater stick-nest rats were translocated into a 14 km^2 paddock within the reserve and subsequently monitored (Moseby et al. 2011). Greater stick-nest rats at Arid Recovery build stick nests beneath shrubs and bushes (such as Umbrella Wattle (*Acacia oswaldii*) and Narrow-leafed Hopbush (*Dodonaea viscosa*)), but have also been observed to shelter in the warrens of

306 burrowing bettongs (*Bettongia lesueur*) (Bolton and Moseby 2004; Moseby and Bice 2004).
307 Burrowing bettongs were reintroduced to the reserve in 1999 and 2000, and have created
308 many warren systems beneath the calcrete layer that provide a thermally buffered
309 environment (Moseby et al. 2011, 2018). Recently, managers at Arid Recovery constructed
310 two hollow rock piles as an alternative thermal refuge for greater stick-nest rats (H.
311 McGregor Pers Comm. 2021). These rock piles were constructed using calcrete blocks and
312 sedimentary stones from within the reserve, with metal pallet frames to create an open space
313 beneath the rocks. The rocks were piled tightly on top of the frames in layers to reach heights
314 of approximately 80 cm, with diameters of roughly 1.5 m. A small gap at the base of each
315 pile was left open to allow greater stick-nest rats to enter the cavity beneath.
316
317 **Data collection**
318
319 10 HOBO MX2301A Temperature/Relative Humidity (RH) data loggers (accuracy ±0.2°C,
320 ±2.5%RH) were deployed at each study location (Reevesby Island and Arid Recovery, 20
321 loggers total), across varying habitat types and nest locations (Figure 1). Nest surveys were
322 conducted, and indicators of activity such as counts of fresh scats and tracks were noted,
323 along with observations of vegetation cover type and density. Loggers recorded temperature
324 and relative humidity at 1 hour intervals. At Reevesby Island, all nests showed some degree
325 of activity (i.e. fresh scats present). At Arid Recovery, not all nests were active at the time of
326 recording but had been used in recent years when the greater stick-nest rat population was
327 higher. At Arid Recovery, loggers were placed on the soil surface inside nests under
328 cottonbush (Maireana aphylla) shrubs (n=2) and Acacia (Acacia ligulata)/Dodonea (Dodonea
329 viscosa) shrubs (n=3), inside burrowing bettong burrows known to be used by greater stick-
330 nest rats (n=3) and inside one artificial rock pile (n=1) (Supplementary Information 1). Given
331 the homogeneity of the landscape, one ambient logger was installed at Arid Recovery ~2 m
332 from the ground inside a ventilated equipment shelter that provided sufficient cover from
333 solar radiation and wind to record true ambient temperatures. At Reevesby Island loggers
334 were placed on the soil surface inside nests underneath boxthorn bushes (n=4) and nests built
335 underneath limestone ledges and outcrops (n=4) (Supplementary Information 1). As the two
336 boxthorn and limestone outcrops habitat types at Reevesby Island varied considerably (inland
337 versus coastal, respectively), an ambient logger was installed in each habitat type. For all
338 analyses comparing internal nest temperature to ambient in the Reevesby Island dataset, the

appropriate ambient temperature was used (i.e. boxthorn nest temperatures were compared to ambient temperature in boxthorn habitat only). The ambient loggers were placed in slatted PVC housing that allowed for airflow but protected the loggers from solar radiation, excess moisture and high winds and were set up in a shaded, south-facing location and installed approximately 30 cm above ground. Infrastructure at Reevesby Island was not available to suspend the ambient logger any higher than 30cm off the ground, without risking exposure to sun and wind that would compromise the results. At Arid Recovery, a shelter was available that would eliminate these risks, but it required the logger to be installed at a greater height where the roof would shelter it from the sun.

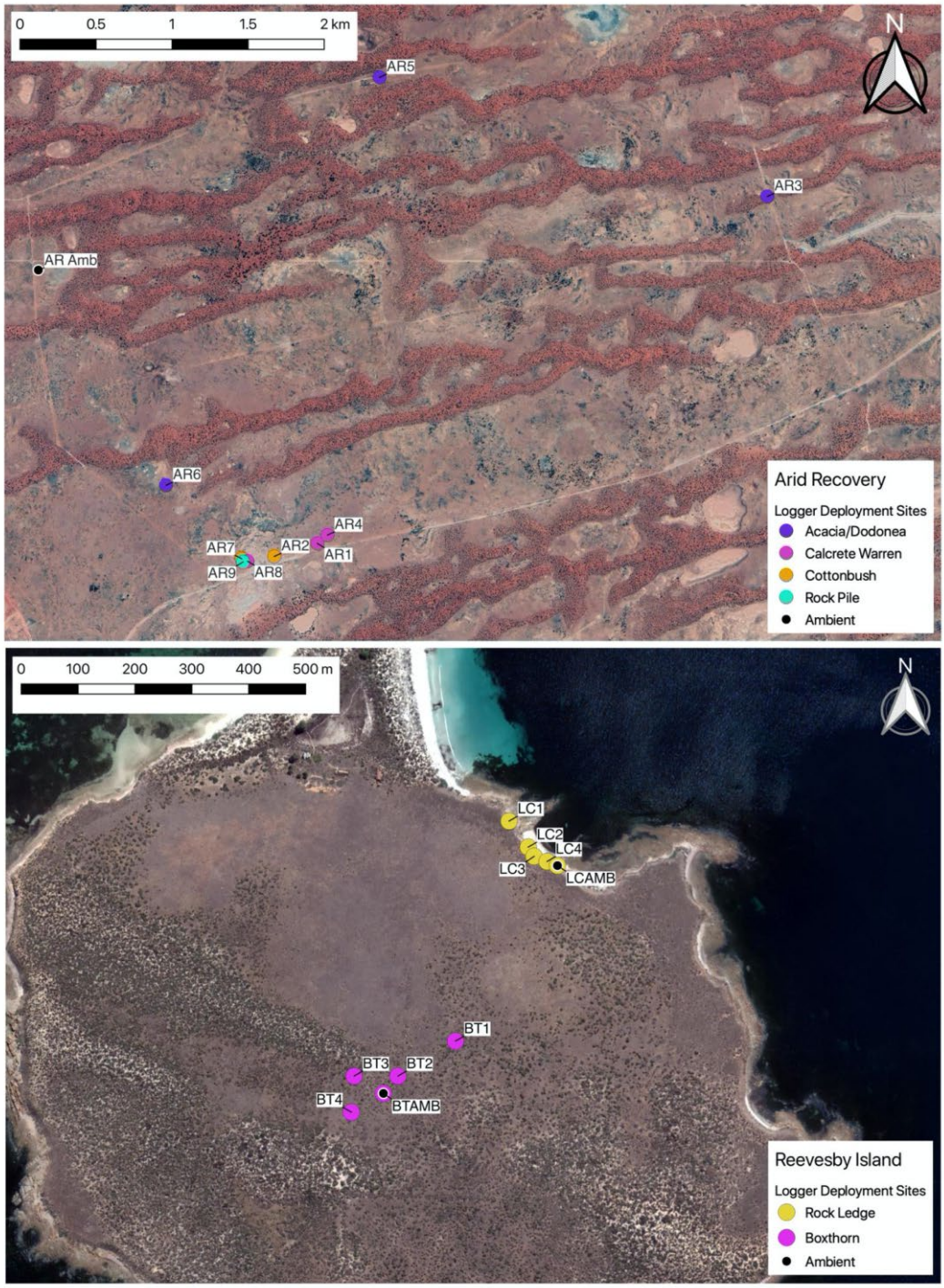


Figure 1: Logger locations at Arid Recovery and Reevesby Island, coloured by nest type.

Data loggers deployed inside nests (n=13) were attached to 2 mm wire to allow for deployment and retrieval with minimal disturbance to the nest and secured to stakes or nearby vegetation. Loggers were installed via rat entry holes to a minimum of 30 cm towards the centre of the nest, at the point of easiest entry that would require minimal disturbance to the nest. In warrens, loggers were deployed to a depth of 30-40cm into the warren and

tethered externally to a stake using wire. GPS coordinates of each nest was recorded. Loggers remained in the field for a period of twelve months (January 2020 – January 2021, summer to summer) at Reevesby Island, and fourteen months (January 2020 – March 2021, summer to autumn) at Arid Recovery to encompass seasonal climatic variation. Upon retrieval of loggers, nest surveys were repeated to determine whether activity patterns of nests had changed. Data from loggers were downloaded onto a mobile device using the Onset HOBO application and exported as a comma delimited file for subsequent analysis.

Data analysis

All data analysis was conducted using the software package R (v3.5.3). To visualise the data, the package "ggplot2" (v3.3.3) was used. Descriptive statistics for loggers at each nest at each site were then calculated using the "dplyr" package (v1.0.2), including mean temperature and standard deviations, as well as minimum and maximum temperatures and daily average temperature range (hereafter "mean range"). As a reflection of the possible amount of time greater stick-nest rats would spend under heat stress sheltering in each nest type, the number of days during the observation period at which internal temperatures reached 40°C or higher were calculated (Liu et al. 2011; Chauhan et al. 2017; Cooper et al. 2020), as well as the number of times internal nest temperatures reached or exceeded 40°C for three or more consecutive days (hereafter "heatwaves") (consistent with the Bureau of Meteorology's (BOM) definition of a heatwave (Bureau of Meteorology 2018)). No specific data on heat tolerance threshold is available for greater stick-nest rats – however, based on the increased mortality observed by Collins (1973) in bush rats (*Rattus fuscipes*) at temperatures exceeding 40°C, we chose 40°C as the threshold for heat stress in this study.

To determine the thermal buffering properties of nests and to capture seasonal variation, we ran linear regression models of each nest with ambient temperature as the independent predictor variable and extracted residuals using the "stats" package (v4.0.2) to determine how closely the internal temperature of the nests matched ambient temperature. Mean daily (7:00-19:00) and nightly (19:00-7:00) residual values were calculated and plotted for each nest type. Negative and positive residual values reflect internal nest temperatures below or above ambient, respectively, with 0 indicating no difference.

Finally, given the number of external factors that may influence internal nest temperature (i.e. vegetation cover, orientation, nest wall thickness), we then conducted linear mixed effects (LME) models using the "nlme" package (v3.1-152) (Pinheiro et al. 2012), comparing ambient temperature variables to all nests at each study site as a function of nest type (i.e. vegetative or rocky), with nest site as a random component to determine whether nest type alone significantly influenced internal temperature.

Results

Five out of twenty of the loggers (four at Reevesby Island and one at Arid Recovery) failed during deployment due to internal water damage, despite the loggers being sold as waterproof. The loggers at Reevesby Island failed in April 2020, August 2020, October 2020, and December 2020 respectively, while the logger at Arid Recovery failed in July 2020. Data collected up until the point of failure were retrieved from all five loggers and included in the analysis, following inspection of the data to ensure that values were not compromised by damage to the sensors. However, due to the high number of logger failures at Reevesby Island and subsequent reduction of the dataset, the recording period used in the analysis for this site was reduced from 12 months to six months, from 22/01/2020 to 30/06/2020 including late summer, autumn and winter.

Descriptive Statistics

Ambient temperature had the widest range at Arid Recovery, with ambient temperature reaching a maximum of 46. 4°C and a minimum of -1.6°C. In comparison, Reevesby Island recorded a maximum of 34.3°C and a minimum of 3.9°C. Temperatures on Reevesby Island did not exceed 40°C during the six month period used in this analysis, nor were temperatures recorded above this threshold during the full twelve month period by the loggers that remained active. However, during the 15 month recording period at Arid Recovery, ambient temperature reached or exceeded 40°C on 19 days, with heatwaves (three or more consecutive days over 40°C) recorded on three separate occasions (Figure 2). Temperatures within warrens and the rock pile at Arid Recovery appeared more stable than nests beneath vegetation, with no heatwave events, fewer days over 40°C and a less thermal variation than other nest types (i.e. lower daily maximum and higher daily minimum) (Figures 2 and 3). A similar trend was apparent at Reevesby Island, where temperatures beneath rock ledges

appeared more stable than those in boxthorn nests, with the exception of LC4 (Figures 2 and 3). Mean temperatures showed little variation between nest location or type at Arid Recovery in general (mean temperatures across all nest sites ranged from 21.3°C to 23.1°C), but nests under rock ledges at Reevesby Island had higher mean daily temperature than boxthorn nests and ambient (18.9-20.0°C compared to 17.7-19.9°C, respectively). A full table of descriptive statistics is available in Supplementary Information 2.

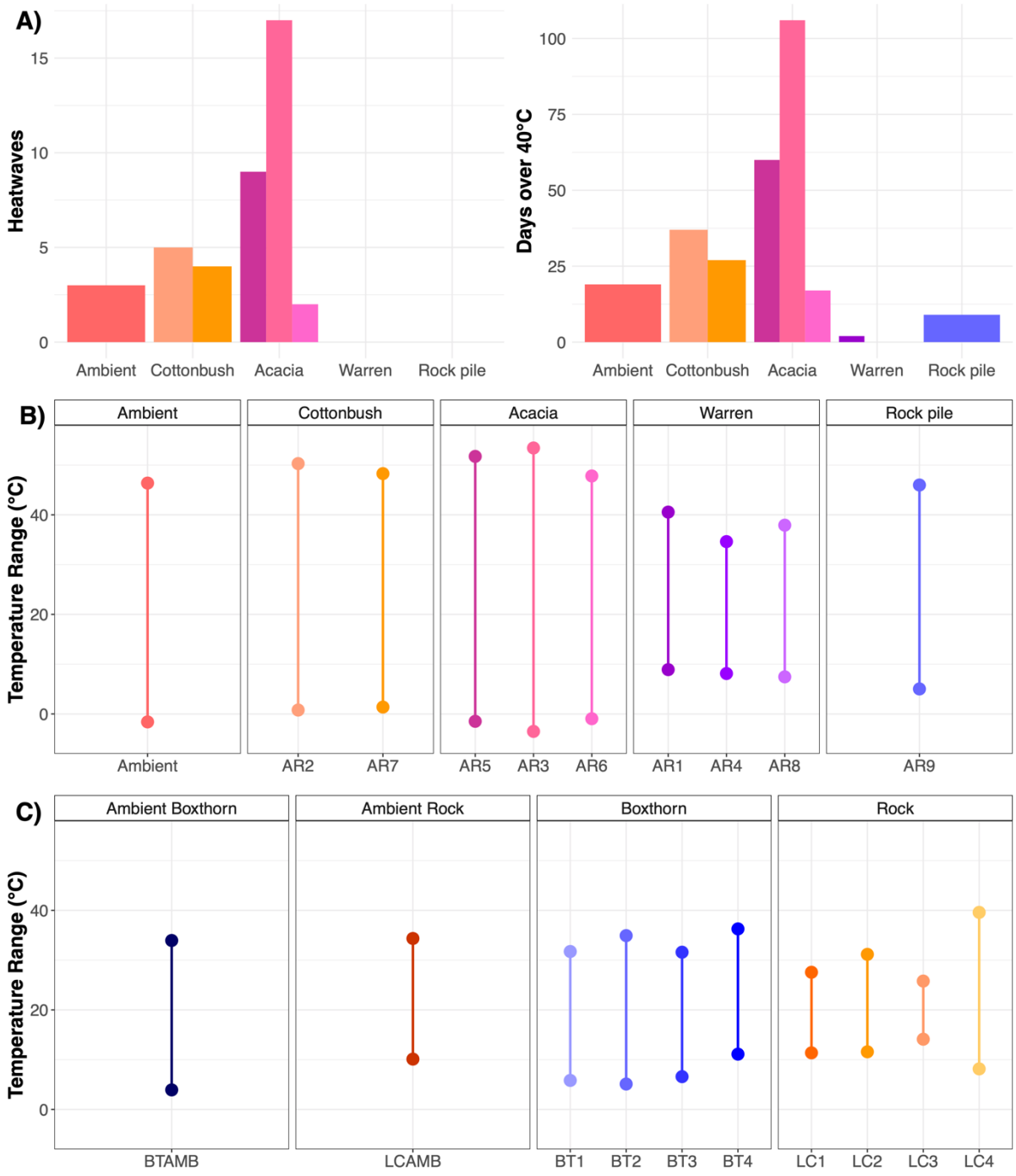


Figure 2: Comparison of A) heatwaves and number of days exceeding 40ºC for each nest by type at Arid Recovery, and overall temperature range for each nest by type at B) Arid Recovery and C) Reevesby Island.

