2021

A genetic investigation of anticoagulant rodenticide resistance in Mus musculus of Western Australia: Implications for conservation and biosecurity

Bridget Judith Maria Lucrezia Duncan

Edith Cowan University

Copyright Warning

A genetic investigation of anticoagulant rodenticide resistance in *Mus musculus* of Western Australia: Implications for conservation and biosecurity

Bridget Judith Maria Lucrezia Duncan

Bachelor of Science (Biological Sciences)

School of Science, Edith Cowan University

31/05/2019

Abstract

Human-wildlife interactions have developed since the agricultural revolution that occurred 10,000 years ago, and the expansion of commensal species' geographical distribution led to conflicts that prompted humans to adopt a wide range of control methods for pest species (Horvitz, Wang, Wan, & Nathan, 2017; Riyahi et al., 2013; Saraswat, Sinha, & Radhakrishna, 2015). The order Rodentia is characterised by a high number of successful invaders, which humans have attempted to manage with the use of anticoagulant rodenticides (ARs) since the 1940s (Capizzi, Bertolino, & Mortelliti, 2014; Ruiz-Suárez et al., 2014). The rise and spread of a genetic mutation that infers AR resistance among mice and rats led to the production of stronger second generation compounds, which are characterised by higher toxicity and longer persistence in the liver tissue (Lohr & Davis, 2018; Ruiz-Suárez et al., 2014). These traits have led to AR residues being detected in a variety of non-target organisms from both terrestrial and aquatic environments (Kotthoff et al., 2018; López-Perea, Camarero, Sánchez-Barbudo, & Mateo, 2019; Rattner, Lazarus, Elliott, Shore, & Van Den Brink, 2014a). Additionally, the impact of ARs on non-target species is exacerbated when rodents are resistant to the poison as they become capable of transmitting high doses to their predators.

To reduce negative effects on the ecosystem, rodent eradication requires information on the presence of resistance within populations; this has been intensely studied in Europe through laboratory feeding trials, blood clotting response tests and genetic screening of the *Vkorc1* gene (Goulois, Lambert, Legros, Benoit, & Lattard, 2016; Grandemange, Lasseur, Longin-Sauvageon, Benoit, & Berny, 2010; Mayumi Ishizuka et al., 2007; Pelz et al., 2012). In Australia, the only available information on resistance of rodent populations comes from a study in 1975 on black rats from Sydney, and another study on *Mus musculus* of Lord Howe Island, New South Wales (Billing, 2000; Saunders, 1978; Wheeler et al., 2018).

Since the house mouse (*Mus musculus)* exhibits a degree of natural tolerance to rodenticides (Cowan et al., 2017), and its eradication has higher failure rate compared to rats (Howald et al., 2007), it is vital to know whether particular populations possess mutations that may infer resistance, and how common the mutations are within the population. The aim of this study was to produce the first data showing whether *Vkorc1* mutations that may provide anticoagulant resistance in house mouse are present in Western Australia by sampling populations from the Perth metropolitan area, which is continuously exposed to ARs, and from Browse Island, which has no history of exposure. Additionally, the mitochondrial D-loop of house mice was sequenced to investigate population genetic structure, identify the origin of Western Australian mice, and to elucidate whether resistance was linked to certain haplotypes.

No resistance-related *Vkorc1* mutations have been detected in either house mouse populations. A genetic introgression in the intronic sequence of the *Vkorc1* gene of Browse Island house mouse was detected and it is thought to have originated through hybridisation with the Algerian mouse (*Mus spretus*). Analysis of the mitochondrial D-loop reported two haplotypes in the house mouse population of Perth, and two haplotypes in the population of Browse Island.

The findings suggest that both house mouse populations exhibit no genetic resistance to ARs, and therefore less strong rodenticides can be employed in pest control and eradication attempts, which will result in a less negative impact on non-target species. Biosecurity measures must be in place to avoid potentially resistant house mice to enter Western Australia, and to prevent the introduction of new house mouse subspecies on mainland, such as the one found on Browse Island.

Copyright and access declaration

I certify that this thesis does not, to the best of my knowledge and belief:

(i) incorporate without acknowledgment any material previously submitted for a degree or diploma in any institution of higher education;

(ii) contain any material previously published or written by another person except where due reference is made in the text; or

(iii) contain any defamatory material

Signed.....  .....

Dated.....31/05/2019.....

Acknowledgements

I would like to express my appreciation to my supervisors Associate Professor Annette Koenders, Dr Quinton Burnham, and Mr Michael Lohr for their valuable knowledge, enthusiastic guidance and constructive feedback during the development of this Honours research project. My grateful thanks are extended to the Edith Cowan University School of Science and the Centre for Ecosystem Management for providing additional funding to promote my research. I would also like to thank the staff of the Department of Biodiversity, Conservation and Attractions for donating house mouse samples to my research and for their useful insights into the history of human movements on Browse Island. Finally, I would like to thank Karl Zwickl for his help with ArcMap, and Alexis Mann for her support.

Table of contents

Table of tables

Table of figures

1 Introduction

The agricultural revolution that occurred 10,000 years ago brought about a substantial change in the environment as humans cleared land and created a new habitat matrix that threatened the existence of certain species while allowing others to thrive and expand (Horvitz et al., 2017; Riyahi et al., 2013). As a result, human-induced biological invasions are an important component of the environmental changes that threaten global biodiversity, and while the impact of urbanisation on native species has been extensively studied, the resulting establishment of commensal species has been scarcely researched (Tang, Low, Lim, Gwee, & Rheindt, 2018).

A wide range of species developed commensal relationships with humans when agriculture techniques arose, such as the granivorous house sparrow (*Passer domesticus*) and the rock pigeon (*Columbia livia*), which were able to exploit the new abundance of seeds as a food source (Riyahi et al., 2013; Tang et al., 2018). Establishing a commensal relationship with humans resulted in the expansion of the geographical distribution of these species, and a substantial increase in population size (Riyahi et al., 2013). Rodents have also been extremely successful at invading the new environments created by humans, in large part due to being *r*-selected species tolerant of a wide range of climates and habitats (Brouat et al., 2014; Yin et al., 2008). Commensal rodents create a wide range of negative issues for humans and the environment, which has led to efforts to eradicate them via physical, biological and chemical means (Clout & Russell, 2006). Rodenticides have become widely used, however, they have their own negative impacts on the environment and mutations have arisen within rodents that infer resistance to the chemicals used (Hindmarch, Elliott, Mccann, & Levesque, 2017; Ishizuka et al., 2008; Ishizuka et al., 2007; Masuda, Fisher, & Jamieson, 2014). This situation is of serious concern for those who are responsible for pest management and biosecurity (Capizzi et al., 2014; Desvars-Larrive et al., 2018; Harris, 2009; Howald et al., 2007).

1.1 Invasive rodents and their impact

The Order Rodentia is characterised by having the largest number of species among all mammal orders, including many pest species (Capizzi et al., 2014). Rodents are ubiquitous on all major landmasses (except Antarctica) (Australian Centre for International Agricultural Research, 2003; Pocock, Hauffe, & Searle, 2005), having been recorded on 80% of the world's large islands (Howald et al., 2007). There is evidence that in relatively recent times numerous species of rats and mice have expanded their geographical distribution, and that this expansion mirrors human phylogeographic patterns (Brouat et al., 2014; Lippens et al., 2017). This pattern of expansion provides a strong indication that the adaptability of rodent species allows them to survive challenging environmental conditions and to exploit resources provided by the human population as a commensal species (Brouat et al., 2014). The link between humans and commensal rodents is so strong that studying the phylogeographic patterns of species of rats and mice has resulted in the discovery of human migration routes. For example, the regional movements of humans during prehistoric times in Southeast Asia have been uncovered through the study of the genetics of the Polynesian rat (*Rattus exulans*), while the distribution of mitochondrial lineages of house mouse (*Mus musculus*) has been used to infer the relationships between human populations around the Mediterranean during the Iron Age (Bonhomme et al., 2011; Matisoo-Smith & Robins, 2004).

The close relationship between humans and commensal rodents generally comes at a cost to the human population and endemic wildlife (Riyahi et al., 2013; Saraswat et al., 2015). Human-wildlife conflict often arises when the niches of humans and wildlife species overlap, or when wildlife threatens the economy or health of humans, such as spoiling crops or carrying zoonoses (Saraswat et al., 2015). The impact of invasive rodents is multifaceted and complex, with effects that range from altering human activities to deteriorating ecosystem health. The most widespread and well-known pest rodents are the rat species *Rattus rattus* and *Rattus norvegicus,* and the house mouse *M. musculus*; these species have severe impacts on economy, food safety, livestock farming, public health, and ecosystems (Capizzi et al., 2014).

It has been calculated that rodents in China are the cause of pre-harvest rice production losses of 5-10% annually, which accounts for more than 30 million tonnes of rice; this amount of produce could be used to feed 180 million people for a year (Singleton, 2003). Rodents can adversely impact urban areas, as they do structural damage to buildings and carry zoonoses (Australian Centre for International Agricultural Research, 2003; Capizzi et al., 2014), such as plague epidemics that human populations experience due to transmission by rodents (Yin et al., 2008). Invasive rodent species affect ecosystems in a variety of ways, including the introduction of disease-carrying fleas, direct predation on indigenous species, and they compete for resources resulting in the exclusion of native species (Wanless, Angel, Cuthbert, Hilton, & Ryan, 2007; Wyatt et al., 2008). Rodents have been found responsible for the extinction of at least 50 species, and for negatively impacting at least another 170 faunal and floral taxa (Howald et al., 2007; Kappes, Bond, Russell, & Wanless, 2019; Russell, Abrahão, Silva, & Dias, 2018). For example, introduced black rats on Christmas Island in the Indian Ocean led to the extinction of the endemic rat *Rattus macleari* and the decline of *Rattus nativitatis* by transmitting fleas infected with a pathogen (Wyatt et al., 2008).