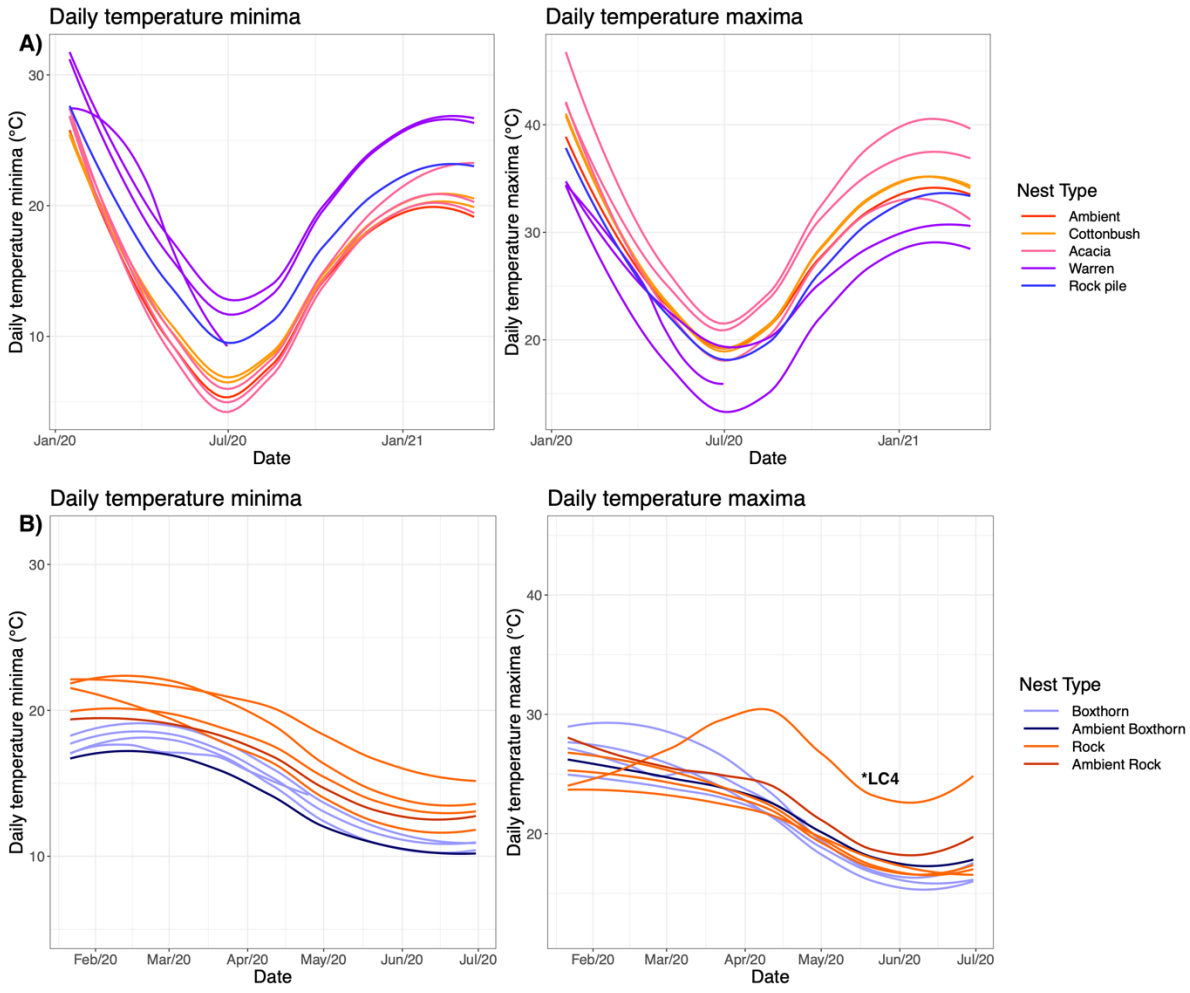
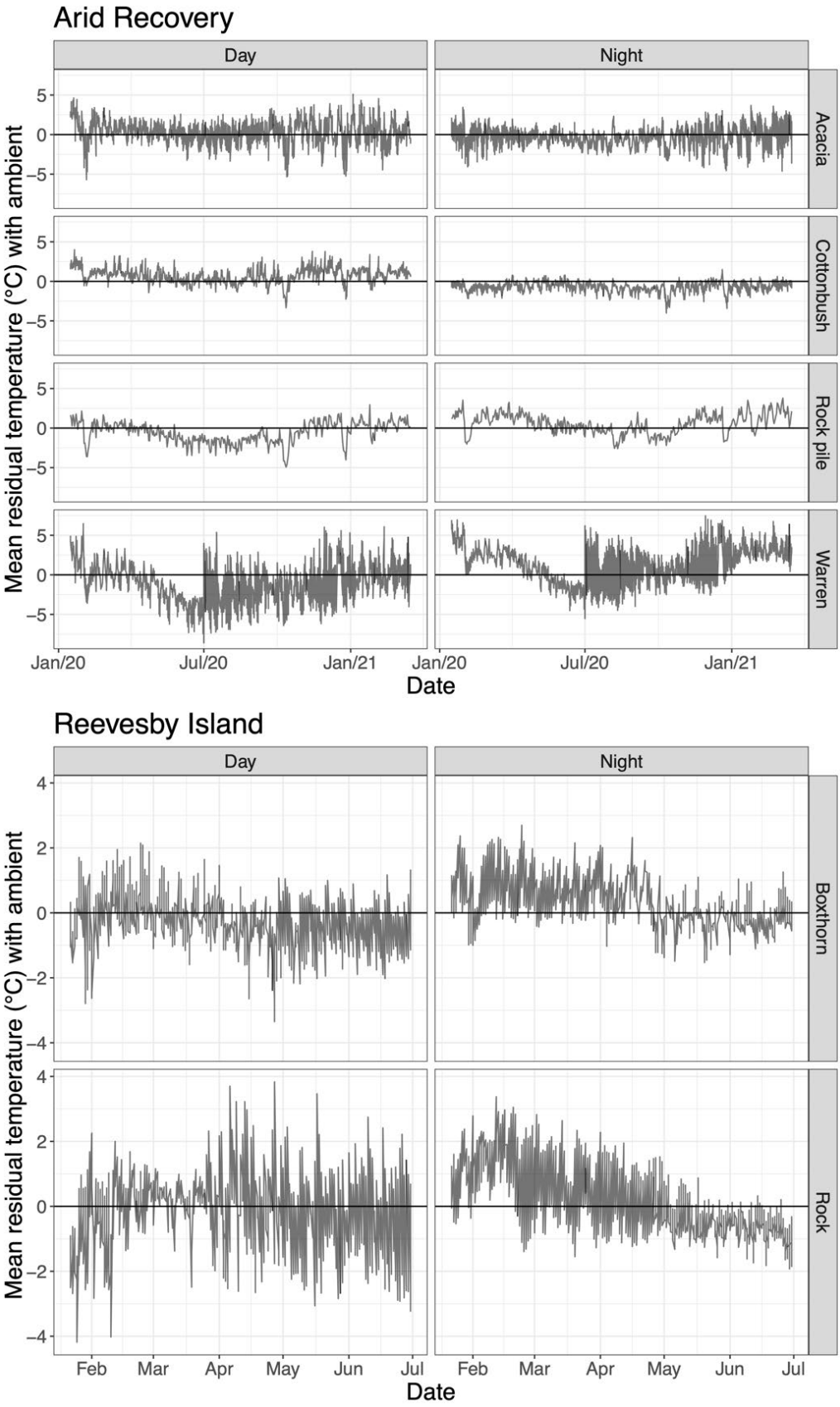


Figure 3: Comparison of smoothed daily minimum and maximum temperatures recorded in each nest by type at A) Arid Recovery and B) Reevesby Island. One rock nest on Reevesby Island, LC4, experienced higher temperatures than other nests.

Residual Internal Temperature vs Ambient

Comparison of residual internal nest temperature with ambient revealed not only seasonal fluctuations, but variation in thermal buffering capacity between nest types. At Arid Recovery, both warrens and rock piles were generally cooler than ambient during the day (mean residual values -1.11 and -0.58 respectively), with the exception of the late summer months, while warmer than ambient at night (mean residual values 1.31 and 0.58 respectively), indicating good thermal buffering most of the year (Figure 4). Conversely, acacia and cottonbush nests were typically warmer than ambient during the day (mean

451 residual values 0.40 and 0.73 respectively), and cooler at night (mean residual values -0.4 and
452 0.73 respectively). At Reevesby Island, residuals for both nest types were similar (mean
453 daytime residual for boxthorn = -0.37 and rock = -0.35, mean night time residual for
454 boxthorn = 0.38 and rock = 0.35), but with some seasonal variation (Figure 4).
455
456
457



458 **Figure 4:** Average daily and nightly residual temperatures of each nest type in comparison to
459 respective ambient temperature (y intercept = 0) at Arid Recovery and Reevesby Island.
460 0=logger is same temperature as ambient.

Mixed Effects Models

LME models revealed no significant effect of nest type on internal nest temperature at Reevesby Island. At Arid Recovery, while variation between the nest types was evident, a statistically significant relationship between internal temperature and nest type was only evident for warrens when evaluating minimum (p-value <0.0001), maximum (p-value 0.038) and daily temperature range (p-value 0.013), and the rock pile in relation to minimum temperature (p-value 0.005). All model outputs are detailed in Supplementary Information 3. The lack of statistically significant relationships between certain nest types and temperature variables when clear variation is evident (e.g. the number of heatwave events in the rock pile in comparison to nests under Acacia) may be caused by the low sample size, particularly for the rock pile.

Discussion

Monitoring of internal temperatures of greater stick-nest rat nests with different nest types at two locations revealed considerable variation in nest type thermal buffering at Arid Recovery, where temperatures within rock and warren shelters were generally more stable than nests located beneath vegetation. A similar trend was observed at Reevesby Island, albeit to a lesser extent.

As hypothesised, internal temperatures of nests at Reevesby Island were lower and less extreme than those recorded at Arid Recovery. Internal temperatures of nests built beneath rock ledges at Reevesby Island proved to be less variable than those inside boxthorn shrubs, with the exception of nest LC4. This nest recorded higher temperatures than all other rock ledge nests, possibly due to solar radiation or radiant body heat from nesting greater stick-nest rats. Overall, rock ledges were more thermally buffered than boxthorn nests. However, LME models determined that the rocky shelters did not have a significant effect on temperature variables at Reevesby Island, possibly due to the absence of extreme temperatures as observed at Arid Recovery. Residual nightly temperatures in rock ledges were lower than boxthorn nests in comparison to ambient temperature during the winter months, indicating poor thermal buffering during this time. This may have been the result of

coastal weather patterns, as the rock ledges monitored in this study were all located on the eastern coastline of the island where they would have been more exposed to coastal winds and sheltered from daily maximum solar radiation, occurring in the afternoon when the sun is in the west (Guan et al. 2013). Internal temperatures of boxthorn nests remained relatively similar to ambient throughout the year, although maximum temperatures were slightly higher in summer and lower in winter, an indication of poor thermal buffering. Again, boxthorn as a nest cover was not determined to have a significant effect on internal temperature by LME models. This suggests that in mesic environments such as Reevesby Island, a variety of nesting habitats may be important across the seasons – in this case, rock ledges may provide good thermal buffering in summer, but boxthorn nests are more favourable in winter.

Although there was no significant variance in mean internal temperature between refuge types at Arid Recovery, clear differences between treatments emerged when analysing nest temperatures during thermal extremes. Bettong warrens and the artificial rock pile exhibited good thermal buffering, with higher daily minimum temperatures, lower maximum temperatures, fewer days over 40°C and no heatwaves in comparison to nests beneath vegetation or ambient temperature, a highly advantageous feature for a nocturnal animal in an extreme desert environment. Although the rock pile did experience a higher number of days over 40°C than warrens, these temperatures were not sustained long enough to be considered a heatwave. LME models showed that nest type had a significant effect on the maximum and minimum daily temperatures of warrens, as well as the daily range. For the rock pile, nest type was significantly associated with minimum daily temperature only, despite this nest also having lower values for maximum temperature and daily range than nests beneath vegetation. This lack of correlation may be the result of low sample size and statistical power for this nest type, which can mask relationships during analysis (Zuur et al. 2009).

Of the nests built beneath vegetation, cottonbush and acacia appeared relatively similar in terms of poor thermal buffering. Both experienced temperatures exceeding 40°C on more days than the ambient temperature in all but one nest, likely due to the exposure of the nests to solar radiation or radiant heat from the ground). Nests beneath both shrub species also experienced more heatwaves, wherein temperatures exceeded 40°C on three or more consecutive days. LMEs did not determine a significant effect of nest type on internal temperature in these nests, suggesting that thermal properties may be influenced by other factors such as nest thickness, size and construction material (Redman et al. 1999; Gedeon et

al. 2010). However, studies on communal nests built by sociable weavers (*Philetairus socius*) found no effect of nest volume on thermoregulatory benefits (van Dijk et al. 2013). Comparison of results from the two study sites suggest that thermal buffering of nests beneath vegetation may not be effective in extreme climates, like the desert environment of Arid Recovery Reserve. However, nests within large shrubs may be important as protection from predators during breeding, as well as providing passive warming of greater stick-nest rats and their young during the cooler winter months. Thus, habitat where large shrubs and rocky warrens are both present may provide the optimum combination for thermoregulation of greater stick-nest rats in arid environments. It should be noted, however, that loggers recording in nests beneath vegetation may not have been placed in the exact location that the greater stick-nest rats were inhabiting, which is a limitation of this study – greater stick-nest rat nests have been recorded as having many chambers at varying heights and depths within the nest (Arid Recovery, unpublished data). Additionally, the presence or absence of greater stick-nest rats inside the nests may also have influenced the temperatures recorded.

Climate refugia are a valuable resource for species living in Australia's arid zone. Many species construct warren and burrow systems, scrapes or nests to act as an environmental buffer (Kinlaw 1999; Riley et al. 2021). These, in turn, create refuges for other sympatric organisms (Read et al. 2008). Reptiles, for example, have been observed to use termite mounds in the Pilbara of Western Australia as shelter from the hot sun and cold nights, as well as predators (Thompson and Thompson 2015). A study on the sheltering behaviour of the sandhill dunnart (*Sminthopsis psammophila*) recently found a preference for constructing burrows under Triodia hummocks rather than sheltering under hummocks alone (Riley et al. 2021). Although the greater stick-nest rat is characterised by its nest building behaviours, accounts of the species prior to its mainland extinction state that greater stick-nest rats in arid areas often built their nests over existing warrens dug by European rabbits (*Oryctolagus cuniculus*), and likely by burrowing bettongs prior to their mainland extinction (Le Souef 1922; Troughton and Wright 1923). Further, greater stick-nest rats on the Franklin Islands have been observed building nests over, and inside, penguin burrows (Troughton and Wright 1923; Robinson 1975). This behaviour has been supported by studies of the greater stick-nest rat population at Arid Recovery, where nest-building over bettong warrens has been observed (Bolton and Moseby 2004; Moseby and Bice 2004; Moseby et al. 2014). This knowledge, combined with the present study, suggests that underground warrens constructed by other

563 species provide important heat refuges for greater stick-nest rats in an arid environment.
564 Further, we present evidence that man-made rock structures provide a good alternative to
565 naturally occurring rocky outcrops. While thermal buffering in the rock pile was not quite as
566 effective as warrens, the rock pile was relatively stable during periods of extreme heat and
567 resistant to heatwaves, an important consideration given the mass wildlife mortality events
568 associated with such climatic events (Ratnayake et al. 2019). Because the loss of vegetated
569 microhabitats as a result of climate change is likely to have a strong impact on arid species
570 (Grimm-Seyfarth et al. 2017), this finding is of significance for future management of greater
571 stick-nest rats and other nesting, arid-dwelling species. As most studies of artificial refuges
572 focus on arboreal species, and only a small percentage measure the thermal properties of
573 these refuges (Cowan et al. 2021), this research is a timely contribution to the literature
574 surrounding heat refuges.
575
576 Heat refuges are becoming an increasingly important resource for managers to consider when
577 planning translocations and population management of threatened species. Managers
578 planning future translocation efforts of greater stick-nest rats to the arid zone or to bioregions
579 predicted to experience highly variable temperatures under climate change, particularly
580 reaching or exceeding 40°C, should ensure that rocky shelters or species that burrow are
581 present in the community so that alternative thermal refuges are made available to greater
582 stick-nest rats in times of heat stress. This will be of particular importance under climate
583 change scenarios, with the number of heatwave days per year predicted to double in certain
584 regions of Australia in the near future (Herold et al. 2018). The ideal habitat for greater stick-
585 nest rats may well be a combination of large shrubs to provide nesting substrate with access
586 to solar warming during winter coupled with burrows in rocky substrate to facilitate thermal
587 buffering in summer. Further research into the thermal properties of burrows in other
588 substrates, such as sand, would also be a valuable contribution to the future management of
589 greater stick-nest rats and other nesting species. If access to rocky outcrops or space in
590 warrens is limited, artificial rock piles present an alternative refuge type. However, the
591 uptake of artificial rock piles by greater stick-nest rats at Arid Recovery has not yet been
592 studied and trials involving optimising the design of artificial rock piles are required.
593
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600

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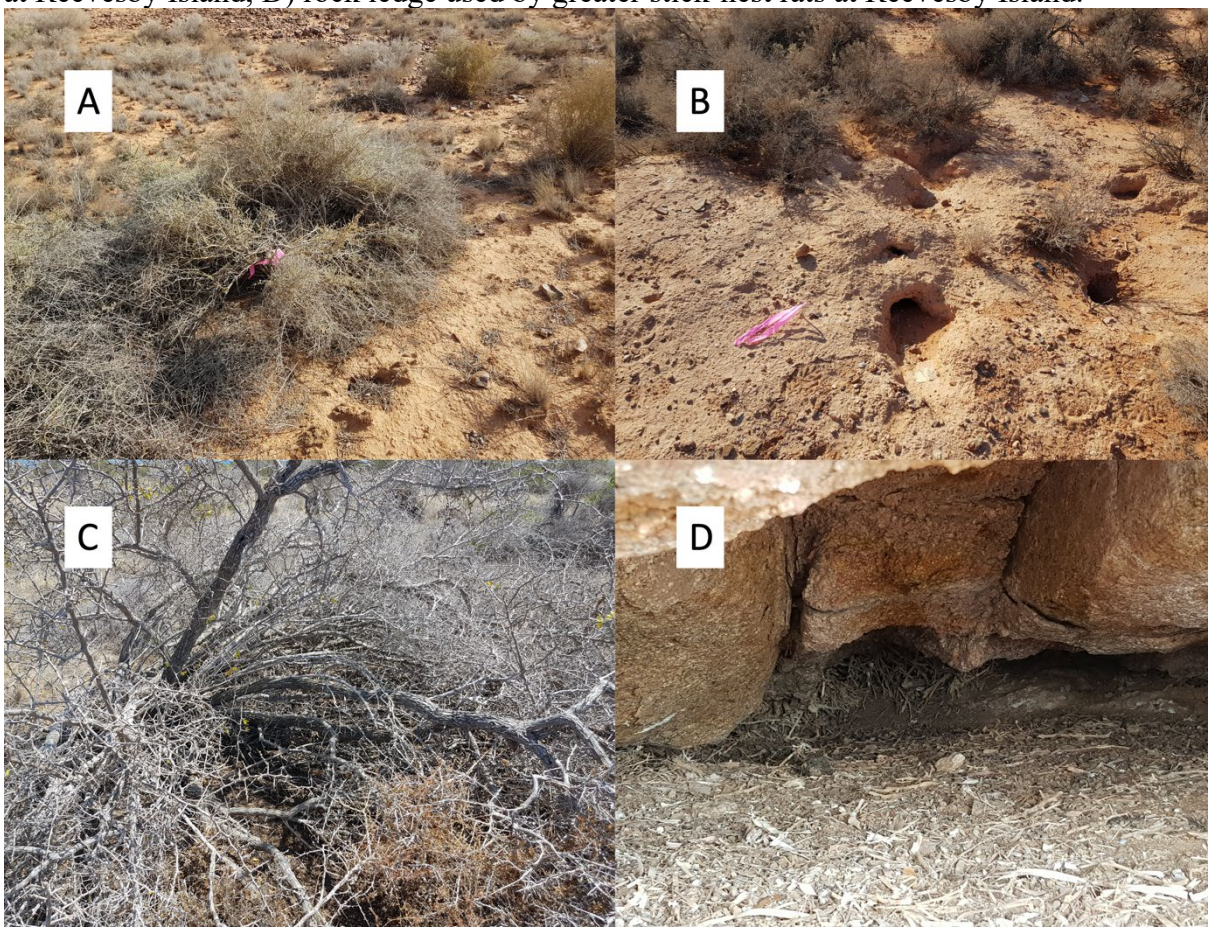
Supplementary Information 1

SI 1: Images of various nest types studied at Arid Recovery and Reevesby Island. Pictured: A) cottonbush over greater stick-nest rat nest at Arid Recovery, B) bettong warren previously used by greater stick-nest rats at Arid Recovery, C) boxthorn over greater stick-nest rat nest at Reevesby Island, D) rock ledge used by greater stick-nest rats at Reevesby Island.



Supplementary Information 2

SI 2: Descriptive statistics of internal nest temperatures per nest at study sites. Ambient loggers were shaded from solar radiation. At Reevesby Island, one ambient logger was installed in each habitat type (boxthorn and rock).

Arid Recovery							
Nest	**Nest Type**	**Mean (°C)±SD**	**Min (°C)**	**Max (°C)**	**Mean Range (°C)**	**Days ≥40°C**	**Heatwaves**
ARAMB	Ambient	21.3±8.45	-1.6	46.4	13.7	19	3
AR3	Acacia	22.3±9.86	-3.5	53.4	18.7	106	17
AR5	Acacia	21.8±9.03	-1.5	51.7	16.0	60	9
AR6	Acacia	21.3±8.09	-1.0	47.8	12.1	17	2
AR2	Cottonbush	21.4±8.48	0.8	50.3	13.8	37	5
AR7	Cottonbush	21.7±8.37	1.4	48.3	13.5	27	4
AR1	Warren	23.1±6.07	8.9	40.5	4.8	2	0
AR4	Warren	21.4±6.25	8.1	34.6	2.2	0	0
AR8	Warren	22.2±6.41	7.4	37.9	4.4	0	0
AR9	Rock pile	22.3±7.31	5.0	46.0	9.4	9	0
Reevesby Island							
Nest	**Nest Type**	**Mean (°C)±SD**	**Min (°C)**	**Max (°C)**	**Mean Range (°C)**	**Days ≥40°C**	**Heatwaves**
BTAMB	Ambient	17.1±4.37	3.9	33.9	7.9	0	0
LCAMB	Ambient	18.9±4.07	10.1	34.3	6.5	0	0
BT1	Boxthorn	17.7±4.05	5.8	31.7	5.1	0	0

BT2	Boxthorn	17.7±5.05	5.1	34.9	8.5	0	0
BT3	Boxthorn	17.4±4.42	6.6	31.6	6.2	0	0
BT4	Boxthorn	19.9±3.61	11.1	36.3	8.1	0	0
LC1	Rock	19.6±3.77	11.4	27.6	2.7	0	0
LC2	Rock	18.9±3.96	11.6	31.2	4.7	0	0
LC3	Rock	20.0±2.74	14.1	25.8	1.4	0	0
LC4	Rock	19.2±4.66	8.1	39.6	9.8	0	0

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762 **Supplementary Information 3**

763

764 **SI 3:** Results of linear mixed effects models for a range of temperature variables at each
765 study site. The relationship between nest type and internal nest temperature in comparison to
766 ambient temperature was tested. Significant p-values are highlighted in bold.

Arid Recovery					
		Estimate	**Standard Error**	**t-value**	**p-value**
Mean Temp (°C)	**(Intercept)**	21.317	0.640	33.289	0.000
	Acacia	0.479	0.739	0.648	0.546
	Cottonbush	0.242	0.784	0.309	0.770
	Rock Pile	1.012	0.906	1.118	0.314
	Warren	0.929	0.739	1.256	0.265
Minimum Temp (°C)	**(Intercept)**	-1.600	0.986	-1.622	0.166
	Acacia	-0.383	1.139	-0.337	0.750
	Cottonbush	2.680	1.208	2.218	0.077
	Rock Pile	6.620	1.395	4.746	**0.005**
	Warren	9.757	1.139	8.566	**0.000**
Maximum Temp (°C)	**(Intercept)**	46.380	2.695	17.212	0.000
	Acacia	4.613	3.112	1.483	0.198
	Cottonbush	2.905	3.300	0.880	0.419
	Rock Pile	-0.390	3.811	-0.102	0.922
	Warren	-8.683	3.112	-2.791	**0.038**
Mean Range (°C)	**(Intercept)**	13.704	2.265	6.050	0.002
	Acacia	1.923	2.615	0.735	0.495
	Cottonbush	-0.056	2.774	-0.020	0.985
	Rock Pile	-4.330	3.203	-1.352	0.234
	Warren	-9.866	2.615	-3.772	**0.013**
Days Over 40 (°C)	**(Intercept)**	19.000	28.336	0.671	0.532
	Acacia	42.000	32.720	1.284	0.256
	Cottonbush	13.000	34.704	0.375	0.723
	Rock Pile	-10.000	40.073	-0.250	0.813
	Warren	-18.333	32.720	-0.560	0.599
Heatwaves	**(Intercept)**	3.000	4.757	0.631	0.556

	Acacia	6.333	5.493	1.153	0.301
	Cottonbush	1.500	5.827	0.257	0.807
	Rock Pile	-3.000	6.728	-0.446	0.674
	Warren	-3.000	5.493	-0.546	0.608
Reevesby Island					
		Value	**Standard Error**	**t-value**	**p-value**
Mean Temp (°C)	**(Intercept)**	18.024	0.671	26.859	0.000
	Boxthorn	0.194	0.822	0.236	0.820
	Rock Ledge	1.411	0.822	1.717	0.130
Minimum Temp (°C)	**(Intercept)**	7.020	2.051	3.423	0.011
	Boxthorn	0.140	2.512	0.056	0.957
	Rock Ledge	4.283	2.512	1.705	0.132
Maximum Temp (°C)	**(Intercept)**	34.145	3.039	11.236	0.000
	Boxthorn	-0.518	3.722	-0.139	0.893
	Rock Ledge	-3.120	3.722	-0.838	0.430
Mean Range (°C)	**(Intercept)**	7.194	1.868	3.851	0.006
	Boxthorn	-0.196	2.288	-0.086	0.934
	Rock Ledge	-2.522	2.288	-1.102	0.307

Chapter 6

Needle in a genomic haystack: searching for signals of selection in a fragmented non-model species

Needle in a genomic haystack: searching for signals of selection in a fragmented non-model species

Abstract

The adaptive potential of threatened species to climate change is of increasing interest to conservation managers. Identifying populations that are well- or maladapted to projected temperature increases may assist with developing adaptive management and breeding programs to encourage resilience. Here I use genotype-environment association (GEA) tests on a translocated population of greater stick-nest rats to determine whether reintroduction to the arid zone has resulted in selection in response to heat stress. While I found evidence of a SNP under selection associated with a heat shock protein in the translocated population, the study was hampered by the lack of reference genome for the species, a high degree of collinearity between environmental variables, and the inconsistent environmental gradient between populations in the dataset. While GEAs can be useful tools when the necessary requirements of the analysis are met, the issues encountered in this study are likely to be faced in many population genetics studies of threatened, bottlenecked species. I therefore highlight the need for a concerted effort towards developing reference genomes for understudied taxa of conservation concern.

Introduction

Current climate change projections, including temperature increases, extreme weather patterns and reduced rainfall (Field et al. 2012; Head et al. 2014; CSIRO and Bureau of Meteorology 2020), imply increasing extinction risk in a broad range of taxa worldwide (Urban 2015). More days with extremely high temperatures are predicted, with longer fire seasons, more time spent in drought coupled with intense periods of heavy rainfall, and rising sea levels (CSIRO and Bureau of Meteorology 2020). Evidence of the impacts of climate change are already being observed, in the form of population declines, selection pressures and phenological and distribution shifts (Hoffmann et al. 2019). Soberingly, in 2016, Australia's first extinction attributed to climate change was recorded; the Bramble Cay melomys (*Melomys rubicola*), a species found only on an island in the Torres Strait, succumbed to habitat loss due to ocean inundation sometime between 2009 and 2011 (Fulton 2017).

Conservation managers are now faced with the challenge of protecting biodiversity under rapidly changing conditions. For many threatened species, adaptation *in situ* to rising temperatures and extreme climatic events is unlikely to keep pace with the speed of environmental change. In this case managers may consider translocation (Burbidge et al. 2011; Thomas 2011) - the facilitated movement of a species from one area to another, with the intention of establishing insurance populations. Alternatively, managers may seek to build adaptive capacity in an existing population via genetic rescue, the introduction of new genetic material via translocation of individuals carrying alleles adapted to projected future climate (Whiteley et al. 2015; Weeks et al. 2017; Hoffmann et al. 2021). While genetic rescue has, to date, predominantly focused on increasing overall genetic diversity and reducing the impacts of inbreeding depression in small populations of threatened species, recent reviews have suggested that it may provide a mechanisms to enhance adaptive response to climate change (Hoffmann et al. 2015, 2021; Prober et al. 2015; Onley et al. 2021). Advances in population genomics allows signals of selection in response to environmental stressors to be identified in a population (Cummins et al. 2019). By identifying individuals or populations that are better adapted to predicted conditions under climate change, managers may soon be able to perform genetic rescue with a specific focus on beneficial alleles, for example, higher thermal tolerance thresholds or improved water retention, thereby encouraging species-wide resilience to anthropogenic climate change (Hoffmann et al. 2021).

Identifying adaptive genetic differentiation between populations of a species can be achieved through the identification of outlier loci with respect to the background level of genomic differentiation (Horscroft et al. 2019). However, such differentiation is not always the result of selection; processes such as genetic drift may cause neutral variation between populations (Wright 1949; Weeks et al. 2016). There are two main mechanisms used to determine whether genetic variation is due to divergent selection; firstly, genomic data may be aligned to an annotated reference genome to determine whether highly differentiated loci are aligned with functional genes, such as heat shock proteins (Ghosh et al. 2020). If no reference genome is available, however, genotype-environment association (GEA) studies may be used to test for correlations between genomic divergence and a variable of interest, such as climate factors or ecological gradients, thereby detecting signatures of local adaptation (Savolainen et al. 2013; Caye et al. 2019; Vranken et al. 2021). These tests are most successful if a reference

genome of a recently diverged species is available to assist in the identification of functional regions prior to testing for selection (Everett et al. 2011).

Most threatened species that are of interest for conservation initiatives under climate change do not have detailed genomic resources including an annotated reference genome (Brandies et al. 2019). In Australia, threatened species are also often highly fragmented, making them primary targets for genetic rescue and assisted gene flow (Aitken and Whitlock 2013; Weeks et al. 2016; Pavlova et al. 2017; Ralls et al. 2018; Hoffmann et al. 2021). Many researchers have called for an increased effort to produce reference genomes for threatened species (Brandies et al. 2019), and the sequencing platforms required are becoming cheaper and more accessible every year. For example, the Earth BioGenome Project was established for this very purpose (Lewin et al. 2018; Exposito-Alonso et al. 2020). However, many reference genomes of threatened species do not yet exist and GEA studies present a useful alternative until such resources are available. In the present study, I applied a GEA test to populations of a non-model species lacking a reference genome, the greater stick-nest rat (*Leporillus conditor*), in order to determine whether translocation to a desert environment has resulted in adaptation in response to extreme arid conditions in the translocated population.

The greater stick-nest rat is a native murid rodent that was once found across most of the southern half of the Australian mainland (Copley 1999a). Its range encompassed a variety of ecological niches, from the arid sandplains of Lake Eyre to mesic coastal islands. With the arrival of European settlers and introduced predators and grazers, however, the greater stick-nest rat suffered a rapid range contraction, and was extinct on the mainland by the 1920s (Copley 1999a). Translocations from the single remaining extant population on the Franklin Islands, South Australia, began in the 1980s to a number of offshore islands and fenced mainland reserves (Short et al. 2019). One such translocation to Arid Recovery Reserve in South Australia's arid zone was considered successful (Moseby and Bice 2004; Moseby et al. 2011; Short et al. 2019), although it was noted that summer heatwaves resulted in increased mortality of greater stick-nest rats despite the region being encompassed by the species' historical range (Bolton and Moseby 2004). This is not surprising, given that the source populations of Reevesby Island and Monarto Zoo (both populations were established using founders from the Franklin Islands, which shares a similar climate to Reevesby Island) experience annual mean maximum temperatures 4-6ºC lower than Arid Recovery Reserve, and considerably higher rainfall (Short et al. 2019; Bureau of Meteorology 2021). A genetic

comparison of greater stick-nest rats at Arid Recovery Reserve with the founding populations 18 years after establishment by White et al. (2018) found six (out of 8,703) differentiated single nucleotide polymorphim (SNP) loci in the genome of the translocated population (note that this research was based on a different dataset to the present study). Although the authors acknowledged that the small effective population size at Arid Recovery Reserve made natural selection an unlikely source of this variation, they note that the high mortality observed as a result of heat stress could be acting as a selective pressure.

Here I applied a GEA test to a single-nucleotide polymorphism (SNP) dataset of greater stick-nest rats sampled at Arid Recovery Reserve, the source populations (Reevesby Island and Monarto) and the extant population of the Franklin Islands to determine whether selection has occurred in the Arid Recovery Reserve population in response to heat stress and climate-associated mortality events. Given the difference in environment experienced by the source populations and the translocated population, I expect that at least some differentiation in the Arid Recovery Reserve genome will have occurred as a result of climate adaptation in response to high temperatures.

Methods

Study populations

Franklin Islands

The Franklin Islands are two islands (East and West) connected at low tide by a small sandbar located off the coast of Ceduna, South Australia (Copley 1988). The islands are dominated by Nitre-bushes and sandy soil, and are believed to have separated from the mainland ~7700 years ago (Robinson et al. 1996). The Franklin Islands are home to the single remaining natural population of greater stick-nest rats, which became the subject of recovery efforts and multiple translocations in the 1980s (Copley 1988, 1999b; Short et al. 2019).

Monarto

Monarto Safari Park, ~80km east of Adelaide, South Australia in mallee bushland, became the site of a captive breeding facility for two greater stick-nest rats sourced from the Franklin Islands in 1985 (Copley 1988). The population was supplemented several times over the subsequent years, eventually totalling 29 wild-caught rats in 1998, and was subsequently maintained as a source for translocations until 2003 (Short et al. 2019). The colony supported

~100 individuals throughout the 1990s in eight breeding aviaries (3 x 7.5 m) that were exposed to the elements (Copley 1988; Short et al. 2019). A breeding colony was reestablished at Monarto Zoo in 2019, again using founders from the Franklin Islands (Australian Wildlife Conservancy 2020).

Reevesby Island

Reevesby Island is a large island located offshore of Tumby Bay, South Australia. The habitat consists mainly of sandplains, low dunes, grasslands and shrublands (Robinson et al. 1996). Greater stick-nest rats were translocated to the island in four stages in 1990 and 1991, sourced from the Monarto captive colony (Pedler and Copley 1993), and is estimated to sustain a population of 600-1,000 individuals (Woinarski and Burbidge 2016).

Arid Recovery

Arid Recovery is a fenced, predator-free mainland reserve located outside of Roxby Downs in South Australia's arid zone. The area is dominated by longitudinal sand dunes and swales with low shrubs (Short et al. 2019). Arid Recovery became the site of a several releases of greater stick-nest rats from 1998-2003, with individuals sourced from both Reevesby Island (55 males and 43 females, translocated in 1998-1999) and Monarto (10 males and 14 females, translocated in 2003) (Moseby and Bice 2004; Short et al. 2019). Only samples that had been collected after 2010 at Arid Recovery were included in this analysis to account for possible selection as a result of heat stress following translocation.

Population genomic analysis of greater stick-nest rat populations from the Franklin Islands, Monarto, Reevesby Island and Arid Recovery is detailed in White et al. (2020).

Samples

187 samples from the four populations of greater stick-nest rats were included in the dataset (Table 1). Tissue samples (tail tips/ear clips) were collected during sporadic trapping and monitoring events, and stored in 70% ethanol at -20°C. DNA extractions were performed prior to sequencing on some samples using the protocols detailed in Barclay et al. (2006) and White et al. (2018), and subsequently stored at -20°C. All samples were sent to commercial sequencing platform Diversity Arrays Pty Ltd (DArT) in Canberra, ACT. DArT uses double-digest restriction-site associated DNA next-generation sequencing to produce a reduced-representation genome sequence while capturing a uniform set of informative markers across all samples (Kilian et al. 2012). The proprietary bioinformatic pipeline process was used to demultiplex, clean, and filter reads, call SNP genotypes and is described in Egea et al. (2017).