Although the black rat (*R. rattus*) is the most widespread rodent pest species worldwide, the house mouse is controlled for the widest variety of impacts (Capizzi et al., 2014). For many years the house mouse was thought to pose little threat compared to the aggressive *R. rattus*, however, studies have reported multiple instances of house mice being solely responsible for population decline of native or protected species, and even for causing local extinction (Cory, Wilson, Priddel, Carlile, & Klomp, 2011; Newman, 1994; St Clair, 2011; Wanless et al., 2007). Predation by *M. musculus* affects many invertebrates, such as snails, stick insects, weevils, millipedes, and flightless moths (Howald et al., 2007; St Clair, 2011) and reptiles, such as skinks and geckos (Newman, 1994). Predation on seabirds by house mouse has been documented; such as on Gough Island and Marion Island where both albatross and petrel population numbers have been hindered by *M. musculus* attacking chicks (Davies, Dilley, Bond, Cuthbert, & Ryan, 2015; Jones & Ryan, 2009; Wanless et al.,

2007). Furthermore, mouse populations have been found to indirectly affect forest birds because of hyperpredation, as the mice constitute a prey base that maintains high numbers of stoats, which also then prey on the birds (Howald et al., 2007; King, 1983). Mice not only affect fauna, they influence plant regeneration both directly and indirectly by harvesting seeds, grazing on seedlings, and through predation of decomposers (Cory et al., 2011; Jackson & Van Aarde, 2003), which alters the composition of the decomposer community, in turn affecting the soil nutrient mineralisation that is needed for plants to thrive (Cory et al., 2011; St Clair, 2011).

1.2 Rodent pest management

Management practices such as pest control and eradication programs have been implemented to reduce the impact of rodents on natural ecosystems and human environments. Commonly employed control methods include trapping, poisoning, habitat management, and repellents (Capizzi et al., 2014), and each method of control presents challenges that need to be evaluated on a case by case basis. For example, traps can affect native rodents or other small mammals, while poisons can have a detrimental impact on organisms higher in the food chain (Australian Centre for International Agricultural Research, 2003). Generally, poisons are the most commonly used method, followed by traps and habitat management (Capizzi et al., 2014). Due to their efficiency, anticoagulant rodenticides have been used to carry out eradications on over 300 islands with a 90% rate of success (Goulois et al., 2017; Howald et al., 2007).

Anticoagulant rodenticides are part of the vitamin K antagonist (AVK) substances, which block the vitamin K cycle (Goulois et al., 2017). AVKs are absorbed through the gastrointestinal tract and enter blood circulation, thereby leading to internal haemorrhage and eventually death of the poisoned rodent (Goulois et al., 2017; Horak, Fisher, & Hopkins, 2017). Anticoagulant rodenticides can be subdivided into two categories; first generation or second generation, according to when they were first synthesised and commercialised (Ruiz-Suárez et al., 2014). The first generation

anticoagulant rodenticides (FGARs) (warfarin, pindone, diphacinone and coumatetralyl) were developed in the 1940s and have been intensively employed globally, while the second generation rodenticides (SGARs) (brodifacoum, bromadiolone, difenacoum, difethialone and flocoumafen) were synthesised in the 1970s and are characterised by more acute toxicities than FGARs, which results in mortality after a single feed (Lohr & Davis, 2018; Ruiz-Suárez et al., 2014). SGARs require a lower bait concentration compared to FGARs as they are more precisely targeted to the proteins of interest and undergo less extensive metabolism compared to the less potent rodenticides (Horak et al., 2017).

1.3 Problems with the use of anticoagulant rodenticides

The use of rodenticides in eradication campaigns is sometimes protested by organisations that are worried about their toxicity potentially affecting the environment, humans and pets; additionally, concerns regarding animal rights might also halt a campaign (Howald et al., 2007). In Europe, there have been environmental concerns around the use of pesticides and rodenticides, such as the contamination of sewage treatment plants and receiving surface waters (Kotthoff et al., 2018), that culminated in the restriction of their use by a European Union directive and, following this new legislation, several insecticides have been removed from the market (Capizzi et al., 2014). For rodenticides, a lack of an environmentally friendly alternative whose efficacy has been demonstrated has meant producers of anticoagulant rodenticides have not followed the trend (Capizzi et al., 2014). This is despite environmental risk assessments that highlighted the high risk of secondary poisoning of non-target wildlife posed by all SGARs, which have been identified as persistent, very toxic and with bioaccumulative traits (Kotthoff et al., 2018). For example, even though brodifacoum use has led to instances of bird and mammal poisoning, the market availability of the poison in both the United States and United Kingdom has only been restricted to professionals but it has not been banned (Berney, Esther, Jacob, & Prescott, 2014; Howald et al., 2007; United States Environmental Protection Agency, 2017).

The issues related to the toxicity and persistence of ARs cannot be overstated as chemical residues have been detected in a wide variety of non-target species, from both terrestrial and aquatic environments (Kotthoff et al., 2018; López-Perea et al., 2019; Rattner, Lazarus, Elliott, Shore, & Van Den Brink, 2014b). The risk of secondary poisoning in non-target species that feed on rodents is high because in addition to the rodents that are killed by the poison and eaten, rodents that consume a sub-lethal amount of ARs can display modified behaviour that renders them easy prey (Lohr & Davis, 2018). Also, liver retention of the AR results in biomagnification and bioaccumulation of the chemicals in the food web (Lohr & Davis, 2018). This can affect a wide variety of animals, for instance in terrestrial environments, residues of rodenticides have been measured in animals including raptors, owls, polecats, foxes, snails, stoats, weasels, and hedgehogs (Alomar, Chabert, Coeurdassier, Vey, & Berny, 2018; Kotthoff et al., 2018).

1.4 Resistance to anticoagulant rodenticides

The presence of AR resistant rodents within populations was discovered in 1958, when resistance to warfarin and diphacinone was observed in *R. norvegicus*, and then in *M. musculus* (Pelz et al., 2005). Although data are still relatively scarce, the presence of resistance within populations appears to have expanded geographically and is likely to have increased in frequency within some populations; with resistance having also now been recorded for both first and second generation compounds (Zhelev, Koev, Dimitrov, & Petrov, 2019). Resistance to ARs in *M. musculus* populations has been linked to mutations in the *Vkorc1* gene, but may also result from changes to the cytochrome P450 genes or via polygenic interactions as has been suggested for rats (e.g. Ishizuka et al., 2007). Testing of *M. musculus* in Britain and continental Europe has been undertaken via feeding trials, blood clotting response tests, and/or genetic screening (Goulois et al., 2016; Grandemange et al., 2010; Pelz et al., 2012).

The most commonly identified cause of resistance is linked to single nucleotide polymorphisms in the *Vkorc1* gene, which results in a slight modification of the VKOR enzyme that impedes the anticoagulant rodenticides' ability to bind to the enzyme, and therefore the poison fails (Baert, Stuyck, Breyne, Maes, & Casaer, 2012). The mutations can arise independently and then be positively selected for in rodent populations that are exposed to ARs, but some mutations have also been found to be present as a result of introgression with other species. For example, hybridisation between *M. musculus* and the Algerian mouse (*Mus spretus*) has resulted in at least four missense mutations in the *Vkorc1* gene that resulted in strong resistance displayed by house mice in France, Spain and Germany (Goulois et al., 2016; Hedrick, 2013; Song et al., 2011a). However, mutations in the *Vkorc1* gene have also been linked to negative pleiotropic effects that are mainly related to defective activity of the VKOR enzyme, and therefore mutations are selected against when exposure to rodenticides is absent (Heiberg, Leirs, & Siegismund, 2006; Ishizuka et al., 2008; Jacob et al., 2012; Markussen, Heiberg, Nielsen, & Leirs, 2003).

1.5 Invasive rodents and anticoagulant rodenticide resistance in Australia

It is thought that *R. rattus*, *R. norvegicus* and *M. musculus* were all introduced to Australia by the first European colonisers ~230 years ago (Australian Centre for International Agricultural Research, 2003; Gabriel, Stevens, da Luz Mathias, & Searle, 2011). Of these, *M. musculus* is the agricultural pest with the most serious negative effects on Australian grain production, while orchards and vegetable crops are significantly affected by *R. rattus* (Australian Centre for International Agricultural Research, 2003). Invasive rodents also negatively impact the recruitment of native plants, with their presence being linked to reduced vegetation cover on islands belonging to the Montebello archipelago (Western Australia) (Lohr, Van Dongen, Huntley, Gibson, & Morris, 2014) and slow re-establishment of seedlings on Montague Island (New South Wales), where the eradication of *M. musculus* is expected to allow the native vegetation to regenerate (Cory et al., 2011). Furthermore, these rodents also pose a high risk to human health and the wellbeing of other wildlife as, for example, *M*. *musculus* in agricultural regions of Australia has

been tested for viruses and been found to be seropositive to eight different viruses, including a potential human pathogen (Smith, Singleton, Hansen, & Shellam, 1993).

Information regarding the presence and distribution of resistance in rodent populations of Australia is scarce, and entirely absent for *M. musculus* populations on the Australian mainland (Wheeler et al., 2018; Zhelev et al., 2019). In 1975, black rats from Sydney were tested for resistance to warfarin, and 80% of the individuals were found to be resistant; this study was critical in evaluating the mechanism of resistance resulting from a genetic mutation rather than acquired from gradual consumption (Saunders, 1978). In Western Australia, neither rats nor mice have been tested to quantify the levels of resistance to ARs despite the pervasive use of poisons by government agencies and the public, whose use of rodenticides is unmonitored and unregulated (Lohr & Davis, 2018). As ARs are the most commonly used class of chemicals to control rodent populations in Australia for both mainland and island populations (Lohr & Davis, 2018), it is critical to evaluate the levels of resistance in rodent populations in order to reduce the impact of ARs on non-target species.