Table 1. Greater stick-nest rat (*Leporillus conditor*) samples included in the SNP dataset.

	n	Period Sampled	Population Type	Source Population
Arid Recovery	17	2012-2017	Translocation	Reevesby Island & Monarto
Reevesby Island	84	1998-2018	Translocation	Franklin Islands
Monarto	56	1994-2003	Captive breeding	Franklin Islands
Franklin Islands	30	1994	Extant	-

Climate data

To obtain covariates suitable for testing genotype-environment associations, five climatic variables were extracted for each population. Given that the sampling periods spanned several years for most populations, annual means for each location were used. The following climate parameters were extracted from the Atlas of Living Australia's Spatial Portal (Belbin 2011) at a resolution of ~1km; mean annual minimum temperature (°C) (CSIRO 2010a), mean annual temperature (°C) (CSIRO 2010b), mean annual maximum temperature (°C) (CSIRO 2010c), mean annual rainfall (mm) (CSIRO 2010d), and mean annual relative humidity (%) (CSIRO 2010e) (Table 2). Where climate information was not available for offshore islands, values from the nearest mainland point were used.

Table 2. Climatic variables extracted for each population of greater stick-nest rats (*Leporillus conditor*) used in this study.

	Arid Recovery	Monarto	Reevesby Island	Franklin Islands
Mean annual minimum temperature (°C)	12.84	8.93	10.86	10.97
Mean annual temperature (°C)	20.2	15.8	16.5	17.3
Mean annual maximum temperature (°C)	26.78	21.22	20.91	22.5
Mean annual rainfall (mm)	13.25	30.89	32.35	26

Mean annual relative humidity (%)	65.92	77.23	80.35	76.47

963
964
965 *Data filtering*
966 Raw demultiplexed sequence data for all samples were obtained from Diversity Arrays in
967 FASTQ format. These reads were mapped to the most recently diverged reference genome
968 available for the greater stick-nest rat, that of the Australian broad-toothed rat (*Mastacomys*
969 *fuscus*) using the bwa-mem algorithm (v0.7.17, (Li 2013)). The resulting SAM files
970 compressed to BAM files using SAMtools (v1.7-1, (Li et al. 2009)). SAMTOOLS was also
971 used to filter out unmapped reads, sort reads by chromosome and position, and index each
972 BAM file. The mapped reads were then passed through ANGSD software (version 0.930,
973 (Korneliussen et al. 2014)) to produce a SNP-by sample matrix with the following read
974 filters; mapping quality: ≥ 20, minimum individual read depth: 5, maximum individual read
975 depth: 100, genotype likelihoods method: SAMtools model, SNP likelihood ratio p-value:
976 1×10-5, and posterior probability: 0.98. The resulting SNP-by-sample matrix was then further
977 filtered using R (version 4.1.0) (R Core Team 2021). The following thresholds were applied
978 in R; samples with >15% missingness, SNPs with >5% missingness, SNPs with minor allele
979 frequencies (MAFs) <0.05, and SNPs with unusually high heterozygosity (>0.6) were all
980 removed from the dataset. Samples and SNPs were filtered alternately using increasing
981 thresholds to retain the most informative samples and SNPs as follows; locus call rate 0.85,
982 individual missingness 0.5, locus call rate 0.88, individual missingness 0.4, locus call rate
983 0.9, individual missingness 0.3, locus call rate 0.92, individual missingness 0.2, locus call
984 rate 0.935, individual missingness 0.15, locus call rate 0.95. The data was also filtered on
985 linkage disequilibrium using the R package "SNPRelate" (version 1.26.0, (Zheng et al.
986 2012)), via the snpgdsLDpruning function using a correlation threshold of 0.5 and a sliding
987 window of 100 kb.
988
989 *Genotype-environment association analysis*
990 To test for a correlation between genetic differentiation and environmental variables across
991 the four populations, I employed a latent factor mixed model (LFMM) approach using the R
992 package "LEA" (version 3.4.0) (Frichot and François 2015). *sNMF* (sparse non-negative
993 matrix factorisation) was used to estimate the number of ancestral populations (*K*) (Frichot et

al. 2014), with ten repetitions performed for each *K* value from 1 to 10. Cross-entropy was then performed on the most appropriate *K* value, with admixture for the best run (i.e. lowest cross-entropy) visualised using a barplot. Missing data was then imputed using the chosen *K* value, the best cross-entropy run and the "mode" (most likely genotype) method. I tested for multicollinearity between climatic predictor variables using variance inflation factor (VIF), implemented in the R package "usdm" (version 1.1-18). VIF analysis of climatic factors revealed a high degree of multicollinearity between predictors, with VIF resulting in a near perfect correlation (*Inf*) for all factors, meaning that all climatic variables were likely to produce the same result. As the use of highly correlated environmental variables can lead to wrong conclusions in GEA studies (Rellstab et al. 2015), I therefore selected only the climatic variable of most relevance to the hypothesis, that of mean annual maximum temperature (°C), for GEA testing.

LFMMs were then run on the imputed SNP matrix and the mean annual maximum temperature (°C) dataset to detect outlier loci. To eliminate false positives, a false discovery rate (FDR) of 0.01 was applied. The positions of the resulting candidate SNPs were then manually searched against the closest annotated reference genome to the greater stick-nest rat, that of the house mouse (*Mus musculus*) (GRCm39), using the National Centre for Biotechnology Information (NCBI) Genome Data Viewer to determine whether any functional genes were associated with the regions.

Results

The initial filtering pipeline from DArT of greater stick-nest rat samples produced 21,792 SNPs. Following alignment to the *Mastacomys fuscus* genome, data filtering and SNP calling, a total of 4,564 SNPs were retained for analysis. Read depths per sample per site had a mean of 14.48 and a median of 12.63. The optimum number of ancestral populations determined by LEA was $K = 2$, consistent with the extant metapopulations of the East and West Franklin Islands.

The LFMM for mean annual maximum temperature (°C) identified three outlier loci (Table 3). When compared to the *Mus musculus* genome, only one SNP had a gene associated with the region. HiC_scaffold_2:119015455 was located within the gene of DNAJ heat shock protein family (HSP40) member C17 (DNAJC17).

Table 3. Outlier loci produced by latent factor mixed models for the climatic variable of interest, annual maximum temperature (°C).

Climatic Variable	Outlier loci (chr:position)	P-value	Associated Gene
Mean annual maximum temperature (°C)	HiC_scaffold_2:119015455	3.308626e-07	DNAJC17
	HiC_scaffold_4:16873031	4.381668e-07	None
	HiC_scaffold_19:97670270	6.194336e-07	None

Discussion

In the present study, I tested for signals of selection in greater stick-nest rats by performing a GEA analysis that tested specifically for climate adaptation due to heat stress. GEA testing of a modest number of SNPs from the translocated population of greater stick-nest rats at Arid Recovery Reserve against those of the source populations revealed high divergence at three SNPs. While two of these SNPs could not be associated with any particular gene due to the lack of annotated reference genome for the species, one SNP was associated with a protein coding gene (DNAJC17), a heat shock protein family member, in the house mouse genome.

All climatic variables gathered for GEA testing in this study were found to be highly correlated, negating the value of testing for signals of selection in response to each predictor. I therefore chose to test only for the climatic variable of most relevance to our hypothesis, mean annual maximum temperature (°C), as mortality events of greater stick-nest rats at Arid Recovery Reserve are believed to have been in response to prolonged periods of extreme heat. Hence, maximum temperature was expected to be the most likely selection pressure in this study. Although this variable was correlated with outlier loci, the multicollinearity between the climatic variables results in uncertainty as to which factor is driving putative selection.

The association of one outlier SNP with a heat shock protein, however, presents some evidence that increased temperature is acting as the selection pressure in the Arid Recovery Reserve population. DNAJC17 is a member of the heat shock protein family HSP40, a group of proteins responsible for a number of functions including protein folding, translocation, degradation, and, importantly, stimulation of HSP70 “chaperone” heat shock proteins (Qiu et

al. 2006). Although DNAJC17's functions are poorly understood (Pascarella et al. 2018), it has been implicated in the function and development of the thyroid gland in mice – more specifically, DNAJC17 has the ability to interfere with thyroid specific genes, resulting in congenital hypothyroidism (Amendola et al. 2010). Reduced thyroid activity, or hypothyroidism, has been found to improve heat stress survival in chickens (Bowen et al. 1984) and livestock (Aleena et al. 2016). Further, a rapid thyrosuppressive mechanism ("Wolff-Chaikoff" phenomenon) induced in laboratory rats alleviated heat stress impacts (Al-Tamimi et al. 2019). While thyroid activity and hormone production can vary throughout an animal's lifetime in response to environmental stressors (Rasouli et al. 2004), prolonged periods of extreme heat stress at Arid Recovery Reserve may have resulted in selection for greater stick-nest rats with upregulated DNAJC17, resulting in reduced thyroid activity and thus improved survival under heat stress.

However, studies have shown that hypothyroidism in rodents can also result in a number of deficiencies, including reduced tactile and sensory processing (Afarinesh et al. 2020) and impaired cognitive function (Amano et al. 2018). Natural selection or mutations resulting in both beneficial and negative consequences for a population are not uncommon. Indeed, Brady et al. (2019) refer to evolution as a "Rubin's vase" illusion, in which most see one component (beneficial adaptation) when in fact, two are present (adaptation and maladaptation). Well-known examples of these types of evolutionary "trade-offs" are malaria resistance in sickle cell disease patients (Ferreira et al. 2011) and the negative correlation between male sexual attractiveness/ornamentation and survival in species such as guppies (*Poecilia reticulata*) (Brooks 2000). A growing number of studies are highlighting the occurrence of these trade-offs in response to climate change (Kelly et al. 2016; Leites et al. 2019); a model system of microalgae in a simulated climate change environment was found to allocate less carbon to growth, while instead increasing resilience to reactive oxygen species, toxic molecules induced by climate stress in plants (Cassia et al. 2018; Lindberg and Collins 2020). Selection for hypothyroidism in greater stick-nest rats may therefore be an adaptive trade-off in response to increased heat stress, with potential costs to other biological functions. Future research on whether the benefits of hypothyroidism under rising temperatures outweigh the negative physiological consequences is required; laboratory gene editing experiments using CRISPR technology may provide further insight.

Differentiation of the DNAJC17 gene in the greater stick-nest rat genome can only be inferred by this study, however. The reference genome used to identify this gene (*Mus musculus*) – although currently the closest functionally annotated reference genome available – shared a common ancestor with the greater stick-nest rat ~10 million years ago (Steppan and Schenk 2017). This divergence must be taken into account (da Fonseca et al. 2016), as it is by no means certain that the functional regions of the greater stick-nest rat genome align perfectly with those of the house mouse; high divergence can significantly reduce gene recovery rate (Ungaro et al. 2017). Indeed, two of the three SNPs found to be under putative selection in the GEA that had been aligned to a genic region in the more recently diverged (but un-annotated) broad-toothed rat genome were not associated with any functional gene in the house mouse. Further, the lack of reference genome for the greater stick-nest rat significantly reduced the number of SNPs that could be identified as belonging to genic regions, and consequently only a small fraction of the greater stick-nest rat genome was analysed for signals of selection. Although less stringent filtering would likely have resulted in more SNPs, it would also have increased the likelihood of false positives. This study could therefore be strengthened by the development of a more recently diverged, functionally annotated reference genome for the greater stick-nest rat, to improve the recovery rate of orthologous genes. The absence of a reference genome is a common issue in conservation genomics (Brandies et al. 2019), and the development of more published genomes for threatened non-model species globally would be highly beneficial.

The results of this study are also confounded by the high degree of multicollinearity between the environmental variables, likely as a result of the absence of an ecological gradient in the dataset. The Franklin Islands, Reevesby Island and Monarto populations all experience a relatively similar, mesic climate, while the environment at Arid Recovery Reserve is decidedly hotter and dryer. The lack of sampling gradient and replication in this study, along with the inability to align all diverged SNPs to a functional gene, make it difficult to parse out signals of selection from population structure and genetic drift, or to determine which, if any, environmental factor is causing divergence (Rellstab et al. 2015). Future studies with greater representation of samples along spatial and climatic gradients may also be expanded by incorporating environmental variables beyond minimum and maximum temperatures that are likely to result in selection pressures, such as the number and duration of heatwaves.

While GEA studies are a useful tool that have been used in many studies of ecologically and commercially important species (eg. Sandoval-Castillo et al. 2018; Cummins et al. 2019; von Takach et al. 2021), this study highlights that the requirements of a statistically sound GEA analysis are not always possible for threatened species. These taxa are often highly fragmented (Ralls et al. 2018), and in some cases, such as that of the greater stick-nest rat, have survived only on offshore islands and in subsequent translocations to mainland reserves (Copley 1988, 1999a; Woinarski et al. 2015; Short et al. 2019). Further, less than 1% of the species listed as threatened by the International Union for Conservation of Nature (IUCN) have a published reference genome (Kitts et al. 2016; Brandies et al. 2019). This is not surprising, given the high cost of sequencing and annotating a high-quality reference genome (Lewin et al. 2018), an often unachievable expense for conservation practitioners and researchers. However, reference genomes are an extremely valuable asset in the genetic management of threatened species. Such tools can not only assist in identifying signals of adaptation and selection, but in effectively managing populations and understanding and controlling disease (Brandies et al. 2019). In recent years, a number of organisations and initiatives have been developed with the goal of generating high-quality, publicly available reference genomes for underrepresented taxa such as the Earth BioGenome Project (EBP), Oz Mammal Genomics (OMG) Consortium and the Global Invertebrate Genomics Alliance (GIGA) (GIGA Community of Scientists 2014; Potter and Eldridge 2017; Lewin et al. 2018; Teeling et al. 2018; Brandies et al. 2019; Exposito-Alonso et al. 2020). These groups are providing vital resources to the conservation community; OMG alone is currently developing reference data for a wide range of marsupials, bats and rodents, and are responsible for publication of the *Mastacomys fuscus* genome used in this study (Eldridge et al. 2020). However, there is still a long way to go before comprehensive genomic analyses can be applied to Australia's threatened species to identify local adaptation and harness that knowledge for conservation management against climate change.

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Chapter 7

Disproportionate admixture improves reintroduction outcomes despite the use of low-diversity source populations: population viability analysis for a translocation of the greater stick-nest rat

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Overall percentage (%)	70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

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Disproportionate admixture improves reintroduction outcomes despite the use of low-diversity source populations: population viability analysis for a translocation of the greater stick-nest rat

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Short title: Skewed admixture improves reintroduction outcomes

Abstract

Translocation is becoming an increasingly important approach to threatened species conservation. Coupled with the knowledge that maximising genetic diversity aids population establishment, the growing use of translocations can place unsustainable harvesting pressure on critical and vulnerable source populations. However, adaptive, genetically-informed modelling tools such as Population Viability Analysis (PVA) can be used to predict translocation outcomes and optimise harvesting strategies. In this study, we use PVAs for the frequently translocated greater stick-nest rat (*Leporillus conditor*) to demonstrate the value of admixing founder populations for translocation, even when one source population is deemed genetically depauperate. This approach not only maximises genetic diversity in the translocated population, but reduces harvesting pressure on critical populations. Further, we show that admixed harvesting ratios can be skewed significantly towards the genetically depauperate population in order to further protect the critical population while still producing

favourable outcomes, providing adequate founder numbers are used. As many threatened species are limited to fragmented and bottlenecked populations, these results are broadly applicable to the science of reintroduction biology, and demonstrate the value of PVAs for preliminary translocation planning and species management.

Key words

Conservation genetics, ecology, population viability analysis, reintroduction biology

Declarations

Funding

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Conflicts of interest

The authors declare no conflicts of interest.

Ethics approval

All samples were collected and sequenced as part of a previous study (White et al. 2020).

Availability of data and material

All de-multiplexed raw sequencing data are available from NCBI's sequence read archive (accession number: PRJNA389954).

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Introduction

Australia's biodiversity faces a growing number of threats associated with land use changes, habitat loss and climate change, and many conservation managers have employed the practice of translocation, the facilitated movement of a species from one area to another, to combat extinctions and secure populations (Seddon 2010; IUCN 2013). Translocation programs face a number of practical challenges both pre- and post-release, including funding shortages, monitoring difficulties, predation, poor habitat quality and lack of baseline knowledge (Clayton et al. 2014; Short et al. 2019; Berger-Tal et al. 2020). Translocation success may often rely on sufficient numbers of genetically diverse individuals. Low founder numbers are associated with high failure rates due to the increased likelihood of inbreeding and founder effects (Weeks et al. 2011; McCoy et al. 2014; Pacioni et al. 2019). Similarly, low genetic diversity (either from founders or due to founder effect/post-release bottlenecks) also places translocations at risk of inbreeding depression or a lack of adaptive potential (Jamieson 2011; Biebach and Keller 2012; Ramstad et al. 2013; Murphy et al. 2019) (but see also Harding et al. 2016).

One of the guiding principles of translocations is to ensure that the source population is not negatively impacted by harvesting (IUCN 2013). The increasing use of translocation programs combined with the importance of maximising genetic diversity for population establishment and persistence means that source populations are under more pressure for conservation reintroductions (Armstrong and Seddon 2008; Jamieson and Lacy 2012; IUCN 2013; Schäfer et al. 2020). As many threatened species have already suffered genetic bottlenecks (Jamieson et al. 2008), it is paramount that harvesting for translocations does not jeopardise the persistence of small and/or genetically depauperate source populations. In some cases, harvesting for translocations can have negative effects on the source population, such as population declines, disruption of social networks, loss of allelic richness and reduced

genetic diversity (Goldenberg et al. 2019; Pacioni et al. 2019; Furlan et al. 2020; Morrison et al. 2020). For example, the sole remaining wild population of redfin blue eye, a small endangered fish endemic to Australia, lost a significant amount of genetic diversity when it was used as a source for eight translocations between 2009-2012, which the authors predicted would reduce adaptive potential in the long term (Furlan et al. 2020). Harvesting of remnant populations of the banded hare-wallaby (*Lagostrophus fasciatus*) in Western Australia has been predicted to result in slower drought recovery within the remnant populations (White, et al. 2020a). Further, population models of threatened *Leiopelma* frog species in New Zealand revealed that harvesting more than 150 individuals from source populations would result in declines in allelic retention (Easton et al. 2020).

One method which has proved helpful in mitigating the unsustainable harvesting of source populations and maximising translocation success is adaptive and genetically informed population modelling (Dimond and Armstrong 2007; Pacioni et al. 2019). These approaches often employ a population viability analysis (PVA), that incorporate population-specific survival parameters, genetic data and environmental variability in order to model demographic stochasticity over time and, ultimately, predict loss of genetic diversity and extinction risk (Morris and Doak 2002). PVAs can be used to predict the impact of harvesting on a source population, while simultaneously determining the likelihood of successful establishment of the translocated population. Well-designed PVAs can be useful in assisting conservation decision-making (Brook et al. 2000; Chaudhary and Oli 2020) and are considered to be of most value when comparing multiple scenarios to determine the most effective management strategy, rather than delivering an absolute result (Akçakaya and Sjögren-Gulve 2000).

Here we aim to incorporate genetically informed population models into planning the translocation of an endemic Australian rodent, the greater stick-nest rat (*Leporillus conditor*) (hereafter GSNR). Once widespread across the southern half of the continent, the combined pressures of land use changes and introduced predators and herbivores reduced the species to a single location (on the East and West Franklin Islands, near Ceduna, South Australia) by the 1930s (Copley 1999*a*). GSNRs were listed as ‘Endangered’ under the IUCN assessment criteria in 1996 but have since been downlisted to ‘Vulnerable’ due to successful translocations to a captive colony at Monarto Safari Park in the late 1980s and several conservation areas since 1990 (Short et al. 2019). All five of the surviving translocated populations have lower genetic diversity than the Franklin Islands individuals (White et al.

2020b), possibly due to founder effects in the Monarto captive population, over- and under-representation of founders in translocated populations, and/or genetic drift after release. As the last remaining wild (and most genetically diverse) population, the Franklin Islands GSNRs represent both an important source for translocation harvesting and a critical population that must be conserved for the ongoing viability of the species. Indeed, White et al. (2020b) identified the Franklin Islands as the most appropriate source population for future GSNR translocations but suggested that other populations with lower diversity were good candidates for cross-translocations. We therefore aimed to use PVAs to determine an optimised harvesting strategy for a new reintroduction of GSNRs on Dirk Hartog Island, Western Australia, whereby natural Franklin Island stock are supplemented with individuals from an additional established translocated population in order to improve the translocation outcome while minimising negative effects on source populations. A former pastoral lease, the majority of Dirk Hartog Island was gazetted as a National Park in 2009. The Dirk Hartog Island National Park Ecological Restoration Project (or 'Return to 1616') aims to return the island to a similar ecological state to how it was when the first Europeans landed there in 1616 (Morris et al. 2017). To achieve this, eradication programs were successfully enacted for sheep (*Ovis aries*; completed in 2010), goats (*Capra hircus*; 2017) (Heriot et al. 2019) and feral cats (*Felis catus*; 2018) (Algar et al. 2019). With these key threats removed, the restoration project is now focused on the reintroduction of 13 locally extinct fauna species, including the GSNR (Algar et al. 2020). Of highest importance for the GSNR translocation is establishing a viable, genetically diverse population via translocation, while minimising harvesting impact on the critical population of the Franklin Islands.

Methods

Study Species & Source Populations

GSNRs are herbivorous, medium-sized rodents (180-450g), feeding predominantly on perennial succulent plants and grasses (Robinson 1975; Copley 1988; Ryan et al. 2003; Procter 2007). They build and inhabit communal stick nests, with females remaining in or nearby their natal nest while males disperse (Onley, et al. in review). Offspring are produced throughout the year, and once born remain attached to the mothers' teats until weaned (Le Souef 1922; Copley 1988). While the species has suffered a rapid decline due partly to predation by cats (*Felis catus*) and foxes (*Vulpes vulpes*), native predators include various

species of owls, kites, snakes, and other reptiles such as monitors (Pedler and Copley 1993; Copley 1999a; Moseby and Bice 2004). Since the 1980s, the species has been the subject of multiple translocation attempts from the single remaining extant population on the Franklin Islands (harvested periodically from 1985-1998, and again in 2011 and 2019 (Page et al. 2011; Short et al. 2019; AWC 2020)) and resulting captive breeding colonies with varying levels of success (see Short et al., 2019). Successful translocations have occurred to Salutation Island (first release 1990) (Copley, 1999b), Reevesby Island (first release 1990) (Pedler and Copley 1993), St Peter Island (first release 1993) (Copley, 1999b), Arid Recovery (fenced reserve) (first release 1998) (Moseby et al. 2011), and Mt Gibson (fenced reserve) (first release 2011) (Short et al. 2019).

The source populations considered in our models were the Franklin Islands (East and West) and Salutation Island (Figure 1). The Franklin Islands – East and West, 225 ha and 247 ha respectively and joined at low tide by a tombolo – populations were chosen because of their relatively high genetic diversity (White, et al. 2020b) and relatively large population size (1000-1200) (Robinson 1975; Copley 1988, 1999a). Genetic comparisons between West and East Franklin GSNRs indicate that the two island populations are weakly genetically distinct, with historical, but little contemporary, gene flow (White, et al. 2020b). We therefore estimated allele frequencies for West and East Franklins separately, with an equal harvesting ratio from both islands. Salutation Island (169 ha) was chosen because it has one of the largest populations of GSNRs (500-1000) (Copley 1999b; Short et al. 2019) and is closest to the release site, thereby minimising travel time for animals. However, it has lower genetic diversity in comparison to other potential source sites (White et al. 2020). Other extant GSNR populations were not considered in this PVA due to either low population sizes (Arid Recovery) or difficult logistics for an overland translocation combined with reduced genetic diversity (Mt Gibson, St Peter Island, Reevesby Island) (White et al. 2020).

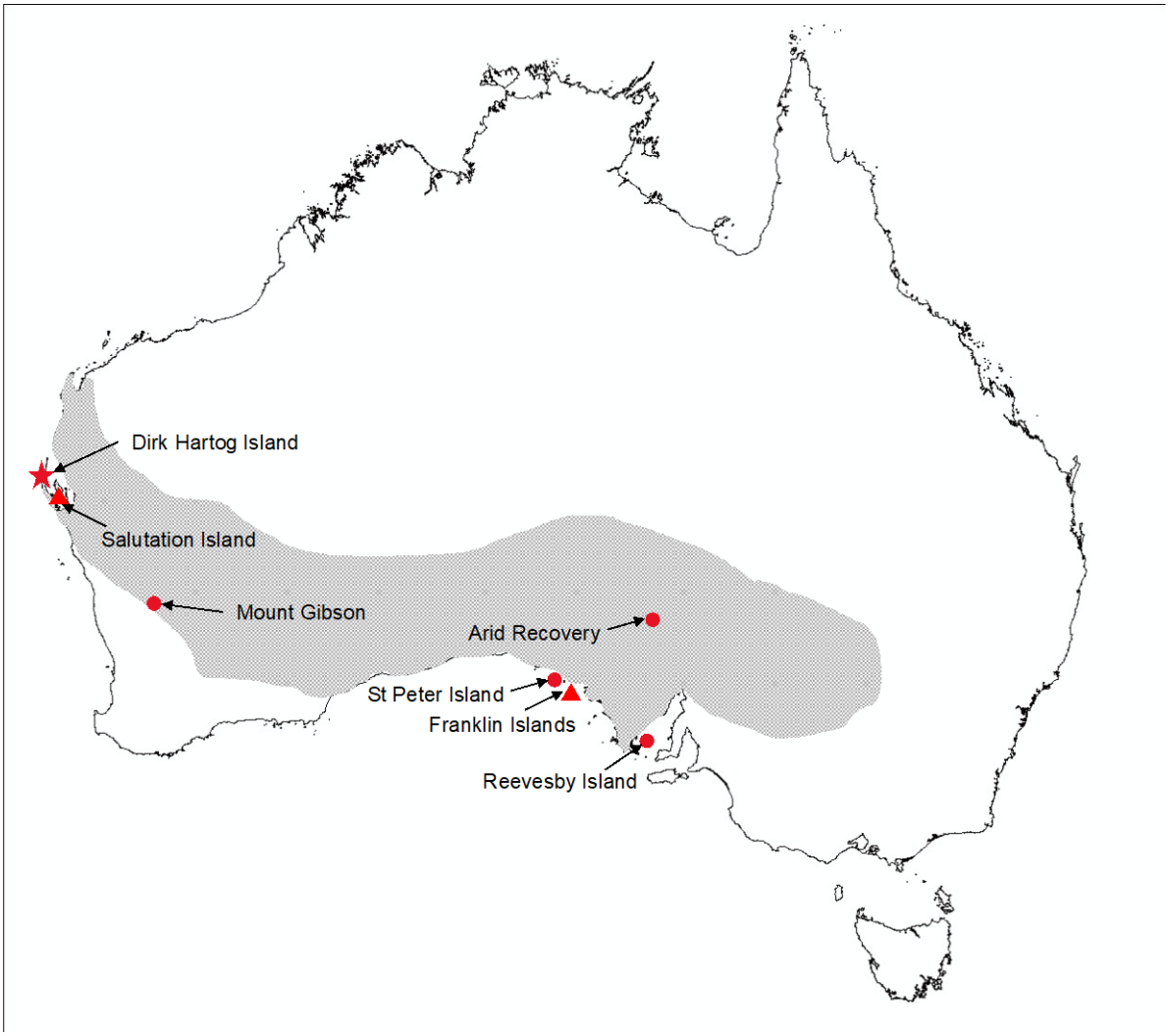

Figure 2 Map of current extant GSNR populations (red circles/triangles), proposed harvesting sites (red triangles), proposed translocation site (red star) and historic GSNR distribution (grey stipple).

Translocation Site

Dirk Hartog Island (DHI) lies at the western boundary of the Shark Bay World Heritage Area in Western Australia and at 63,300 ha in size it is the state's largest island. DHI has a semi-desert Mediterranean climate, with most rainfall (mean annual rainfall 224 mm) occurring in the winter and mean maximum summer temperatures of 31.8 ºC (Bureau of Meteorology 2018). Avian predators of GSNRs, such as eastern barn owl (*Tyto javanica*) and Australian boobook (*Ninox boobook*), are infrequently encountered on DHI but known reptilian predators such as sand monitors (*Varanus gouldii*) are common and widespread (Moseby and Bice 2004; Cowen et al. 2018, 2020). The western quoll (*Dasyrurus geoffroii*) is locally extinct on DHI and is planned for translocation, once prey species have established

populations predicted to be sufficiently large to withstand predation by quolls. A trial reintroduction of western quolls to Arid Recovery found GSNRs were not frequently found in quoll scats (West et al. 2020) but sample size was low and observations at rat nest sites suggest it is likely that quolls represent a significant predator of stick-nest rats (Arid Recovery unpublished data). Furthermore, successful establishment of GSNRs on DHI may lead to increased presence of avian predators. Given the relatively large size of the island and extensive areas of suitable habitat, it is anticipated that the carrying capacity of GSNRs is significantly higher than any extant populations – we therefore estimate the carrying capacity as 10000 in our models. Successful establishment of GSNRs on DHI would therefore represent an important outcome for the recovery of the species (Woinarski et al. 2014).

Genetic Data

To incorporate genetic information into our PVA, we used single nucleotide polymorphism (SNP) data generated and first published by White et al. 2020b. These data were generated using ddRAD-seq (Poland et al. 2012) from ear or tail clips sampled from GSNRs trapped on the Franklin Islands in 1994 and on Salutation Island in 2016. SNPs with minor allele frequencies of <0.05 and more than 25% missing data were removed (White et al. 2020b). Demultiplexed and adapter-trimmed sequencing data are available from NCBI's sequence read archive (accession number: PRJNA389954) and more detailed methodology regarding sampling, library preparation and bioinformatic processing can be found in White et al. (2020b). We chose to not identify and remove close-kin from this dataset as we have no evidence that sampling on the Salutation and Franklin Islands was non-random with respect to relatedness (Waples and Anderson 2017; Wang 2018). Thus, we assume relatives are present in the sample in proportion to their prevalence in the populations and that our sample is representative.

The SNP dataset includes 8723 loci genotyped from 19 individuals from Salutation Island, and 15 individuals from the Franklin Islands (8 from East Franklin and 7 from West Franklin). From this total dataset, SNPs were randomly subset to 500 loci as a representative sample of the genetic diversity of each population, and an allele frequency table was created using the R package "adegenet" (version 2.1.5) as per the requirements of the population modelling software.

Given that genetic samples from the Franklin Islands were collected in 1994, we first modelled a 25-year scenario of the Franklin Islands, including periodic harvesting for translocation, to ensure that no significant changes to allele frequency were likely to have occurred since sampling (Supplementary Information 1). Changes in genetic diversity were minimal (<0.005 expected heterozygosity), and were not considered significant enough to impact the outcome of PVAs.

Population Modelling

Population modelling software Vortex (version 10.3.6.0) was used to conduct the PVA (Lacy and Pollak 2017). Vortex uses Monte Carlo simulations based on life history and population parameters and incorporates uncertainty and stochastic events in order to predict demographic changes over time. Life history parameters (Table 1) were developed using a combination of published literature and observations by conservation managers with decades of experience in GSNR husbandry. A full description of life history parameters and rationale is detailed in Supplementary Information 2.

It should be noted that the GSNR is a relatively understudied species, and reported breeding and mortality rates vary between environments and conditions. Many reproductive rates and life span parameters available in the literature and used in this PVA are based on data from captive populations. While we may not expect wild populations exhibit identical traits to captive animals, this information was still informative in developing realistic parameters, especially when releasing individuals into a new environment (such as DHI), where resources are not likely to be limiting in the medium term at least. Inevitably though, some uncertainty around the parameters used remains, and future PVAs for this species would benefit from further life history studies, the chosen parameters were developed and validated in consultation with experienced practitioners specialising in the species in question. Furthermore, as the present study was a comparative analysis of harvesting techniques, absolute values are of less importance to our models than if they were to be used to predict actual extinction risk of a real-life population, and more conservative estimates would cloud the central question of the influences of founder size and source population on translocation outcomes.

1720 *Table 1* Life history parameters used in population modelling of GSNR translocation. EV denotes environmental variation. SD denotes standard deviation.

Population Parameters		Male	Female	Sensitivity Testing Range (min-max)	Reference (see also Supplementary Information 2)
Species Description	Lethal equivalents	3.14		2-6.5	(Ralls et al. 1988)
	Percent due to recessive lethal alleles	50			
	EV correlation between reproduction and survival	1			
	EV correlation among populations	0.8			
Reproductive System (monogamous)	Age of first offspring (years)	1	1		
	Maximum age of reproduction (years)	5	5		(Procter, 2007; K. Branch, *pers. comm.* 2021)
	Maximum lifespan (years)	5	5		
	Maximum number of broods per year	-	3		(Copley, 1988; K. Branch, *pers. comm.* 2020)
	Maximum number of progeny per brood	-	3		(Copley, 1988; Copley, 1999*a*; Pedler and Copley, 1993).
	Sex ratio at birth (%)	50	50		
Reproductive Rates	Adult females breeding (%)		=(80-((80-50)*((N/K)^2)))*(N/(1+N))		(Barclay et al., unpublished data)

	SD in % breeding due to EV		8		
	Number of broods per year (% distribution)		0 broods – 0 1 broods – 10 2 broods – 60 3 broods – 30		(Copley, 1988; Copley, 1999*a*; Pedler and Copley, 1993).
	Number of offspring per brood (% distribution)		1 offspring – 52 2 offspring – 41 3 offspring – 7		(Copley 1988)
Mortality Rates	Mortality from age 0 to 1 (±SD) (%)	36±11	36±11		(Barclay et al., unpublished data)
	Annual mortality after age 1 (±SD) (%)	15±4	16±4	10-20	(Barclay et al., unpublished data)
Catastrophes (drought)	Frequency (%)	16			(White et al., 2020a)
	Reproduction (% of normal rate)	15			(Copley, 1999b; Barclay et al., unpublished data)
	Survival (% of normal rate)	70			(Copley, 1999b; Barclay et al., unpublished data)
Mate Monopolization	Males in breeding pool (%)	100		70-100	

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Harvesting scenarios

Eleven different scenarios were modelled based on various harvesting numbers and source populations (Table 2). These scenarios were chosen to reflect the outcome of translocations using both single and multiple source populations with a range of founder numbers and ratios. Simulations (hereafter "Sims") 1 and 2 and Sims 3 and 4 represent single source translocations with baseline (n = 120) harvesting numbers and low (n = 64) harvesting numbers respectively. Sims 5 to 7 represent multiple sourced translocations with baseline, low and high (n = 240) founder numbers. Sims 8 and 9 and Sims 10 and 11 are multiple sourced translocations with skewed harvesting ratios, and baseline and high founder numbers respectively. The number of baseline founders was determined following Weeks et al. (2015), who advocated for sampling up to 50 unrelated individuals to capture 95% of genetic diversity. Accounting for related individuals and mortality following translocation, we chose 120 individuals (60 from each population) as our baseline harvest number. Survival during and after translocation was estimated at 70%, based on monitoring results from translocation of GSNRs to Mount Gibson (Short et al. 2019). GSNRs have been observed to demonstrate some mortality during trapping and transportation, as well as post-release (Pedler and Copley 1993; Short et al. 2019). Each scenario was simulated 1000 times over a 50 year period. Carrying capacity (K) for DHI was estimated to be 10000 individuals, but this is likely to be conservative given the carrying capacity of Salutation Island (just 169ha in size) appears to be 500-1000 individuals (Short et al., 2019). Salutation Island's K and initial population size was set to 600 individuals (K. Branch, *pers. comm.* 2020). Based on density estimates (Copley 1988) and the fact that both East and West Franklin Islands are larger than Salutation Island, we estimated K of each of the Franklin Islands to be 800, but the current population size was set to 500 individuals on East and West respectively.