1.6 Aim and study design

> The aim of this study is to investigate whether *Vkorc1* mutations that have been linked to anticoagulant rodenticide resistance are present within populations of *M. musculus* in Western Australia.

In order to achieve this, *M. musculus* will be sampled from two contrasting populations: one with high exposure to ARs in Perth, the capital city of, and largest city in, Western Australia; and a low exposure population on Browse Island, an uninhabited nature reserve situated on the Sahul Shelf approximately 160 km northwest from Western Australia's Kimberley coastline and 450 km from Broome (Figure 1; Clarke, 2010; Grund, 1996). This design allows for an investigation into

whether selection pressures for this potential resistance mechanism may be influencing its prevalence in mouse populations. The phylogeography of these populations will also be investigated (using DNA sequence data from the mitochondrial D-loop), as the distribution of mutations may be related to genetic connectivity within and between populations.

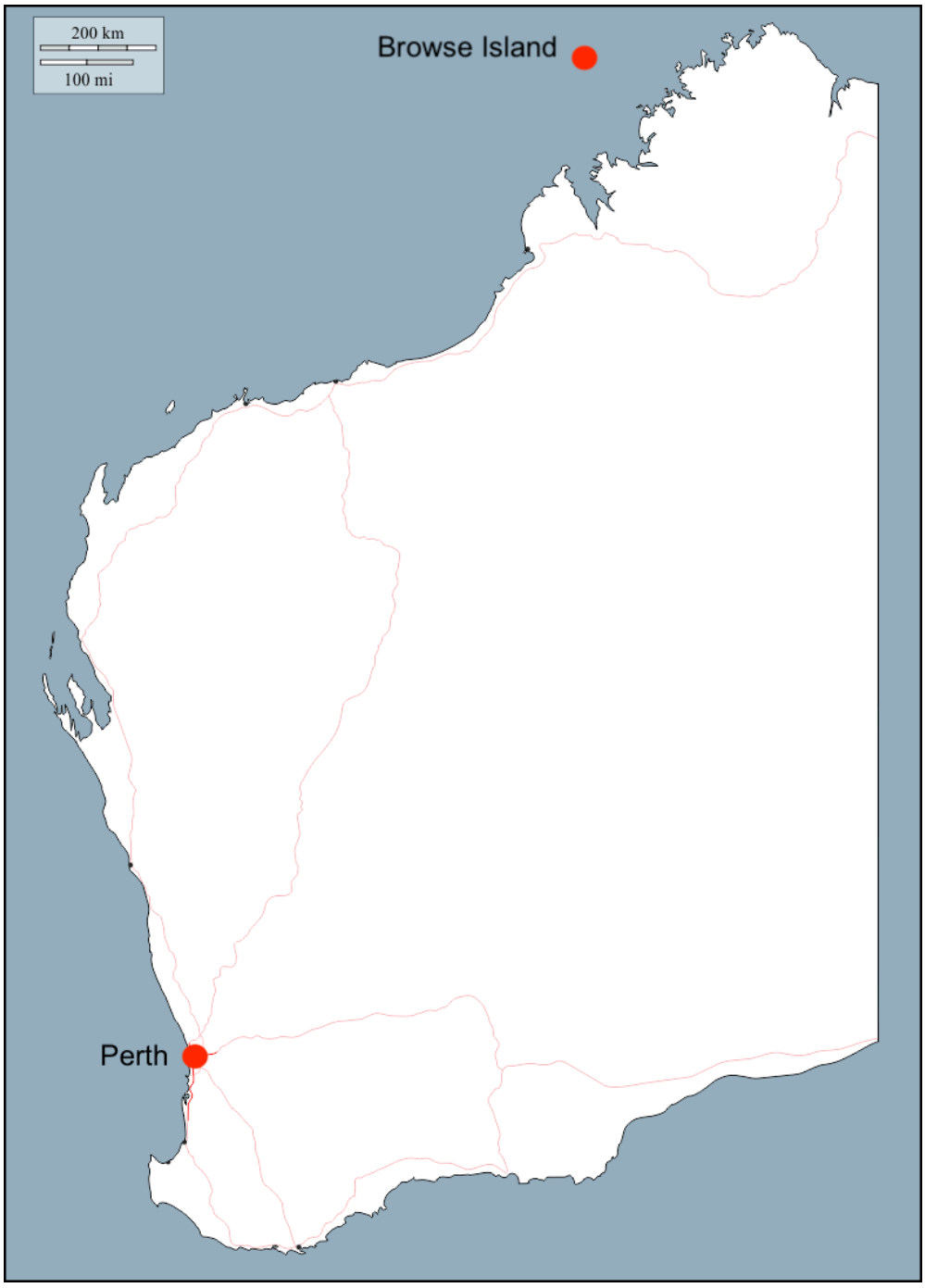


Figure 1 Map of Western Australia depicting locations for Perth and Browse Island.

Perth is the most isolated capital city in the world and is surrounded by the Indian Ocean to the west and 1.89 million ha of desert to the east (Christensen & Burrows, 1994). The city is connected to major commercial ports through the harbour town of Fremantle (Stannage, 2015). The isolated position of Perth resulted in the area being free of many pests found elsewhere in Australia as quarantine inspectors ensure that biosecurity measures are in place to protect the environment (Department of Primary Industries and Regional Development, 2015) and safeguard agricultural production and exports (Craik, Palmer, & Sheldrake, 2017). The natural environment is one of the main assets underpinning Australia's economy and trade being valued at $6 trillion in 2017 (Craik et al., 2017), and the economy of Perth is aided by the natural resources and ecosystem services provided; for example, it has been calculated that property prices are positively associated with proximity to wetlands and bushland, which are involved in carbon sequestration and removal of air pollutants as well as being aesthetically pleasing (Pauli & Boruff, 2016).

Despite the human population of Perth having reached over two million people at the end of 2018 (Australian Bureau of Statistics, 2018), the city still retains numerous areas of conservation importance including wetlands, coastal dune systems and bushland reserves recognised as containing extraordinary levels of biodiversity and endemism of both flora and fauna (Pauli & Boruff, 2016). Over 2000 species of plants have been recorded in the region and 10% of flowering plant species in the metropolitan area are listed as being of conservation concern (Pauli & Boruff, 2016). The western house mouse (*Mus musculus domesticus*) is present in Perth and likely has been since Europeans settled in the area. Therefore, it is vital to eradicate any resistant house mice that are present and/or to avoid the introduction of resistant house mice to Perth.

Browse Island is a small platform reef of 17 ha whose shore consists of eroded coral (Clarke, 2010; Moro, Palmer, Greatwich, Dickinson, & Anderson, 2018). The island supports populations of breeding seabirds and migratory shorebirds, as well as

endangered green turtles (Clarke, 2010; Limpus, 2002). There is evidence of human activities on the island since the 1800s, both in the presence of structures, and the introduction of alien species (such as weeds, cats and mice), which led to a profound alteration of the vegetation (Moro et al., 2018). The island has been protected by the Department of Biodiversity, Conservation and Attractions (DBCA) since 1991 (Moro et al., 2018). As the island is remote, the last recorded visit to the island prior to a visit from DBCA in August 2018 occurred in 2005 (Moro et al., 2018), however, there has been evidence of Indonesian fishermen landing on the island (Stacey, 2007).

House mice on the island have been trapped by the Department of Biodiversity, Conservation and Attractions in 2018, and were identified as being south-east Asian house mouse (*Mus musculus castaneus*) via genetic studies and morphological identification (Moro et al., 2018). Mouse densities on the island are high and resources are stressed, evidenced by the staff from DBCA observing cannibalism (Moro et al., 2018). For these reasons, house mice on Browse island are a risk to the multiple protected species as well as posing a biosecurity threat to Australia should they reach the mainland where they have not previously been recorded (Gabriel et al., 2011). At present, no eradication programs have been attempted on Browse Island.

The *Vkorc1* gene of mice from the Perth metropolitan area will be sequenced to measure the frequency of mutations in a location characterised by high exposure to rodenticides, while mice from Browse Island, where there is no history of rodenticide use, will be used to test the frequency of mutations in a population that has not been exposed to ARs. The mitochondrial D-loop of mice will be sequenced to investigate whether the presence or lack of mutations is influenced by genetic diversity and population structure. Additionally, the D-loop data will be used to confirm the species present and the likely origin of the populations. Finally, the results will be interpreted to assess the resistance levels of Western Australian house mouse populations, and based on this measures for conservation and pest management will be suggested.

2 Methods

2.1 Sample collection and preparation

Western house mice (*Mus musculus domesticus*) from the Perth metropolitan area (Western Australia) were acquired through donations from people involved with pest management, as well as members of the public. Therefore, the geographic distribution of samples was influenced by the availability of suitable samples (Figure 2). Only mice without visible signs of decomposition were accepted, to maximise DNA quality and yield. Mice were collected by use of traps or other methods not involving anticoagulant rodenticides. The date and location where each mouse was collected were recorded by the collector. The mice were stored at -20°C at Edith Cowan University until they had their liver extracted, and the livers were then stored in 100% ethanol at -8°C until DNA extraction. During dissection, the sex of each individual was determined by the presence or absence of male primary sex characteristics, and was recorded to reduce bias resulting from overrepresentation of either sex during analyses. Furthermore, no signs of internal haemorrhage were detected in the samples during dissection, which excluded death by anticoagulant rodenticides.

Liver samples of south-east Asian house mouse (*Mus musculus castaneus*) were donated by the Department of Biodiversity, Conservation and Attractions. These mice had been collected from Browse Island using snap traps and there have been no anticoagulant rodenticides used on the island. Liver samples from these mice were preserved in salt saturated dimethyl sulfoxide (DMSO) and stored at -8°C at Edith Cowan University until DNA extraction.