Table 2 Harvesting scenarios used in population modeling for GSNR translocation to Dirk Hartog Island. Symbols denote the following; *single source, †multiple source, ‡low founder numbers, §baseline founder numbers, ¶high founder numbers, #skewed harvesting ratio.

Scenario	Harvest Strategy (50:50 sex ratio)	Total *n*
Sim 1*§	60 from Franklin Islands in Year 1; 60 from Franklin Islands in Year 2	120

Sim 2*§	60 from Salutation Island in Year 1; 60 from Salutation Island in Year 2	120
Sim 3*‡	32 from Franklin Islands in Year 1; 32 from Franklin Islands in Year 2	64
Sim 4*‡	32 from Salutation Island in Year 1; 32 from Salutation Island in Year 2	64
Sim 5†§	60 from Salutation Island in Year 1; 60 from Franklin Islands in Year 2	120
Sim 6†‡	32 from Salutation Island in Year 1; 32 from Franklin Islands in Year 2	64
Sim 7†¶	120 from Salutation Island in Year 1; 120 from Franklin Islands in Year 2	240
Sim 8†§#	40 from Salutation Island in Year 1; 80 from Franklin Islands in Year 2	120
Sim 9†§#	80 from Salutation Island in Year 1; 40 from Franklin Islands in Year 2	120
Sim 10†¶#	200 from Salutation Island in Year 1; 40 from Franklin Islands in Year 2	240
Sim 11†¶#	180 from Salutation Island in Year 1; 60 from Franklin Islands in Year 2	240

Data Analysis

All Vortex outputs were collated using the package "vortexR" (Pacioni and Mayer 2017) in R Studio (version 4.0.2). Post-hoc analysis of translocated populations was conducted using the package "stats" (version 4.0.2) (R Core Team 2020). Since data was determined to be abnormally distributed, we conducted a non-parametric analysis of variance (ANOVA; Kruskal-Wallis test) model followed by a pairwise Wilcoxon rank sum test of all 1000 iteration outputs for population size, expected heterozygosity, inbreeding and probability of extinction averaged over each year of the PVA in order to test for significant differences between translocation scenarios. Finally, to determine relative impact to founder populations,

expected heterozygosity, inbreeding coefficient and size of each population were compared at years 1 and 5 under each scenario.

While a reasonable amount of data on breeding and survival rates was available for this species (strengthened by consultation with leading practitioners), it is possible that variation to breeding and survival rates may occur in the population following reintroduction. We therefore used sensitivity testing in Vortex to determine the impact of variation in three key parameters on population establishment, represented by probability of extinction, inbreeding, heterozygosity and population size. These parameters were lethal equivalents, % males in the breeding pool and % mortality after age 1 (Table 1). Sensitivity testing was performed on the source population of East Franklin Island, due to computing restraints encountered when attempting sensitivity testing on multiple populations with extremely large carrying capacity (eg. 10000 individuals on DHI). The results of the sensitivity tests were analysed using a binomial logistic regression, with all parameters of the sensivity test included as predictor variables (Rayner et al. 2021).

Results

Population Growth

All scenarios, regardless of founder source population, reached a stable population size just below the estimated carrying capacity within 35 years of translocation to DHI (Figure 2A).

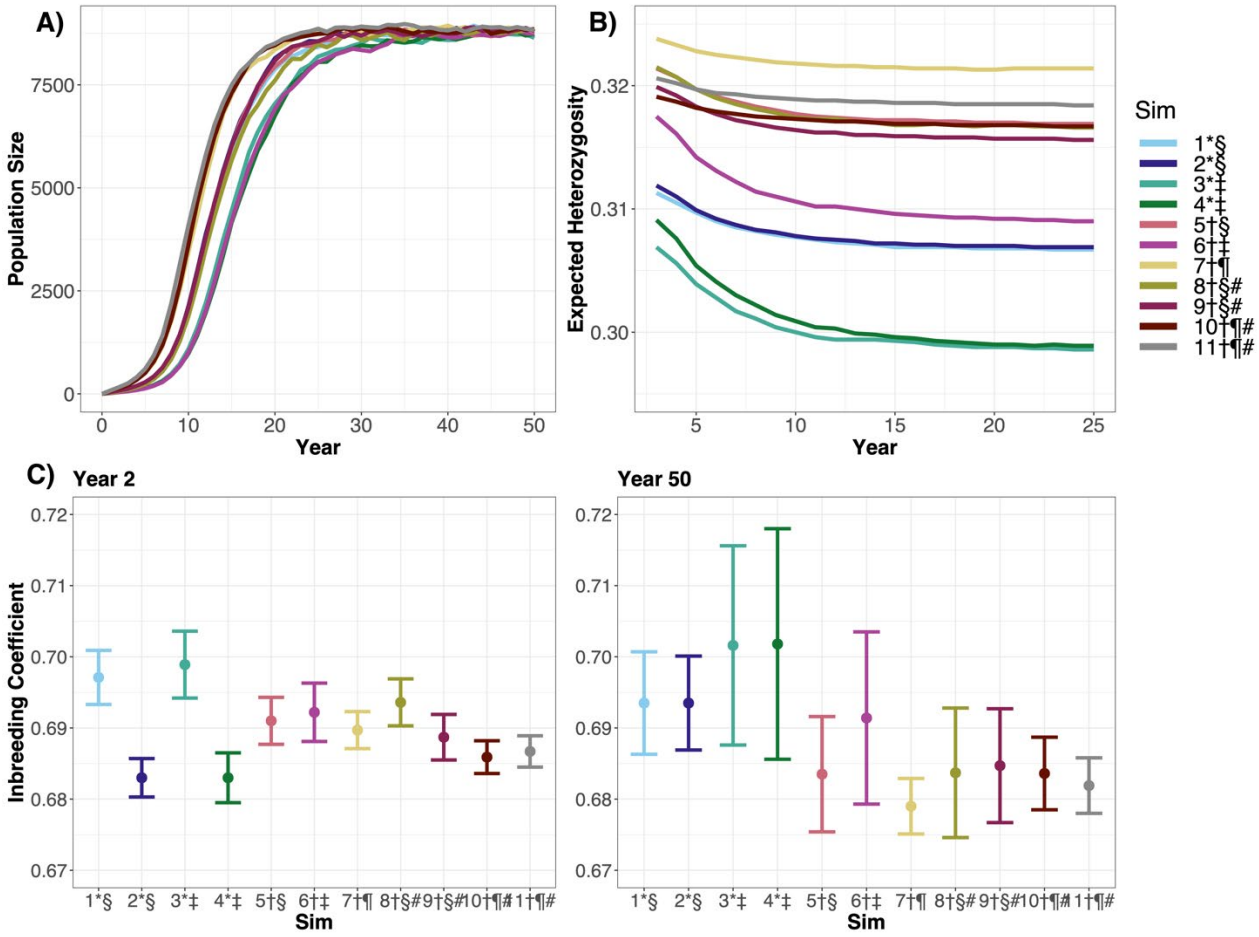

Figure 3 A) Population size, B) expected heterozygosity as a measure of genetic diversity, and C) comparison of inbreeding coefficients (mean and SD) of greater stick-nest rats at Dirk Hartog Island under each scenario over 50 years. Symbols denote the following; *single source, †multiple source, ‡low founder numbers, §baseline founder numbers, ¶high founder numbers, #skewed harvesting ratio.

Genetic Diversity

Scenarios resulting in the lowest expected heterozygosity were those with single source populations and low founder numbers (Sim 3 Franklins only and Sim 4 Salutation only),

followed by single source populations with baseline founder numbers (Sim 1 Franklins only and Sim 2 Salutation only) (Figure 2B). Multiple sourced translocations with low numbers performed better (Sim 6), but not as well as multiple source populations with baseline and high numbers (Sims 5, 7, 8, 9, 10 and 11), even when the ratios were skewed. Whether the harvesting was skewed towards the critical population (Franklins) or not had little impact on the outcome.

Inbreeding

Inbreeding coefficients for each scenario were relatively similar at the beginning of the translocation, with the exception of single sourced translocations from the Franklin Islands (Sims 1 and 3), which had a higher inbreeding coefficient than all other scenarios initially. By year 50, however, single sourced populations (Sims 1 to 4) and the population with two sources but low founder numbers (Sim 6) had the highest degree of inbreeding, while all others remained relatively constant (Figure 2C).

Probability of Extinction

In 1000 iterations, all scenarios had a low probability of extinction (□1.5%). Of these scenarios, single sourced translocations with low founding numbers (Sims 3 and 4) had the highest probability of extinction (Table 3).

Table 3 Year and probability of extinction of Dirk Hartog Island stick-nest rat population for each PVA scenario over 50 years and 1000 iterations. Symbols denote the following; *single source, †multiple source, ‡low founder numbers, §baseline founder numbers, ¶high founder numbers, #skewed harvesting ratio.

Scenario	Years Population Went Extinct	Probability of Extinction
Sim 1*§	11, 12, 18, 27	0.4%
Sim 2*§	9	0.1%
Sim 3*‡	6, 8, 10, 12, 13, 14, 18, 20, 26, 27	1.5%

Sim 4[*‡]	5, 7, 8, 10, 11, 12, 16, 23	0.9%
Sim 5[†§]	6, 7, 9, 15	0.4%
Sim 6[†‡]	9, 10, 12	0.5%
Sim 7[†¶]	21	0.1%
Sim 8[†§#]	11, 12, 17, 20	0.5%
Sim 9[†§#]	7, 12	0.2%
Sim 10[†¶#]	-	0%
Sim 11[†¶#]	-	0%

Statistical Differences

Non-parametric ANOVA models of four key outputs – population size, expected heterozygosity, inbreeding coefficient and probability of extinction – averaged across 1000 iterations for each year of the 50 year PVA per scenario revealed a significant difference between scenarios in outcome for all parameters (Table 4).

Table 4 P-values of output parameters for all PVA scenarios determined by non-parametric ANOVA models.

	Population size	Expected Heterozygosity	Inbreeding Coefficient	Extinction Probability
P-value	< 0.001	<0.001	<0.001	<0.001

Pairwise testing revealed that differences in inbreeding coefficients and expected heterozygosity were statistically significant between all models except Sims 1 and 2, Sims 3 and 4, and Sims 8 and 10 (Supplementary Information 3). Sims 5 and 10 were not significantly different in terms of inbreeding coefficient, but were significantly different in expected heterozygosity. Single source populations with low and baseline founder numbers had therefore higher inbreeding values and lower expected heterozygosity than all multiple sourced translocations, even when low founder numbers were used.

Sensitivity testing did not reveal significant impacts of variation of lethal equivalents, % males in the breeding pool or % adult mortality rates on GSNR populations (Supplementary

Information 4). Of the life history parameters we examined, % mortality after age 1 appeared to have the strongest effect on heterozygosity and extinction probability, however these effects were not statistically significant.

Impact of Harvesting on Source Populations

Harvesting did not appear to have any impact on the source populations long term, regardless of numbers removed from the population; 10 years after harvest, expected heterozygosity for all founding populations and harvesting scenarios decreased <0.003, inbreeding values increased by <0.002, and population size remained constant. Values for these outputs for each founder population at years 1, 5 and 10 of each harvesting strategy are detailed in Supplementary Information 5.

Discussion

PVAs are a valuable tool in conservation planning, management and decision-making (Chaudhary and Oli 2020). Population modelling of eleven different scenarios for the translocation of GSNRs to DHI revealed that sourcing founders from multiple populations improved the outcome of reintroductions in comparison to single sourced translocations. In translocated populations with multiple sources, inbreeding coefficients were, on average, lower, while expected heterozygosity was higher than single sourced populations. Inbreeding values for single sourced translocations were higher initially, but this is likely due to a Wahlund Effect resulting from the slight genetic divergence between the East and West Franklins (Hartl 1988; Frantz et al. 2006). Founder numbers also contributed to the outcome of translocations; where multiple sources were used, those scenarios with higher harvesting rates produced higher genetic diversity and lower inbreeding in the long-term. Skewing the harvesting strategy towards either source did not appear to change the outcome of the translocation, particularly when overall founder numbers were high. Interestingly, impact on source populations did not appear to vary between harvesting strategies, regardless of number of individuals taken in the scenarios we tested. Sensitivity testing on variable values of mate monopolisation, lethal equivalents, and % mortality after age 1 did not reveal a significant impact on population parameters of interest. This may be due to the large population sizes and carrying capacities of the populations considered within this study.

Value of skewed admixture for translocations

The results of our PVA support previous studies indicating that sourcing founder individuals for translocation programs from multiple populations not only reduces the risk of placing harvesting pressure on critical source populations, but can improve the outcome of the reintroduction as a whole (Biebach and Keller 2012; Wirtz et al. 2018; McLennan et al. 2020). Both genetic diversity and levels of inbreeding were significantly improved in the DHI GSNR population when founders were sourced from both Salutation Island and the Franklin Islands, in comparison to single sourced translocations from either location. This pattern has been observed in real-world translocations of other taxa, such as sea otter (*Enhydra lutris*) (Bodkin et al. 1999; Albrecht and McCue 2010; Robinson et al. 2021), Tasmanian devils (*Sarcophilus harrisii*) (McLennan et al. 2020) and bighorn sheep (*Ovis canadensis*) (Olson et al. 2013; Jahner et al. 2019; Poirier et al. 2019). The improved genetic diversity outcomes in the admixture scenarios is particularly interesting given that Salutation Island is considered a genetically depauperate population (White et al., 2020b), demonstrating that even populations of low diversity can act as valuable sources for reintroductions when combined with other populations. Further, skewing the proportion of animals harvested towards either the Franklin Islands or Salutation Island did not appear to significantly alter the outcome of the translocation. Skewing towards Salutation Island when founder numbers were high (Sim 10) had similar outcomes to Sim 8, where there was a skew towards the Franklin Islands. This key finding indicates that the critical population of GSNRs can be protected in future translocations by admixing with a high proportion of animals from the genetically depauperate population of Salutation Island.

Although we found little difference in the likelihood of population persistence/extinction or population growth across the simulated scenarios, admixture may still improve population sustainability for the DHI GSNRs through positive fitness effects. Our simulations modelled inbreeding depression through the inclusion of a number of lethal equivalents equal to the average for diploid organisms. It is possible that the true number of lethal equivalents in the GSNR populations is higher than this average – for example, GSNRs have been observed to suffer from cataract formation in both captivity and the wild, though it is unknown whether this is associated with genetics or diet (Robertson 2007)). If this is the case, the probability of positive fitness effects in admixed individuals through the reversal of inbreeding depression (i.e. genetic rescue, (Frankham et al. 2010; Frankham 2015; Whiteley et al. 2015)), may also

increase. This result has been demonstrated in practice for several taxa, including genetic rescues of the South Island robin (*Petroica australis*) and the mountain pygmy possum (*Burramys parvus*) (Heber et al. 2013; Weeks et al. 2017). Future investigation on the potential fitness benefits associated with translocation would be valuable for the management of GSNRs and other threatened species.

Role of founder numbers in translocation success and source population impacts

Our models support previous findings that founder numbers play a role in conservation outcomes (Weeks et al. 2011; McCoy et al. 2014; Pacioni et al. 2019). While scenarios with multiple source populations performed better overall, of these scenarios, those with higher founder numbers appeared to be the most successful in terms of retaining genetic diversity and minimising inbreeding over time. The positive impact of increased founder numbers has been reported on many times in recent years (Griffith et al. 1989; Lee et al. 2019; Furlan et al. 2020; White, et al. 2020a), while low founder numbers have been attributed to a number of failed reintroductions, including several translocations of woylies (brush-tailed bettong) (*Bettongia penicillata*) where the genetic effects of small founder numbers were further compounded by predation and drought (Fischer and Lindenmayer 2000; Mawson 2004; Germano and Bishop 2009; Short 2009). However, given the importance of conserving critical source populations, a trade-off must be reached between optimising translocation outcomes and minimising impacts to existing populations. Although we found no noticeable impact of higher harvesting numbers on source populations, detrimental effects of overharvesting have been observed (Goldenberg et al. 2019; Furlan et al. 2020), and the possibility of this occurring should be avoided where possible. Our PVAs showed similar genetic outcomes between Sims 5 and Sims 8 and 9, wherein 120 total founders were used in both, but the harvesting ratios from Salutation Island and the Franklin Islands were 50:50 and ~70:30/30:70 respectively. Further, increasing the founder numbers to 240 individuals but heavily skewing the harvesting towards the genetically depauperate population (Salutation Island) as in Sims 10 (~85:15) and 11 (75:25) also produced favourable results. Our results indicate that managers may consider alleviating harvesting pressure on critical source populations by heavily supplementing translocations with individuals from other, less diverse, populations, as long as a high number of founders are used.