All protocols were approved by the Edith Cowan University Animal Ethics Committee (approval 21776).

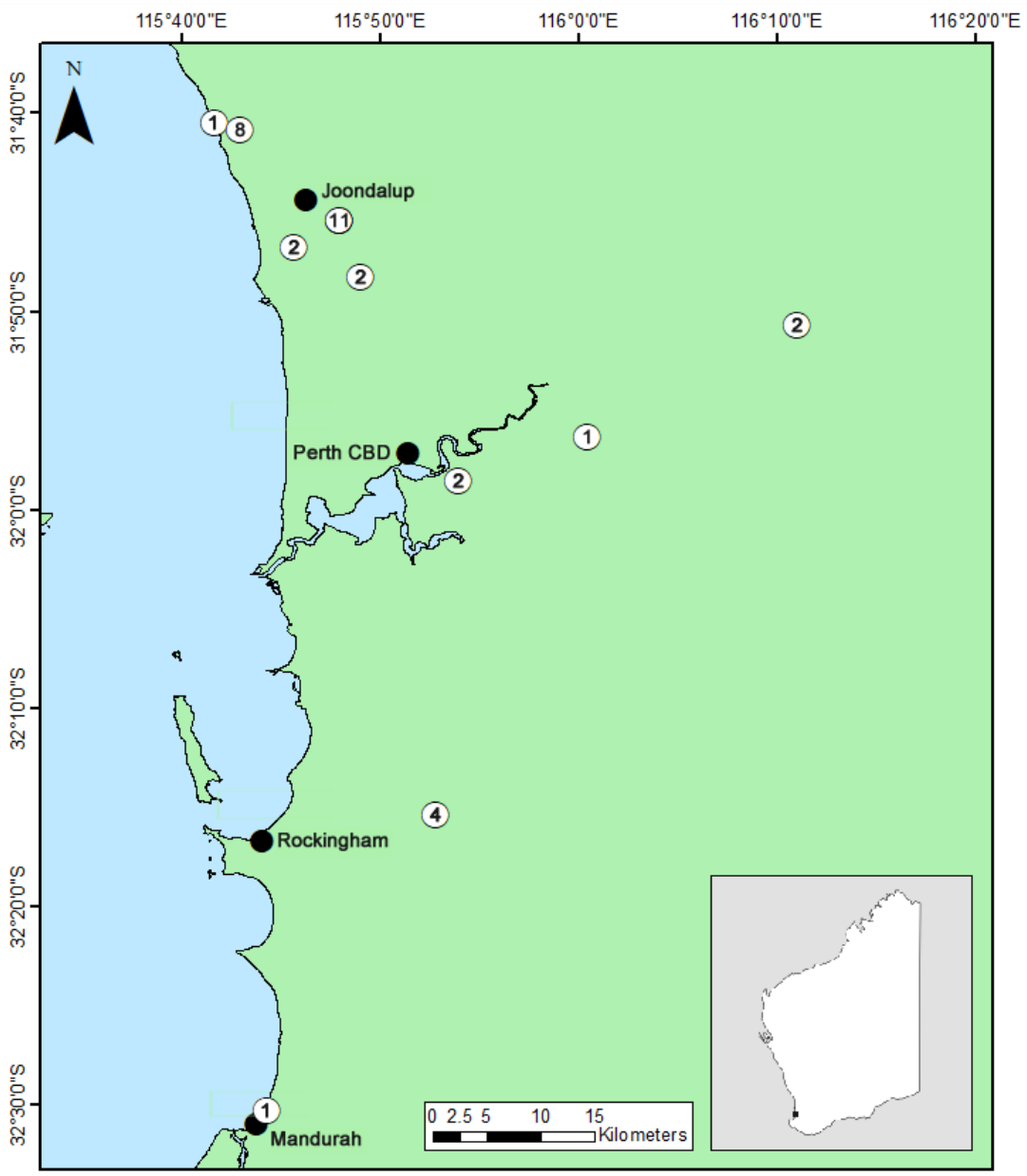


Figure 2 Sample collection locations from the Perth metropolitan area are indicated by clear circles in which the number correlates to sample size from that location. Major towns located in proximity of samples collection sites are indicated by black circles.

2.2 DNA extraction and PCR

Prior to DNA extraction, the liver was placed in Milli-Q water for 5 minutes on a rocking platform at room temperature, then the water was drained and replaced, and the process repeated under the same conditions for 10 minutes, in order to clean the sample of preservation liquid. Genomic DNA was then extracted from the liver using a DNeasy Blood & Tissue kit following the manufacturer's instructions (Qiagen). Concentration and purity of the DNA were measured using a NanoDrop (Thermo Fisher Scientific), and then concentration of DNA in all samples was standardised to 10 ng/µL by dilution with Milli-Q water unless they were already below 10 ng/µL.

Polymerase chain reaction was used to amplify the nuclear *Vkorc1* gene region (Goulois et al., 2016) and the complete mitochondrial D-loop and flanking regions (Gabriel et al., 2011). Reactions included 20 ng of DNA template, 12.5 µL of Platinum HotStart master mix (Thermo Fisher Scientific), 0.5 µL of both forward and reverse primers, and were made up to 25 µL with DNA-free H_2O. DNA from Perth metropolitan mice did not amplify well for primers VKORC1-S1 and VKORC1-AS1, so PCR was repeated using Platinum II HotStart (Thermo Fisher Scientific) as this amplifies longer sequences in a shorter time compared to Platinum HotStart. The mitochondrial D-loop required only a single set of primers, whereas to cover the three exons of the *Vkorc1* gene two sets of overlapping primers were required (Table 1). Cycling conditions for PCR are shown in Table 2. The PCR products were run on an electrophoresis gel to ensure the PCR conditions were amplifying the target gene at a satisfying quality prior to sending the products for sequencing.

Table 1 Primers used for amplification of the nuclear Vkorc1 gene region (Goulois et al., 2016)*, and the mitochondrial D-loop and flanking regions* (Gabriel et al., 2011).

Gene region	Forward/ Reverse	Primer Name	Sequence
Vkorc1 (exon 1 & 2)	F	VKORC1-S1	GATTCTTCCCTCCTGTCC
	R	VKORC1-AS1	AGACCCTGTCTCAAAACCTA
Vkorc1 (exon 3)	F	VKORC1-S2	GAAAGCAGAACACTTAGCAGG
	R	VKORC1-AS2	AACCAACAGCAGAATGCAGCC
D-loop	F	L15774	TGAATTGGAGGACAACCAGT
	R	H2228	TTATAAGGCCAGGACCAAAC

Table 2 PCR parameters for Vkorc1 and D-loop amplification. All conditions included 40 cycles. Conditions were adapted from Goulois et al. (2016).

Gene region	Master Mix	Initial denaturation	Denaturation	Annealing	Extension	Final extension
Vkorc1	Platinum HotStart	2 min @ 94°C	30 sec @ 94°C	30 sec @ 58°C	110 sec @ 72°C	10 min @ 72°C
	Platinum II HotStart	2 min @ 94°C	30 sec @ 94°C	30 sec @ 60°C	30 sec @ 72°C	10 min @ 72°C
D-loop	Platinum HotStart	2 min @ 94°C	30 sec @ 94°C	30 sec @ 55°C	1 min @ 72°C	10 min @ 72°C

Successfully amplified PCR products were purified and sequenced at the Australian Genome Research Facility in Perth, Western Australia. The amplified products of the first set of *Vkorc1* primers were sequenced dual direction, and the products of the second set of *Vkorc1* primers were sequenced on the reverse strand only as this was sufficient to cover the third exon of the gene. The products of the D-loop primers were sequenced on the forward strand only as this allowed subspecies identification.

2.3 Sequence construction

The chromatogram of each sequence was manually checked and edited using the freeware program FinchTV (Geospiza) to remove sequencing error or poor-quality sequences. Edited sequences were aligned in Geneious Prime. Sequences derived from the forward and reverse primer set covering exons 1 and 2 of the *Vkorc1* gene were aligned to obtain a consensus sequence for each sample. Where the two sequences did not overlap, the consensus was obtained by mapping the forward and reverse sequences on the *Vkorc1* reference sequence (accession number 27973) and by extracting the resulting consensus sequence; however, this method resulted in sequences with only partial coverage of exon 2. A single *Vkorc1* gene sequence for each mouse sample was obtained by assembling the consensus sequence for exons 1 and 2 with the sequence covering exon 3, which had been amplified by primer VKORC1-AS2. The D-loop sequences were blasted in GenBank to confirm species identification. All sequences were then aligned in Geneious to be compared to multiple reference *Mus musculus* and *Mus spretus* sequences published in GenBank (Appendix A) in order to confirm (sub)species identification, identify haplotypes, and

detect *Vkorc1* SNPs. Reference sequences for the *Vkorc1* gene included wild type (susceptible to ARs) *M. m. domesticus*, the Algerian mouse *M. spretus*, and the hybridised (and resistant to ARs) *M. m. domesticus*/*M.spretus*.

2.4 Haplotype network construction

The free population genetics software PopART (Population Analysis with Reticulate Trees) was used to generate haplotype networks using D-loop sequences. The D-loop sequences of Perth metropolitan mice were aligned with 578 *M. m. domesticus* D-loop sequences retrieved from GenBank to analyse mitochondrial phylogeography (Appendix A). The D-loop data from Browse Island specimens of this study were aligned with 246 *M. m. castaneus* D-loop sequences available on GenBank, which covered the geographical distribution of *M. m. castaneus* to analyse the phylogeography of this subspecies (Appendix A). Both datasets had non-unique sequences filtered out, where the sequences were from the same country, and the length of sequences standardised. All aligned datasets were then exported from Geneious and haplotype networks created from them using the minimum spanning network method in PopART (Bandelt, Forster, & Rohl, 1994; Leigh & Bryant, 2015).

3 Results

Sequences were obtained for house mouse from the Perth metropolitan area (n=34) and Browse Island (n=15) (Table 3). The low number of sequences covering the third exon of the *Vkorc1* gene appears to be due to nonspecific binding of primer set VKORC1-S2 and -AS2 as, in multiple instances, sequencing of DNA product amplified with this primer set resulted in segments of chromosomes other than seven being amplified (including chromosomes four, five, fourteen and fifteen). Furthermore, electrophoresis of the product amplified from the primer set VKORC1-S1 and -AS1 displayed two bands of different molecular weight on the electrophoresis gel. Attempts to optimise the PCR, by modifying temperature and time parameters of the annealing and extension periods of PCR and the use of a more efficient Taq polymerase (Platinum II HotStart), did not yield better results as the two bands were still visible after electrophoresis. However, the sequences were clear and a conservative approach was used to select the sequences to analyse.

Table 3 Genetic sequences for the Vkorc1 gene and mitochondrial D-loop obtained from 34 Mus musculus domesticus from the Perth metropolitan area, and 15 M. m. castaneus from Browse Island.

		Perth metropolitan area			Browse Island	
Gene		♀ (n)	♂ (n)	Juvenile (n)	♀ (n)	♂ (n)
Vkorc1	Exon 1 & 2	7†, 5‡	7†, 8‡	4†, 1‡	5†, 1‡	8†
	Exon 3	6†	2†, 1‡	3†	8†	7†
D-loop		13	16	5	8	7

†Complete coverage of the exons, ‡Partial coverage of the exons

3.1 *Vkorc1* mutations

No mutations were detected in the coding regions of the *Vkorc1* gene in western house mice (*Mus musculus domesticus*) from the Perth metropolitan area. All mice from this area displayed 100% homology to the exons of the reference sequence (27973). All *Vkorc1* gene sequences of south-east Asian house mice (*Mus musculus*

castaneus) from Browse Island displayed two single nucleotide polymorphisms in exon 1. The clear signals on the chromatograms represent mutations at amino acid positions 10 (Appendix 2) and 37 (Appendix 3) of exon 1 of the *Vkorc1* gene. Both mutations are silent and the latter one, named E37E, at nucleotide position 111, where an adenine is replaced by a guanine, and the resulting glutamic acid is retained as the mutation is silent. The mutation E37E was also exhibited by the reference sequence for the *Vkorc1* gene of the Algerian mouse (*Mus spretus*). The single nucleotide polymorphism (SNP) at nucleotide position 10 involves the replacement of a cytosine by a guanine, which results in the amino acid leucine being unchanged.

3.2 *Vkorc1* insertions and deletions

The *Vkorc1* gene of all 15 *M. m. castaneus* from Browse Island contained a 68 base pair insertion at nucleotide position 1454, which is located in the intron following exon 2 (Appendix 4). The insertion was not identical across individuals from the island. When compared to published sequences it was found that for twelve mouse samples from Browse Island the insertion was 100% homologous to a section of DNA in published *M. spretus* sequence as well as certain *M. m. domesticus* sequences that hybridised with the Algerian mouse (GQ905709-11) (Song et al., 2011a). The remaining three samples differed from the published sequence by three point mutations each; all were different to each other. No variability was detected in the location of the insertion across individuals. The Browse Island mice shared a silent mutation (E37E) in exon 1 with the three published sequences that carried the insertion. However, the Browse Island mice did not carry an additional three mutations in exon 1, and one mutation in exon 2 that were displayed by the reference mice. The *Vkorc1* gene of specimens from Browse Island did not exhibit any of the mutations linked to the *M. spretus* allele in the coding sequence, despite all displaying the intragenic sequence (Song et al., 2011a). Furthermore, all 15 Browse Island samples reported an eight bp deletion at position 1143 that is shared with the published sequences previously mentioned (Appendix 5). One mouse from Browse Island displayed a seven bp and an 11 bp insertion at nucleotide position 939 and 1266 respectively, which were not exhibited by any of the published *Vkorc1*

sequences (Appendix 6). All mice specimens from the Perth metropolitan area lacked the insertion.

3.3 *Mus musculus* D-loop haplotypes in Western Australia

Mitochondrial D-loop sequences were obtained for all house mice in this study (n=49), with sequenced PCR products varying in length, but being mostly between 900 and 1100 base pairs. All D-loop sequences from the Perth metropolitan area but one corresponded with known *M. m. domesticus* haplotypes, which had previously been found in Australia. There were two haplotypes found within metropolitan area mice, with the most common being 100% homologous to published sequences of *M. m. domesticus* from Australia. Perth metropolitan mice belong to haplotype AUSTRALIA.01 (JF277281), which is part of the most widespread clade E haplotype in Australia (Gabriel et al., 2011). The sequences had 99% identical sites, with two sequences each having a single deletion (one at position 797 and the other at position 828). One mouse from the metropolitan area displayed a different haplotype, which did not match any published D-loop sequences (MM019); this mouse was most closely related to haplotypes from Australia and The Netherlands (JF277281, JF277295), but differed from them in a single base pair change at position 718.

The haplotype network (Figure 3) shows that western house mice from the Middle East, West Europe and the British Isles were represented by the highest number of unique haplotypes, which were broadly distributed throughout the network. Geographical clustering was observed in the sequences from Madeira, while the New Zealand sequences were widely distributed across the network. A similar pattern was observed in haplotypes from Cyprus. The haplotype to which most Perth mice belong to was grouped with haplotypes from West Europe, New Zealand, and the British Isles, as well as Southeast Asia, Africa and Oceanic Islands. The western house mouse from Perth with a unique haplotype was most closely related to *M. m. domesticus* from the Middle East, Cyprus, and Europe.

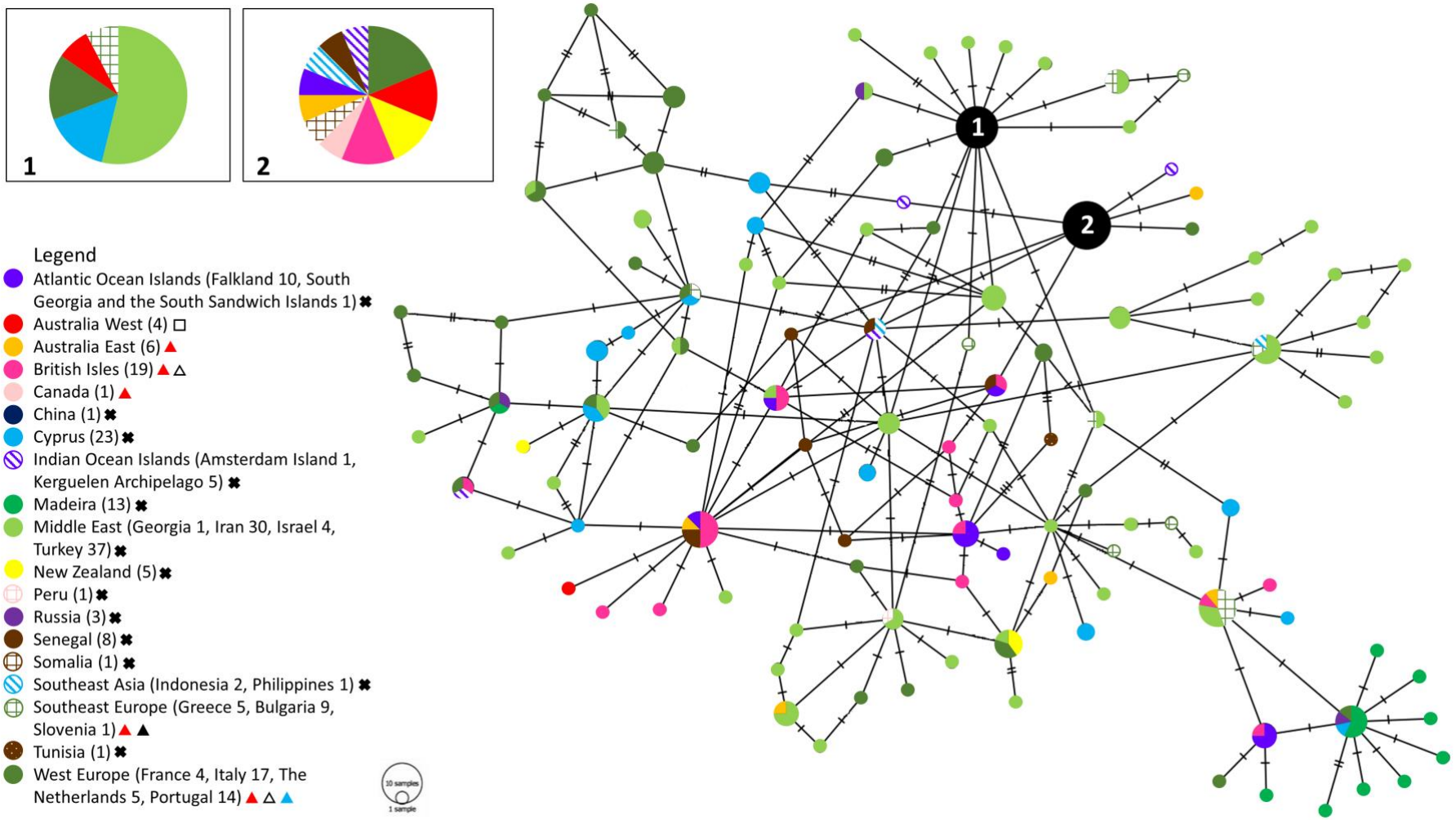


Figure 3 Minimum spanning haplotype network from Mus musculus domesticus D-loop data. The numbers in the legend are representative of the number of unique haplotypes included in the analysis. The size of each circle indicates the frequencies of the haplotype, and each colour represents the geographical origin of the sequences. The numbered nodes in the haplotype network correspond to the numbered inset boxes. A black cross in legend indicates that M. m. domesticus in that area has not been tested for anticoagulant rodenticide (AR) resistance (Fisher, 2005). *A white square indicates that M. m. domesticus has been tested for Vkorc1 mutations related to AR resistance and was found to be negative (this study). A red triangle indicates that M. m. domesticus has been tested for resistance through a lethal feeding period test and was found to be resistant to ARs* (Goulois et al., 2017; Pelz et al., 2005; Prescott et al., 2018; Wheeler et al., 2018; Zhelev et al., 2019). *A white triangle indicates that M. m. domesticus has displayed Vkorc1 mutations related to AR resistance* (Prescott et al., 2018). *A black triangle indicates that a blood clotting response test has been performed and M. m. domesticus was found to be resistant to ARs* (Zhelev et al., 2019). *A blue triangle indicates that an assay of the VKOR enzyme has been carried out and resistance to ARs was determined* (Goulois et al., 2017).