Limitations and considerations

While PVAs are a valuable, and often highly accurate, method of predicting translocation outcomes (Brook et al. 2000), they are not infallible. The single-species focus and inability to account for all survival factors mean that there will always be some uncertainty associated with the results. Here, all scenarios produced a very low risk of extinction (≤1.5%). In reality, the likelihood of translocation failure is far higher; a study of Australian macropod translocations found between 51% and 61% of translocations to be successful, depending on the criteria (Clayton et al. 2014). Similarly, Short (2009) collated 380 translocations of 102 Australian species and identified 54% as successful. For GSNR translocations specifically, the success rate is 40% (Short et al. 2019). It is therefore unlikely that the extinction probability for the DHI translocation of GSNRs is as low as our models predict due to the inability to include all potential risk factors, and the values should be considered as relative, rather than absolute (Akçakaya and Sjögren-Gulve 2000). Furthermore, understudied species often have limited demographic data available; for example, in our analysis we assume that all males have equal breeding success. While no data currently exist for GSNRs that suggest otherwise, it should be acknowledged that the potential for unequal reproductive success rates may have genetic impacts on translocated populations. However, in this comparative analysis of translocation scenarios we feel it is unlikely that greater certainty around variation in male breeding success would result in any changes to our conclusions. The results of the sensitivity testing support this.

Conclusions and recommendations

Our models show that skewed harvesting ratios towards genetically depauperate source populations can produce favourable outcomes following translocation, highlighting a promising approach to protect critical populations without jeopordizing reintroduction programs. These results are broadly applicable, as many native species have suffered range contractions and genetic bottlenecks similar to those of greater stick-nest rats. Disproportionate admixed harvesting, rather than a single-source approach, has the potential to lessen harvesting impacts on the genetic diversity of critical naturally-occurring populations, even if one source population is genetically suboptimal. These findings are a timely contribution to the growing science of reintroduction biology. Managers working with other species should take a case-by-case approach and consider species-specific life-history parameters such as reproduction rates, brood size and breeding age to determine appropriate founder numbers. Tailored, species-specific PVAs are a valuable tool for incorporating this

information into conservation planning, and should be used to assist with decision-making for future reintroductions.

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Supplementary Information 1

Changes in expected heterozygosity over 25 years of population modelling for GSNRs on the Franklin Islands.

	Year 0	Year 25
East Franklin Island	0.3051	0.3005
West Franklin Island	0.2908	0.2863

Supplementary Information 2

Description and rationale of life history parameters used in GSNR population modelling

Species description

One Vortex "year" was deemed to be 365 days, therefore all reproductive and mortality rates are annual. Inbreeding depression was estimated at 3.14 lethal equivelants per diploid gene, the mean value for mammals (Ralls et al. 1988).

Reproductive system and rates

GSNRs have been observed to breed up to four times per year in captivity and in good conditions in the wild (Copley, 1988; K. Branch, *pers. comm.* 2020), although high summer temperatures may limit breeding to annual events in cooler months in semi-arid and arid areas (Moseby and Bice 2004). This is more likely to occur on Salutation and Dirk Hartog Islands than the Franklin Islands, given the lower latitude and warmer climate of the translocation sites. We therefore estimated an average of three broods per year (K. Branch, *pers. comm.* 2020). Litters may contain three offspring in captivity, but in the wild are typically limited to one or two (average 1.32 offspring per litter) (Copley, 1988; Copley, 1999*a*; Pedler and Copley, 1993). We estimated the distribution of offspring per litter to reflect this (Table 1). The maximum age of reproduction is approximately five years old in captivity (Procter, 2007; K. Branch, *pers. comm.* 2021).

Mortality rates and catastrophes

Mortality rates and standard deviations were calculated by Sean Barclay (unpublished data) using mortality estimates reported in Copley (1988) and further refined using Vortex sensitivity testing. DHI is an offshore island with no introduced predators, and few avian predators, and predation risk is therefore negligible. Reptilian predators such as sand monitors are present on DHI; Short et al. (2019) proposed that predation pressure from monitors may have been at least partially responsible for some poor translocation outcomes, and there is some evidence that GSNRs are frequently preyed upon by this species (Bolton and Moseby 2004). However, on DHI, a limited study of monitor diet found that small mammals made up a relatively small proportion of prey items (<12% of samples), compared to invertebrates such as beetles and cockroaches (~75%) (Cowen et al. 2019), despite sandy inland mice (*Pseudomys hermannsburgensis*) being abundant on the island (Cowen et al., 2020). GSNRs have demonstrated increased mortality during hot summer periods (Moseby and Bice 2004) and we therefore modelled "drought" as a stochastic environmental process. The frequency of a drought event was determined to be 16% per year (1 in 6.25 years) based on drought frequency predictions of nearby Western Australian islands Bernier and Dorre (White et al., 2020a). Mortality and proportion of animals reproducing during drought was estimated by analysing the under-representation of juveniles in trapping data from Reevesby Island in 1995 compared to 1994, which recorded a population decline during this period due to drought (Copley, 1999b; Barclay, unpublished data).

Supplementary Information 3

Pairwise Wilcoxon rank sum test comparisons of Vortex results for population size, expected heterozygosity, inbreeding coefficient and extinction probability of each simulation. Statistically significant values (p value <0.05) appear in bold.

Inbreeding Coefficient										
	DHI Sim 1	**DHI Sim 2**	**DHI Sim 3**	**DHI Sim 4**	**DHI Sim 5**	**DHI Sim 6**	**DHI Sim 7**	**DHI Sim 8**	**DHI Sim 9**	**DHI Sim 10**
DHI Sim 2	0.26040993	NA	NA	NA	NA	NA	NA	NA	NA	NA
DHI Sim 3	**6.09E-12**	**3.43E-13**	NA	NA	NA	NA	NA	NA	NA	NA
DHI Sim 4	**3.14E-08**	**2.43E-09**	0.73756548	NA	NA	NA	NA	NA	NA	NA
DHI Sim 5	**3.62E-16**	**7.13E-15**	**3.62E-16**	**6.55E-15**	NA	NA	NA	NA	NA	NA
DHI Sim 6	**9.88E-11**	**4.69E-08**	**2.99E-15**	**1.33E-10**	**9.94E-12**	NA	NA	NA	NA	NA
DHI Sim 7	**3.62E-16**	**3.76E-16**	**3.62E-16**	**3.63E-16**	**3.86E-12**	**7.74E-16**	NA	NA	NA	NA
DHI Sim 8	**2.71E-15**	**1.52E-13**	**3.62E-16**	**1.06E-14**	**0.02977523**	**6.64E-11**	**3.14E-12**	NA	NA	NA
DHI Sim 9	**3.62E-16**	**1.75E-13**	**3.62E-16**	**7.78E-14**	**3.79E-07**	**5.97E-11**	**7.13E-15**	**9.52E-06**	NA	NA
DHI Sim 10	**3.62E-16**	**1.06E-14**	**3.62E-16**	**7.13E-15**	0.07957477	**1.14E-11**	**1.19E-14**	0.3609373	**3.50E-06**	NA
DHI Sim 11	**3.62E-16**	**3.62E-16**	**3.62E-16**	**3.62E-16**	**2.18E-07**	**4.98E-14**	**3.16E-13**	**7.97E-08**	**4.43E-10**	**3.12E-10**
Expected Heterozygosity										
	DHI Sim 1	**DHI Sim 2**	**DHI Sim 3**	**DHI Sim 4**	**DHI Sim 5**	**DHI Sim 6**	**DHI Sim 7**	**DHI Sim 8**	**DHI Sim 9**	**DHI Sim 10**
DHI Sim 2	0.44952744	NA	NA	NA	NA	NA	NA	NA	NA	NA
DHI Sim 3	**6.59E-15**	**4.73E-15**	NA	NA	NA	NA	NA	NA	NA	NA
DHI Sim 4	**8.30E-13**	**7.37E-13**	0.98662069	NA	NA	NA	NA	NA	NA	NA
DHI Sim 5	**1.61E-16**	**1.61E-16**	**1.61E-16**	**1.61E-16**	NA	NA	NA	NA	NA	NA
DHI Sim 6	**9.14E-11**	**2.21E-10**	**1.61E-16**	**5.83E-15**	**1.41E-14**	NA	NA	NA	NA	NA
DHI Sim 7	**1.61E-16**	**1.61E-16**	**1.61E-16**	**1.61E-16**	**6.96E-14**	**1.61E-16**	NA	NA	NA	NA
DHI Sim 8	**1.72E-16**	**1.82E-16**	**1.61E-16**	**1.61E-16**	**0.00403423**	**9.51E-14**	**9.28E-14**	NA	NA	NA
DHI Sim 9	**1.61E-16**	**1.61E-16**	**1.61E-16**	**1.61E-16**	**1.38E-09**	**1.11E-13**	**1.97E-15**	**8.62E-09**	NA	NA
DHI Sim 10	**1.61E-16**	**1.61E-16**	**1.61E-16**	**1.61E-16**	**0.01666205**	**1.52E-14**	**1.78E-15**	0.37094742	**7.81E-09**	NA

DHI Sim 11	**1.61E-16**	**1.61E-16**	**1.61E-16**	**1.61E-16**	**1.18E-09**	**1.70E-15**	**2.76E-15**	**4.96E-10**	**3.02E-12**	**1.32E-12**
Population Size										
	DHI Sim 1	**DHI Sim 2**	**DHI Sim 3**	**DHI Sim 4**	**DHI Sim 5**	**DHI Sim 6**	**DHI Sim 7**	**DHI Sim 8**	**DHI Sim 9**	**DHI Sim 10**
DHI Sim 2	0.33604951	NA	NA	NA	NA	NA	NA	NA	NA	NA
DHI Sim 3	0.14307435	**0.04874016**	NA	NA	NA	NA	NA	NA	NA	NA
DHI Sim 4	0.16153061	0.06231325	0.9919901	NA	NA	NA	NA	NA	NA	NA
DHI Sim 5	0.96145691	0.28476298	0.09993078	0.14631337	NA	NA	NA	NA	NA	NA
DHI Sim 6	0.16728272	0.05293725	0.903242	0.90804473	0.15858551	NA	NA	NA	NA	NA
DHI Sim 7	0.1123439	0.4180602	**0.0097982**	**0.01179315**	0.09778416	**0.01085642**	NA	NA	NA	NA
DHI Sim 8	0.79849807	0.24578519	0.19060443	0.25376609	0.79849807	0.25376609	0.06231325	NA	NA	NA
DHI Sim 9	0.70279408	0.59705509	0.06231325	0.09778416	0.69998155	0.09778416	0.16728272	0.54445743	NA	NA
DHI Sim 10	0.16919354	0.73663166	**0.0097982**	**0.01228571**	0.14001151	**0.01179315**	0.70279408	0.09778416	0.29432204	NA
DHI Sim 11	**0.03577634**	0.08647022	**0.00741652**	**0.00741652**	**0.02393829**	**0.00741652**	0.28476298	**0.01542926**	0.05293725	0.16153061
Probability of Extinction										
	DHI Sim 1	**DHI Sim 2**	**DHI Sim 3**	**DHI Sim 4**	**DHI Sim 5**	**DHI Sim 6**	**DHI Sim 7**	**DHI Sim 8**	**DHI Sim 9**	**DHI Sim 10**
DHI Sim 2	0.30275853	NA	NA	NA	NA	NA	NA	NA	NA	NA
DHI Sim 3	0.1954	**0.03589532**	NA	NA	NA	NA	NA	NA	NA	NA
DHI Sim 4	0.35148892	0.09044384	0.62412539	NA	NA	NA	NA	NA	NA	NA
DHI Sim 5	1	0.30275853	0.1954	0.35148892	NA	NA	NA	NA	NA	NA
DHI Sim 6	0.80824867	0.44130498	0.146375	0.30275853	0.80824867	NA	NA	NA	NA	NA
DHI Sim 7	0.30275853	1	**0.03589532**	0.09044384	0.30275853	0.44130498	NA	NA	NA	NA
DHI Sim 8	1	0.30275853	0.20185406	0.370503	1	0.80824867	0.30275853	NA	NA	NA
DHI Sim 9	0.51104919	0.66621051	0.09044384	0.14675744	0.51104919	0.72275943	0.66621051	0.51104919	NA	NA
DHI Sim 10	0.146375	0.44130498	**0.02587765**	**0.03589532**	0.146375	0.20185406	0.44130498	0.146375	0.30275853	NA
DHI Sim 11	0.146375	0.44130498	**0.02587765**	**0.03589532**	0.146375	0.20185406	0.44130498	0.146375	0.30275853	NA

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Supplementary Information 4

Results of binomial logistic regression analysis of sensitivity testing for three life-history parameters (lethal equivalents, % males in breeding pool and % mortality after age 1) and their impact on GSNR populations.

	Parameter	Estimate	Std. error	z-value	P-value
Population size	Lethal equivalents	3.879e-14	6.937e-02	0	1
	% Males in breeding pool	1.507e-14	9.150e-03	0	1
	% Mortality after age 1	1.512e-14	2.965e-02	0	1
Heterozygosity	Lethal equivelants	-0.0003	0.076	-0.004	0.996
	% Males in breeding pool	0.0004	0.01	0.039	0.969
	% Mortality after age 1	-0.014	0.033	-0.435	0.664
Inbreeding	Lethal equivalents	-0.01	0.084	-0.113	0.91
	% Males in breeding pool	-0.0003	0.011	-0.023	0.98
	% Mortality after age 1	0.015	0.036	0.411	0.68
Extinction probability	Lethal equivalents	0.221	3.542	0.062	0.95
	% Males in breeding pool	-0.043	0.522	-0.082	0.934
	% Mortality after age 1	0.247	1.916	0.129	0.897

Supplementary Information 5

Expected heterozygosity, inbreeding coefficients and population size of each founder population under different harvesting scenarios at years 1 and 5. Symbols denote the following; *single source, †multiple source, ‡low founder numbers, §baseline founder numbers, ¶high founder numbers, #skewed harvesting ratio.

Source Population	Model	Expected Heterozygosity			Inbreeding Coefficient			Population Size		
		Year 1	**Year 5**	**Year 10**	**Year 1**	**Year 5**	**Year 10**	**Year 1**	**Year 5**	**Year 10**
East Franklin Island	**Sim 1***§	0.305	0.3042	0.3033	0.6945	0.6949	0.6959	654.84	707.29	705.8
	Sim 3*‡	0.305	0.3043	0.3033	0.6946	0.6949	0.6958	676.03	706.88	711.65
	Sim 5†§	0.305	0.3043	0.3034	0.6945	0.6949	0.6958	676.02	716.98	715.8
	Sim 6†‡	0.305	0.3043	0.3033	0.6945	0.6949	0.6958	687.84	710.45	703.8
	Sim 7†¶	0.305	0.3043	0.3034	0.6945	0.6949	0.6959	688.05	714.28	716.07
	Sim 8$^{†§#}$	0.305	0.3043	0.3033	0.6945	0.695	0.6958	687.37	706.8	711.63
	Sim 9$^{†§#}$	0.305	0.3043	0.3033	0.6946	0.6949	0.6958	673.11	705.06	703.42
	Sim 10$^{†¶#}$	0.305	0.3043	0.3034	0.6945	0.6949	0.6957	687.51	712.82	710.89
	Sim 11$^{†¶#}$	0.305	0.3043	0.3034	0.6945	0.6949	0.6958	684.47	717.41	710.39
West Franklin Island	**Sim 1***§	0.2906	0.29	0.2891	0.7089	0.7093	0.7101	654.4	703.68	710.16
	Sim 3*‡	0.2906	0.29	0.2891	0.7089	0.7093	0.7102	674.22	705.47	712.48
	Sim 5†§	0.2903	0.2897	0.2888	0.7092	0.7096	0.7104	683.15	709.76	703.71
	Sim 6†‡	0.2903	0.2897	0.2887	0.7092	0.7096	0.7105	688.11	711.67	709.75
	Sim 7†¶	0.2903	0.2896	0.2887	0.7093	0.7097	0.7105	689.55	709.6	711.06

	Sim 8[†§#]	0.2903	0.2896	0.2887	0.7093	0.7097	0.7105	680.57	713.01	703.95
	Sim 9[†§#]	0.2903	0.2896	0.2887	0.7093	0.7096	0.7105	683.4	705.3	701.27
	Sim 10[†¶#]	0.2903	0.2897	0.2888	0.7093	0.7096	0.7105	679.4	711.88	707.46
	Sim 11[†¶#]	0.2903	0.2897	0.2888	0.7093	0.7096	0.7104	681.07	709.47	706.19
Salutation Island	**Sim 2**[*§]	0.3165	0.3154	0.3139	0.6831	0.6835	0.6849	551.38	526.45	529.89
	Sim 4[*‡]	0.3165	0.3154	0.3139	0.683	0.6835	0.6848	556.42	531.33	527.6
	Sim 5[†§]	0.3165	0.3156	0.3143	0.683	0.6834	0.6845	556.59	527.57	524.99
	Sim 6[†‡]	0.3166	0.3156	0.3143	0.683	0.6833	0.6846	563.68	522.67	531.87
	Sim 7[†¶]	0.3165	0.3156	0.3143	0.683	0.6834	0.6845	531.14	530.84	529.89
	Sim 8[†§#]	0.3165	0.3156	0.3143	0.683	0.6834	0.6846	559.13	527.05	534.07
	Sim 9[†§#]	0.3165	0.3156	0.3143	0.683	0.6834	0.6846	543.51	530.06	536.09
	Sim 10[†¶#]	0.3165	0.3155	0.3142	0.683	0.6834	0.6846	522.01	525.88	525.48
	Sim 11[†¶#]	0.3164	0.3155	0.3142	0.683	0.6835	0.6846	513.68	527.47	530.38

Chapter 7

General Discussion

Thesis Discussion

Soulé (1985) defined the goal of conservation biology as providing principles and tools for preserving biological diversity. The discipline seeks to identify vital questions about the biology and ecology of a species, and to provide answers that can be harnessed and applied in a management context. Some of the major challenges encountered by proponents of conservation biology include deficiencies in species-specific demographic knowledge for threatened taxa, the management of genetic diversity in fragmented and bottlenecked populations, and the added pressures of a rapidly changing climate (McCarty 2001; Kim and Byrne 2006; Root and Schneider 2006; Frankham 2010; Conde et al. 2019). In this thesis, I have taken a multi-disciplinary approach to a broad ecological study of a species of conservation concern, the greater stick-nest rat (*Leporillus conditor*), to provide tools and principles required for its ongoing conservation. I have used a combination of field studies, genetics, morphology, and population modelling to resolve previously unanswered biological questions about this species, and to make informed suggestions for its ongoing management under the pressures of projected climate change. I have shown the importance of genetic considerations for effective, long-term threatened species recovery programs, and have highlighted the need for adaptive management under climate change. Ultimately, these tools will assist in the implementation of more informed translocation and conservation initiatives, increasing resilience to climate change and improving genetic diversity in threatened, bottlenecked species (Figure 1). In this chapter I will summarise my findings and their significance to conservation biology as a whole, and call attention to areas requiring further research.