The house mice on Browse Island were previously identified as the south-east Asian house mouse (*M. m. castaneus*) (Moro et al., 2018). The D-loop sequences of 15 mice from Browse Island were identical to published *M. m. castaneus* D-loop sequences from Asia and Kenya. One mouse displayed a different haplotype that was 100% homologous to published *M. m. castaneus* sequences from Asia, Russia and Kenya which differed from the other haplotype by one base pair at position 101. However, the current version of PopART (1.7) masks any columns in the alignment containing gaps or ambiguous characters (such as 'N' and '?'), and therefore the haplotype diversity resulting from deletions and insertions in sequences is not represented in the network, therefore all Browse Island samples appear in the same haplotype node.

On the haplotype network (Figure 4), south-east Asian house mice from Iran, India and China were represented by the highest number of unique haplotypes. Geographical clustering was observed for haplotypes from both Iran and India, while sequences from China were arranged in a star-like pattern with 17 haplotypes stemming directly from the most common haplotypes (merged into a single node numbered one in Figure 4). Mitochondrial D-loop sequences of *M. m. castaneus* from Browse Island were grouped with sequences from China, Bangladesh, Japan, Indonesia and Kenya, as well as other Asian countries.

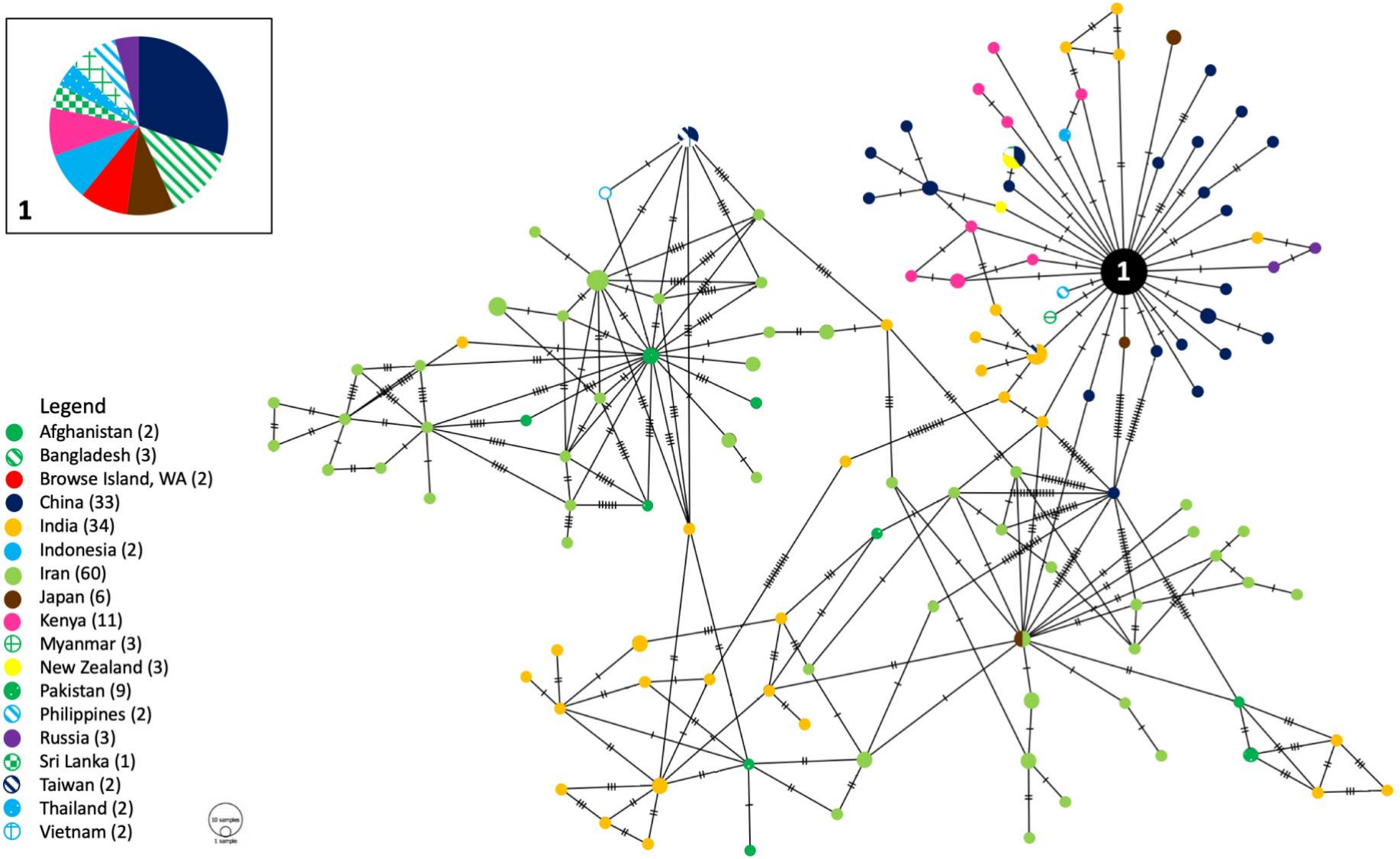


Figure 4 Minimum spanning haplotype network from mitochondrial D-loop data of Mus musculus castaneus. The numbers in the legend are representative of the number of unique haplotypes included in the analysis. The size of each circle indicates the frequencies of the haplotype, and each colour represents the geographical origin of the sequences. The numbered node in the haplotype network corresponds to the numbered inset box.

A comparison of haplotype diversity (i.e. the number of haplotypes per population size) could not be undertaken as the sampling strategy and scale of sampling varied across studies. For instance, Gabriel et al. (2011) reported 12 unique haplotypes (and deposited 12 corresponding sequences on GenBank) from 77 mice across Australia, whereas in this study 34 mice sampled from one city within Australia had two haplotypes. The difference in sample sizes and geographic regions covered also impacts on the haplotype network, as some countries appear in many different haplotypes and/or more common haplotypes; it is difficult to interpret the biological significance of the haplotype diversity because of uneven sampling.

4 Discussion

4.1 *Mus musculus domesticus* of Perth lacks *Vkorc1* mutations

Resistance to ARs is usually recorded in populations of house mice and rats that have been exposed to toxins, especially when exposure is repeated and involves intensive use of first generation anticoagulant rodenticides such as warfarin (Cuthbert, Visser, Louw, & Ryan, 2011). ARs have been intensely used by the public and professionals for conservation outcomes, or in agricultural, residential and commercial settings in Western Australia (Lohr & Davis, 2018), and professional pest managers have anecdotally observed resistance to the anticoagulant rodenticides warfarin, coumatetralyl and bromadiolone exhibited in Perth house mice (Piggott D 2019, written communication, 21st February 2019). The resistance of mice reported by professionals and the intensive use of rodenticides by the public are in agreement with the documentation of exposure in non-target wildlife being correlated with human population density (Lohr, 2018; Lohr & Davis, 2018). When mice are resistant, it encourages people to use larger quantities or stronger ARs, increasing the likelihood of non-target animals being affected directly through feeding on baits and indirectly through feeding on resistant mice that have ingested higher doses of poison (in addition to consuming dead susceptible mice).

The susceptibility of Australian *Mus musculus* to anticoagulant rodenticides (ARs) has been tested only on Lord Howe Island (700 km off the coast of New South Wales), and never on Western Australian populations nor on populations located elsewhere on mainland Australia (Billing, 2000; Wheeler et al., 2018). The studies of Lord Howe Island mice were via laboratory no-choice feeding tests, and suggested that the mice were resistant to warfarin (Billing, 2000; Wheeler et al., 2018). In Australia, the *Vkroc1* gene of mice has not previously been sequenced, and therefore the results of this study are the only available information on genetic resistance of mice on mainland Australia.

Although there are anecdotal reports of resistance in Perth, and the feeding trial on Lord Howe Island, in the present study no mutations that would indicate resistance to first generation anticoagulant rodenticides such as warfarin were detected in the *Vkorc1* gene of western house mice (*Mus musculus domesticus*) from the Perth metropolitan area. Previous *Vkorc1* screening in Europe has recorded a high frequency of mutations related to anticoagulant rodenticide resistance (Goulois et al., 2016; Liu et al., 2015; Mooney et al., 2018; Pelz et al., 2012; Song, Lan, & Kohn, 2014). In France, 70% of house mice tested were found to display *Vkorc1* mutations, with high level of homozygosity (Goulois et al., 2016), while in Ireland, 84% of mice samples tested presented point mutations associated with AR resistance (Mooney et al., 2018) (Table 4). There has been no research published finding populations free from resistance-related mutations, therefore the absence of *Vkorc1* sequence variants in the Perth region is a unique result. That *M. m. domesticus* inhabiting the Perth region displayed no *Vkorc1* mutations poses interesting challenges for pest management and biosecurity.

Table 4 Testing of Mus musculus populations worldwide for anticoagulant rodenticide resistance conferred by mutations in the Vkorc1 gene.

Location	N	Percentage of resistance (%)	Reference
France (65 locations)	266	70	(Goulois et al., 2016)
United Kingdom (5 counties)	53	88.7	(Prescott et al., 2018)
Germany (25 locations)	352	90	(Pelz et al., 2012)
Ireland	50	84	(Mooney et al., 2018)
Switzerland (3 locations)	48	100	(Pelz et al., 2012)
Spain	29	93.1	(Song et al., 2011a)

While the main known mechanism for resistance is carrying mutations in the *Vkorc1* gene, the absence of these mutations does not rule out the possibility of the mice being resistant through polygenetic factors. For example, another mechanism of resistance suggested for rats is accelerating the cytochrome P450 systems that are involved in the metabolism of ARs (Zhelev et al., 2019). Furthermore, *M. musculus* is known to display a natural level of tolerance to anticoagulant rodenticides compared to the rats *Rattus rattus* and *Rattus norvegicus* (Cowan et al., 2017; Grandemange et al., 2010; Prescott et al., 2018; Wheeler et al., 2018). Other tests to determine resistance, such as feeding trials and blood clotting response tests have been implemented worldwide, and resistant *M. musculus* have been found in many European countries, and also in Canada (Pelz et al., 2005). For these reasons, house mice from the Perth region should be tested through feeding trials or blood clotting response tests in order to assess the presence of resistance to anticoagulant rodenticides via another mechanism.

The absence of mutations in the coding regions of the *Vkorc1* gene of *M. m. domesticus* of Western Australia could be the result of six different scenarios (Table 5). The simplest scenario is that the mice in Australia have never had mutations in the *Vkorc1* gene due to founder effect. As *M. m. domesticus* was probably introduced in Australia by European settlers on the First Fleet in the late 1700s, the absence of SNPs in the exons of *Vkorc1* might be the result of these founder animals not carrying any mutations (Cory et al., 2011; Gabriel et al., 2011). The mitochondrial D-loop data supports this, as the low haplotype diversity may indicate rapid expansion from a small founding population (Tang et al., 2018). It is possible that the founder population did not carry any resistant alleles as no selection pressure from exposure to ARs was present since anticoagulant rodenticides had not been invented until the 1940s, and the first evidence of resistance in rodents dates to 1958 (Grandemange et al., 2010). Therefore, the highly conserved coding regions of *Vkorc1* have been maintained free of mutations because the initial rodent population that colonised Western Australia did not exhibit any.

If western house mice of Western Australia have possessed mutations but no longer do, it may be that heavy use of SGARs has removed them from the population, or the result of selection against the mutations because of pleiotropy. In Australia, eight anticoagulant rodenticides are commercially available for rodent control, and the poisons are readily available to the public who can purchase them from popular retail stores and do not require government permits; additionally, ARs are intensively used in Australia, and poorly regulated and restricted compared to other countries globally (Lohr & Davis, 2018). While there is no information regarding the volume of use of rodenticides in Australia, evidence of secondary poisoning and AR residues in non-target species is an indication of heavy use of rodenticides in Western Australia, both by professionals and the public (Lohr & Davis, 2018). As the most common resistance is exhibited towards the anticoagulant warfarin, the use of stronger SGARs may have removed any individuals carrying SNPs related to warfarin resistance from Western Australian populations (Lohr MT 2019, oral communication).

The absence of resistance-related mutations in the *Vkorc1* gene of Western Australian *M. m. domesticus* could be explained by the negative selection that acts on these alleles due to pleiotropy. Dysfunctional *Vkorc1* activity has shown to come at a cost, as blood clotting requires the enzyme to perform well (Ishizuka et al., 2008). Resistant rodents that carry mutations in the *Vkorc1* gene display a lower activity of the VKOR enzyme, and the reason for survival of these individuals is yet to be described (Ishizuka et al., 2008). To maintain blood clotting, these animals might be consuming exogenous vitamin K to overcome the defective enzyme activity (Markussen et al., 2003). Furthermore, vitamin K is essential during foetus bone formation and therefore females with normal VKOR activity are better breeders (Heiberg et al., 2006). Due to these negative pleiotropic effects, *Vkorc1* mutations are selected against in conditions where rodenticides are not used and rodent populations are not exposed to the poisons (Heiberg et al., 2006; Ishizuka et al., 2008; Jacob et al., 2012; Markussen et al., 2003); however, continuous exposure of house mice to ARs in the Perth Metropolitan area poses a selection pressure to maintain any resistant mutations, and therefore it is unlikely that mutations in the *Vkorc1* gene

are absent due to negative pleiotropic effects. Mutations in the *Vkorc1* gene are selected against when exposure is absent, which is not the case for the Perth metropolitan region (Ishizuka et al., 2008; Lohr & Davis, 2018).

It is also possible that they have resistance through other mechanisms (adaptation or other genes encoding for resistance), which would result in there being no selection in favour of *Vkorc1* mutations (and they may be actively selected against as per the pleiotropy scenario). Previous studies have shown that rodents adapted to living in arid environments have a tolerance to anticoagulant rodenticides, and this could be the method of resistance exhibited by western house mice of Western Australia (Bradfield & Gill, 1984; Gryseels, Leirs, Makundi, & Gouy De Bellocq, 2015; Mahmoud & Redfern, 1981). A laboratory feeding test on golden hamsters (*Mesocricetus auratus*) confirmed high levels of resistance to warfarin and a considerable level of tolerance to brodifacoum (Bradfield & Gill, 1984). The Egyptian spiny mouse (*Acomys cahirinus*) was also found to be resistant to warfarin and difenacoum with some level of tolerance to brodifacoum (Mahmoud & Redfern, 1981). The mechanism of resistance of these two rodent species is unknown, however three SNPs in the third exon of the *Vkorc1* gene were detected in a sub-Saharan mouse (*Mastomys natalensis*) (Gryseels et al., 2015). These three rodents are naturally distributed in arid environments that are characterised by a scarcity of available vitamin K. As Australia is renowned for its arid environment that led to many adaptations of the fauna and flora, it is possible that *M. m. domesticus* in Australia have evolved a tolerance to depleted vitamin K levels and this does not necessarily result in house mice carrying *Vkorc1* mutations. As this tolerance has been linked to resistance in other rodents, they may also be expected to have resistance to ARs, but as the resistance mechanism is unknown in some of these species it may not correlate with SNPs in the *Vkorc1* gene.

The absence of resistance-related alleles in the *Vkorc1* gene does not rule out the presence of resistance in Western Australia as this gene is not the only described mechanism of resistance to anticoagulant rodenticides. The gene encoding for the enzyme CYP2C9 (cytochrome P450 2C9) is responsible for metabolising anticoagulant rodenticides, and variants of its gene are associated with the need for a different dosage to take effect (Gryseels et al., 2015; Zhelev et al., 2019). It has been documented that resistant rodents are able to metabolise a first generation compound in half the time compared to susceptible individuals (Ishizuka et al., 2007). As Cytochrome P450 also detoxifies the organism from other pollutants, such as pesticides, the presence of environmental pollutants may lead to an increase of cytochrome P450 activity and render the rodents pre-adapted to rodenticides (Ishizuka et al., 2007). Other unidentified genes could be involved in rodenticide resistance as well, and resistance to certain ARs, such as difenacoum, has been shown to be polygenic (Cowan et al., 2017; Limdi & Veenstra, 2008). Until resistance to anticoagulant rodenticides is tested in *M. m. domesticus* of Western Australia through other laboratory tests such as feeding trials and blood clot response tests, it cannot be said whether the mice of this region are resistant to ARs even though this study has shown that they do not carry resistance-linked alleles in the *Vkorc1* gene. Pest managers have anecdotally reported resistance in the Perth region, and therefore it is possible that house mice of Western Australia are resistant due to other genes encoding an adaptation.

A final possibility is that mutations in the *Vkorc1* gene of Perth house mice are present but were not detected. As a sample is a subset of the total population, non-detection of *Vkorc1* mutations is possible if the allele is not fixed in the Perth population. If only a subset of mice from the Perth metropolitan area carries mutations in the *Vkorc1* gene, then it is possible that sample design did not include suitable samples to detect mutations. Measures were taken to avoid sample collection bias according to sex (equal number of male and female samples were used), location (a geographical range spanning through the entire Perth metropolitan area was preferred), and sample collection was designed to not underestimate the

frequency of *Vkorc1* mutations (i.e. no mice killed with rodenticides were collected). Furthermore, testing of European populations of house mouse has reported high percentages of resistance (between 70% to 100%), and all other tests performed on house mouse populations worldwide detected alleles associated with resistance (Goulois et al., 2016; Mooney et al., 2018; Pelz et al., 2012; Prescott et al., 2018; Song et al., 2011a) so the sample size of this study is expected to be sufficient for detection.

It also needs to be acknowledged that there may be combinations of these scenarios occurring (potentially in different places and at different times); for example, if the initial colonising population did not have mutations in the *Vkorc1* gene (scenario founder effect), any mutations arising independently could have been quickly removed from the population by the intensive use of strong anticoagulant rodenticides that killed those individuals (scenario SGARs).

Table 5 Possible scenarios that could have led to the absence of SNPs in the Vkorc1 gene of Mus musculus domesticus of the Perth metropolitan area, Western Australia.