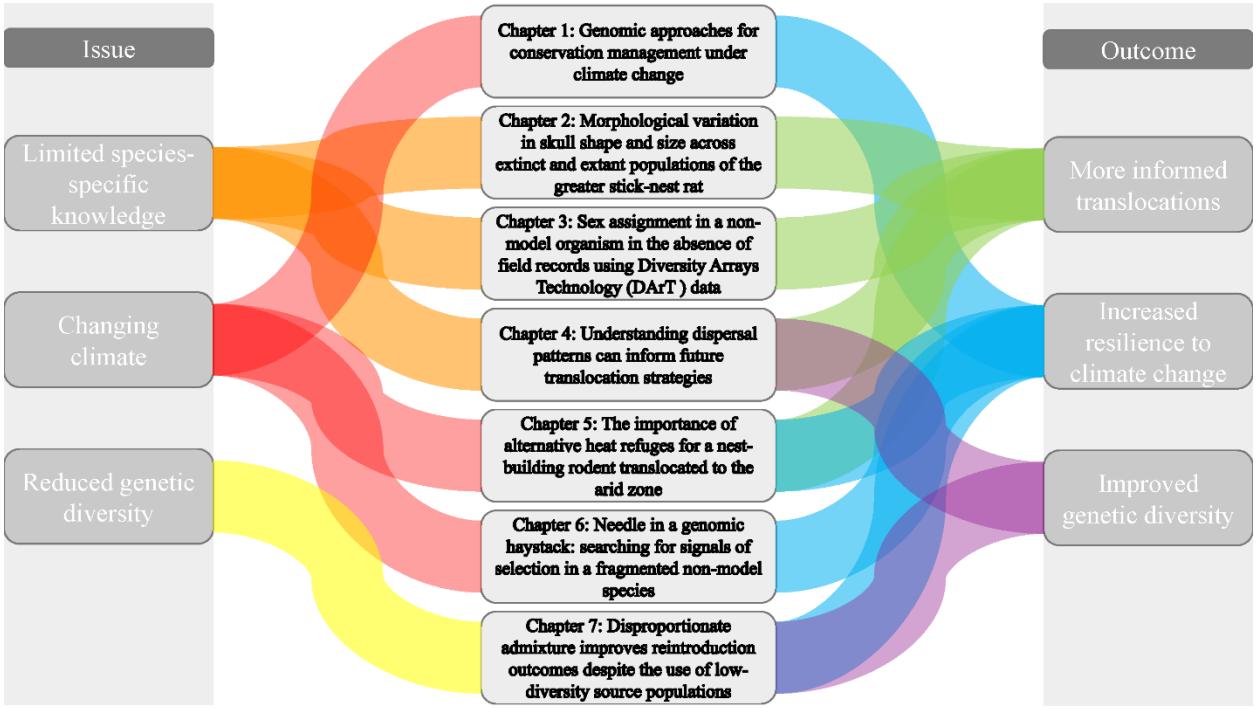

Figure 1. A summary of the chapters contained within this thesis, the common issues associated with conservation biology that they address, and the combined outcomes of this research.

Summary of Findings

Quantifying the past diversity of greater stick-nest rats

Quantifying past diversity in threatened species is a critical step towards designing recovery plans. This information allows conservation managers to set goals for the recovery of the species, as well as determining appropriate reintroduction sites and identifying any local adaptations that may influence persistence following translocation. The results of Chapter Two provide insight into the historical diversity of greater stick-nest rats. The species was once found across a variety of habitats and bioclimates, from offshore islands to the central arid zone. While limited cranial shape variation existed between populations that may indicate local adaptation to food sources or environmental pressures, the remaining extant population on the Franklin Islands was significantly larger in size than most mainland populations, apart from those in central Australia. Encouragingly, this may be evidence that the greater stick-nest rat conforms to an evolutionary pattern observed in many Australian rodents in which a generalised skull shape allows for success in a variety of habitats, and so local morphological adaptation may not be of concern for translocated populations.

Understanding contemporary populations

Effective, tailored and adaptive threatened species management requires detailed understanding of the biology and demography of the species in question. This thesis comprises of a number of studies that have contributed to a better understanding of the social structure, dispersal behaviours and habitat requirements of extant greater stick-nest rat populations, and provided tools for genomic studies of non-model species. Chapter Three demonstrated that a bioinformatics pipeline originally designed for sex determination of shotgun sequencing samples could be successfully applied to ddRAD-seq data generated by Diversity Arrays Technology Pty Ltd (DArT), a popular platform for conservation genomics studies. Furthermore, this study showed that the pipeline could be applied to a non-model species using a diverged reference genome, and is therefore a valuable tool for understudied and threatened species. Informed by the results of Chapter Three, Chapter Four used SNP data and field monitoring data to show the first empirical evidence of sex-biased dispersal and female philopatry in the greater stick-nest rat. Chapter Four quantified the average dispersal distance of male and female greater stick-nest rats at Arid Recovery Reserve, and used the findings to provide clear direction for future spatially-sensitive harvesting strategies when implementing a translocation. Implementing these recommendations will help future conservation managers to maximise genetic diversity and establishment in new populations of greater stick-nest rats.

Chapter Five used field monitoring data and statistical analysis to compare internal nest temperatures of greater stick-nest rat sites at two locations, one coastal and one arid. This study showed that thermal capabilities of nests were of much greater importance under the extreme temperature variation of the arid zone, and that bettong warrens provided an effective climate refuge during heat waves. Further, it demonstrated that man-made rock refuges in the arid environment were also effective thermal buffers of both extreme cold and extreme heat, more so than nests built beneath vegetation. This study has important implications for the management of the species under increasingly frequent heat waves and climatic extremes, and highlights the importance of alternative climate refuges for greater stick-nest rats and other small mammals experiencing such conditions.

Planning for the future

The goalposts of conservation management are rapidly shifting under climate change. The aim is no longer to simply restore and recover what has been lost, but to safeguard populations against the rapid environmental changes of the near future. For greater stick-nest rats and other fragmented species, this means that adaptive management is required to build resilience against climate change. Chapter Six of this thesis applied genome-environment association tests (GEAs) to determine whether greater stick-nest rats at Arid Recovery had undergone adaptation to heat stress in the two decades following their translocation from the more mesic source region, with the hope that this may indicate adaptive resilience to the projected conditions under climate change and therefore an ideal source population for targeted gene flow (explored in Chapter One). A signal of selection was detected on a genomic region associated with hypothyroidism in the house mouse (*Mus musculus*) reference genome. Hypothyroidism has been associated with improved heat stress survival in several taxa, and may therefore be evidence of heat stress adaptation in the Arid Recovery population of greater stick-nest rats. However, given that no reference genome exists for the greater stick-nest rat, and the population is highly fragmented, GEAs are unlikely to be a reliable method of determining adaptive genomic responses in greater stick-nest rats or, indeed, many other Australian threatened species that are similarly fragmented and data-deficient.

Maximising genetic diversity is another way in which populations can be safeguarded against climate change, as the chances of heat or drought resistant alleles existing within a population are increased. Chapter Seven incorporated genetic data into a population viability analysis (PVA) to predict the optimal harvesting strategy for a translocation of greater stick-nest rats to Dirk Hartog Island, Western Australia, that would maximise genetic diversity in the new population and minimise harvesting pressure on the single remaining extant ("critical") population. The results of the PVA supported the use of high founder numbers and multiple source populations for improving genetic diversity and reducing extinction risk in the new population. Further, it revealed that skewed admixture, wherein a small proportion of individuals were sourced from the critical population and the remainder from a genetically depauperate population, could still produce favourable outcomes whilst protecting the critical source population from the pressures of overharvesting.

Implications for Conservation Biology

Wildlife conservation is a multi-disciplinary science, and must take into consideration species' life history, ecology and genetics. Further, when planning for future climate change, it must be acknowledged that adaptation can come in many forms, be it behavioural, phenotypic, or at the genetic level. Taken together, the results of this thesis provide a multi-faceted toolkit for the effective management of a threatened endemic species under climate change. For greater stick-nest rats, future translocations and management programs should consider a spatially sensitive harvesting strategy with multiple source populations and high founding numbers, with a smaller proportion of individuals taken from the relict source population of the Franklin Islands. Conservation efforts for the species should ensure that adequate nesting materials are available for the establishment of family groups, and that climate refugia such as warrens and rock piles are accessible during periods of climatic extremes. Recovery efforts for the species should work towards reconstructing the historical geographic range of the species and maximising its genetic diversity while monitoring morphological change post-translocation. Finally, a concerted effort should be made towards sequencing a reference genome for this species, so that detailed analyses targeting responses to climate change at the genetic level can be undertaken in order to further improve conservation management of the greater stick-nest rat. Access to a reference genome for this species would allow researchers to search for functional adaptations in response to selection pressures associated with climate change, such as heat or drought tolerance, and use this knowledge to inform future management of the species.

These findings are also applicable on a broad scale. Many taxa in Australia and worldwide exist in small, fragmented populations with reduced genetic diversity, making them all the more vulnerable to the pressures of climate change. With so much of our precious biodiversity at risk of extinction, we can rarely afford to be experimental – this thesis demonstrates that an adaptive management approach guided by data and sound biological knowledge is extremely valuable for conservation success in a changing environment.

Firstly, reconstructing or restoring lost diversity is not possible without understanding the historical diversity and range of the species in question. For this purpose, natural history collections are an invaluable resource, and should be utilised wherever possible to inform conservation practices. Many reintroduction programs are planned and operated without

incorporating knowledge of the morphological and genetic diversity of extirpated, with potentially adverse consequences. We are learning more and more about the cryptic nature of taxonomy, particularly in Australia; recent studies on bandicoots, for example, have resulted in the reclassification of subspecies and the identification of new species (Travouillon and Phillips 2018; Travouillon et al. 2019). Individual populations of a species are not always equal in terms of local adaptations and habitat requirements; it is therefore important for future reintroductions to consider past diversity and conduct "like-for-like" translocations wherever possible.

Further, for translocations to be executed successfully at both the harvest and release stages, knowledge of a species' dispersal behaviours is critical. Sex-biased dispersal was found to contribute to fine-scale genetic heterogeneity across the landscape in our study population of greater stick-nest rats, an important consideration for future translocation harvesting and management strategies. Further, the nesting behaviour of the species clearly contributes to the spatial genetic structure and dispersal patterns. Might such patterns be of importance in other species, particularly those that use shelters such as nests or burrows? The greater bilby (*Macrotis lagotis*) or the Shark Bay mouse (*Pseudomys fieldi*), for example? Combining biological and genetic data with decision-making tools such as Pacioni et al.'s (2020) spatial trapping design or the decision-tree approach presented in Ebrahimi et al. (2015) when planning harvest, holding, and release stages of a translocation could significantly improve the outcome of translocation programs in the future.

Populations, translocated or otherwise, experiencing heat stress are unlikely to persist without adequate habitat and climate refugia, so managers must also ensure that a variety of heat-resistant microclimates are available that suit the species' sheltering preferences (e.g. burrows, tree hollows, vegetation or rocky outcrops). More than 300 Australian species utilise tree hollows for nesting or shelter (Gibbons and Lindenmayer 2002), with many others relying on burrows, caves, rocky outcrops and nests. As such, the availability of thermally buffered refugia should be a consideration for most conservation programs. Where habitats are degraded, the provision of such shelters is of particular importance, and can be extremely effective. In a study by Croak et al. (2010), artificial rocks crafted from cement provided similar thermal regimes to natural rocks, and were colonised by many invertebrate and reptile species within 40 weeks of installation. Not all shelters are equal, however; for example, a

recent study on two types of artificial hollows provided for the Leadbeater's possum (*Gymnobelideus leadbeateri*) found that temperatures were far more stable in chainsaw hollows than nest boxes, which reached temperatures as high as 48.5°C and as low as -5.5°C (McComb et al. 2021). Higher temperatures in nest boxes and other shelters with poor thermoregulatory buffering increase the likelihood of dehydration and heat-stress in the inhabiting taxa (Rowland et al. 2017), ultimately leading to higher mortality. It is therefore of utmost importance that managers test and monitor microclimates within sheltering sites, both natural and artificial, in order to optimise the refugia available to threatened species, particularly under the increasingly frequent heat waves predicted under climate change.

Lastly, with adequate biological data and genetic information, population modelling can be used to plan best-practice translocation and supplementation programs and minimise the risk of population crashes or loss of genetic diversity, in both the source and translocated populations. A recent review of published studies on wild-sourced translocations found that only 11% estimated the impact of harvest on the source population (Mitchell et al. 2021). The application of population modelling tools such as PVA should, in future, be a priority for translocation planning in order to ensure source sustainability.

Areas for Future Research

Most of the approaches discussed in this thesis require at least some degree of genetic knowledge to be performed in-depth. Fortunately, high-throughput next-generation sequencing is becoming more accessible every year. Commercial platforms such as Diversity Arrays Pty Ltd provide a means for conservation researchers and practitioners to gain access to whole or reduced-representation genome sequencing of individuals and populations. This genetic information can inform on not only genetic diversity and levels of inbreeding, but on adaptive traits that may increase resilience to climate change. Such technology could even be combined with other valuable conservation resources, both new and old, to enhance our management capabilities in the future. The results of Chapter Two could be further explored in the future using genetic analysis to determine whether the historical variation in size of greater stick-nest rats represents phenotypic plasticity or genetic variation. Further, next-generation sequencing could be used to identify heat-adapted genes in extirpated populations of species which are represented by specimens in natural history collections (Card et al.

2021). These genes could then be inserted into the genome of contemporary populations that are maladapted to high temperatures using CRISPR gene-editing technology. Such an approach is already being employed for de-extinction research into the passenger pigeon (*Ectopistes migratorius*) (Hung et al. 2013; Servick 2013) – it follows that gene editing is a tool that could be utilised to prevent, as well as reverse, extinction.

Given the complex nature of reintroduction biology and the many considerations required for successful establishment (a number of which have been explored in this thesis), future translocations should be guided by two main questions at the outset. Firstly, is the site appropriate? Many translocations occur on the peripheral edge of the species in question's historical range, with little consideration for the suitability of the site under climate change projections. Species distribution models (SDMs) combined with future climate scenarios can be applied here to great effect. The second consideration should then be, is the species appropriate? There is a significant lack of equal representation of native species in conservation reserves. During the period of 2010-2017, 11 "safe havens" were established that provided protection for 16 native species susceptible to predation – however, these species were already well represented in conservation reserves and did not add any new taxa to the haven network (Ringma et al. 2018). Ringma et al. (2019) present a systematic framework to address and close this representation gap by creating more reserves and adding predator-susceptible, underrepresented taxa in a fair way. The role of the species in the ecological community is also important to consider; if the goal is reconstructing a diverse, functional ecosystem, managers should carefully consider trophic levels and ecological niches, and the order in which these should be filled. Native meso- and top order predators, for example, should not be reintroduced into communities that do not have an adequate abundance of appropriate prey species.

Finally, and perhaps most importantly, there is a need for greater transparency and timely reporting of translocation failure. Translocation failures, as well as challenges faced during translocation programs, are rarely reported in peer reviewed journals, making it difficult for future reintroduction programs to be informed by problems from the past and subsequently improved (Germano and Bishop 2009; Berger-Tal et al. 2020). Fischer and Lindenmayer (2000) found that 49% of reviewed reintroduction case studies did not explicitly identify causes of decline. Further, 40% of translocations reviewed by Short (2009) had an

"indeterminate outcome". The accumulated knowledge from failed translocations is equally important to the science of reintroduction biology as that of successful translocations. It is therefore vital that conservation managers are accountable for reporting translocation outcomes even when they are unfavourable, and that scientific journals seek to address the publication bias that hinders the dissemination of results considered to be "negative" or "nonsignificant" (Scargle 1999). To encourage transparency, the conservation community needs to work towards removing the stigma and judgement associated with negative outcomes when managers have worked to the best of their knowledge and ability towards population establishment. Translocation approvals issued by government bodies should also incorporate a data sharing agreement which obliges proponents to report translocation outcomes either in a peer reviewed journal or on a public repository such as bioRxiv.

Conclusion

In a time of unprecedented biodiversity loss and environmental change, adaptive conservation strategies are more vital than ever before. A "preservationist" approach to threatened species management is no longer possible or appropriate – instead, conservationists must move to build resilience and adaptability in the populations they protect through methods such as translocation, habitat provision, assisted gene flow and genetic rescue. In this thesis, I have contributed to the biological knowledge of one threatened species, the greater stick-nest rat, and explored a number of tools to further inform its future conservation. The findings of this research can be extrapolated to other Australian endemics with similar histories of range contraction, fragmentation and genetic bottlenecks, and will contribute to the ongoing protection of threatened species in the face of anthropogenic climate change.

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