Scenario	Description	Likelihood
Founder effect	Mutations have never been in Western Australia because the founder population did not carry *Vkorc1* mutations.	Possible
SGARs	The use of strong SGARs removed any warfarin resistance (the most common kind of resistance inferred from *Vkorc1* mutations) from the Western Australian house mouse population.	Very likely
Pleiotropy	Defective VKOR enzyme activity posed a negative selection pressure on *Vkorc1* mutations related to rodenticide resistance.	Not likely
Adaptation	The arid Australian environment selects for natural tolerance to vitamin K depleted conditions, which result in AR resistance.	Possible
Other genes	The metabolising action of cytochrome P450 and the function of other unidentified genes can be encoding for resistance to ARs in Western Australian mice.	Possible
Detection failure	As only a subset of the Western Australian house mouse population was tested, the sample could have excluded any individual carrying *Vkorc1* mutations, which are present in the population but were not detected.	Not likely

4.2 *Vkorc1* introgression between *Mus* species

Amplification of the *Vkorc1* gene detected an intragenic insertion in *M. m. castaneus* inhabiting Browse Island, which was 100% homologous with the GenBank *Mus spretus* reference sequence, as well as some *M. m. domesticus* that have hybridised with *M. spretus* (Song et al., 2011a). The mitochondrial D-loop sequences identified the island mice as the *castaneus* subspecies of house mouse despite the insertion being present in the *Vkorc1* gene. For the past 30 years, mitochondrial DNA markers have been used for phylogenetic studies to infer the structure of species and to identify evolutionary events (Morando, Avila, Baker, & Sites, 2004). However, inferences about a species tree can be inaccurate due to introgression or incomplete lineage sorting (ILS), as the species tree does not always match the gene tree (Morando et al., 2004). In this case, we have an assumed introgression event in *M. m. castaneus,* with the Browse Island population appearing closely related to *M. spretus* when analysed for the *Vkorc1* gene, however, based on D-loop data, morphological characteristics and geographic distribution we can reasonably assume the Browse Island population is *M. m. castaneus* and therefore more closely related to the subspecies *M. m. domesticus*.

Both ILS and introgression have been documented to occur regularly in a variety of phyla, and they are hard to differentiate; recent studies have begun using coalescent and simulation methods to attempt to distinguish between the two (Rheindt, Christidis, & Norman, 2009; Staubach et al., 2012). There is evidence of a current ongoing process of hybridisation between the *M. musculus* subspecies, which, combined with the retention of ancestral polymorphism and introgression in the wild, renders it difficult to distinguish between subspecies (Yang et al., 2011). Some of the genetic variability found in lab strains could indeed have originated in the wild due to hybridisation, and it may not be possible to define a single, wild-type genome for each subspecies either in captive strains or in nature (Staubach et al., 2012). While the availability of voluminous genome information on *M. musculus* and its defined phylogenetic history allows researchers to distinguish between introgression and ILS more easily compared to other species (Staubach et al., 2012), the high occurrence

of introgression between *M. musculus* subspecies poses a degree of complexity in defining population models and identifying influences on the genomes.

The *M. musculus* subspecies that have been most researched are *M. m. musculus* and *M. m. domesticus,* which are found in east and west Europe, respectively (Rajabi-Maham, Orth, Boursot, Siahsarvi, & Bonhomme, 2011). Two studies investigated the hybridisation between these two subspecies and found evidence for introgression in at least 10% of the genome, as well as introgression in multiple mitochondrial restriction sites, and that the patterns of introgression were not compatible with an ILS model (Bozikova et al., 2005; Staubach et al., 2012). When compared to *M. m. musculus* and *M. m. domesticus*, *M. m. castaneus* is more polymorphic, and its retention of ancestral polymorphisms might be the reason behind the complexity of defining its lineage and identifying introgression (Rajabi-Maham et al., 2011), making it difficult to interpret the history of the Browse Island mice in regards to the observed insertion.

Prior to the introgression between *M. spretus* and *M. musculus* species being documented, several studies had misinterpreted the introgressed regions as missing data or they made hasty conclusions due to poor study design, which placed *M. spretus* as an inadequately performing outgroup (Keane et al., 2011; Yang et al., 2011). The study by Liu et al. (2015) used a statistical inference method to identify introgression in genomes compared to ILS; furthermore, the authors used genome-wide variation data to compare allopatric and sympatric samples of *M. spretus* and *M. m. domesticus*. Distinguishing between introgression and ILS was not in the scope of this study, and therefore the *Vkorc1* insertion discovered on Browse Island is defined as introgression in agreement with previous studies that have used simulation and statistical methods to reach this conclusion (Liu et al., 2015; Song et al., 2011a; Ullrich, Linnenbrink, & Tautz, 2017).

The intragenic insertion detected in this study in the *Vkorc1* gene has been previously found to have transferred from *M. spretus* to *M. m. domesticus* in areas of sympatry in Spain and allopatry in Germany, where hybridisation and introgression between allopatric species might have been caused by human interaction that increased the dispersal of the *Mus* species (Song et al., 2011a). Although there is a selective disadvantage connected with the hybridisation between *M. musculus* and *M. spretus*, including sterile male offspring and the possibility of sterile female offspring depending on the direction of the backcross (Dejager, Libert, & Montagutelli, 2009), the introgression of the *M. spretus* haplotype between distant populations has been possible because it involved an adaptive gene such as *Vkorc1* and its spread has been driven by the selection pressure posed by the common use of ARs (Ullrich et al., 2017).

Previous studies identified three distinct introgression events between *M. m. domesticus* and *M. spretus*, including an ancient event that occurred more than 2000 years ago prior to the colonisation of Europe by *M. musculus* (Liu et al., 2015). The other more recent introgression events are dated around 50 to 60 years ago, since the introduction of ARs (Song et al., 2011a). Introgression between the two species in other genes has been documented in allopatric mice populations in France and Germany; most of the genes involved in the introgression events are adaptive and they are linked to immune defence (Ullrich et al., 2017). Furthermore, introgression in the *Vkorc1* gene was present but in low frequencies and it was therefore considered far from becoming fixed and the details were not reported (Ullrich et al., 2017). The 68 bp insertion detected in the present study was displayed in 100% of the Browse Island mice and it can be concluded that the introgression event was likely to be fixed in this population. Therefore the region of the *Vkorc1* gene from *M. spretus* that has been detected in the *M. m. castaneus* sample from Browse Island is considered a comet allele (*sensu* Staubach et al., 2012) that has migrated from one evolutionary lineage to another and its frequency has increased to reach fixation.

According to the D-loop marker, the mice on Browse Island are most closely related to mice from China, Indonesia, and other Asian countries, but as no other studies have sequenced the *Vkorc1* gene region in this subspecies or attempted to identify introgression in different *Mus* species from Asian countries, we cannot make inferences on the process and location of the hybridisation between the two *Mus* species. Hybridisation between *M. m. musculus* and *M. m. castaneus* in Japan resulted in the *Mus musculus molossinus* subspecies (Yang et al., 2011), however hybridisation involving *M. m. castaneus* is generally less studied compared to the European subspecies (Jing et al., 2014). Introgression in the *Vkorc1* gene between *M. spretus* and *M. m. castaneus* has not previously been described. Additionally, a study from Jing et al. (2014) investigated the phylogeography of *M. m. castaneus* in China, but used *M. spretus* as an outgroup, which could have concealed evidence of introgression between the two species.

The length of the insertion in the *Vkorc1* gene is 68bp, which could be an indication of an ancient introgression event (>2000 years ago) that is characterised by continued backcrossing, drift and recombination (Liu et al., 2015); however the insertion is not fragmented, and is identical across most individuals from Browse Island and the reference sequences from GenBank. The fact that the region is highly conserved is evidence of its adaptive attribute as selection would maintain it over time, but this affects the length-based method used to determine the age of the hybridisation. The location of the insertion on the gene could influence the way it is conserved, as despite it being in an intron, it is close to the flanking region of exon 2. Noncoding intron regions are usually conserved when the exons are alternatively spliced, however conserved intronic sequences are also observed in 17% of exons that are constitutively spliced (Sorek & Ast, 2003). In humans, the *Vkorc1* gene is alternatively spliced, and three isoforms have been described, while the *Vkorc1* of *M. musculus* has a single described isoform and two potential isoforms that have been computationally mapped (The UniProt Consortium, 2018). If the *Vkorc1* gene of *Mus musculus* is indeed alternatively spliced, the possibility of the intron being conserved is much higher, as this phenomenon is observed in 77% of the conserved

exons (Sorek & Ast, 2003). Furthermore, the insertion was carried by all individuals on Browse Island, which could be explained by the introgression being ancient. However, the population is isolated on an island far from the mainland, and the low genetic diversity could be the result of a small number of individuals arriving on the island, which generated a founder effect, or be the result a bottleneck event post-colonisation.

The *Vkorc1* gene is highly conserved across organisms and no south-east Asian house mice from Browse Island carried mutations in the coding sequence that are linked to the *M. spretus* allele. The absence of these adaptive mutations is consistent with the absence of exposure to ARs, which would select for them (Song et al., 2011b). As Browse Island has not been subject to eradications aided by anticoagulant rodenticides, the absence of mutations in the coding region could have been caused by the lack of positive selection.

4.3 Low genetic diversity of *Mus musculus* of Western Australia

Mus musculus domesticus of Western Australia belongs to two closely related haplotypes that differ from each other by a single base pair change, and are most closely related to mice from European countries, such as The Netherlands and United Kingdom. A phylogeographic study of mice in Australia discovered 12 haplotypes, with Western Australian house mice belonging to the two major clades E and F (following nomenclature by Gabriel et al., 2011). The clade occurring geographically closest to Perth was clade E, to which the mice analysed in this study also belong. As well as being the most widespread clade in Australia, this haplotype group is also the most common in the United Kingdom, which suggests the origin of Western Australian *M. m. domesticus* could be from the British Isles (Gabriel et al., 2011).

Apart from mice from Madeira, which were grouped closely in the haplotype network, no countries were characterised by pockets of diversity. The resulting network was highly reticulate with few missing haplotypes (represented by mutational steps on the network), indicating western house mice are closely related worldwide. The genotypic analysis of the mitochondrial D-loop of house mice from the Perth metropolitan area indicated the presence of a single panmictic population, with the lack of population structure suggesting high gene flow and a large effective population size (Tang et al., 2018; Van Hooft, Cosson, Vibe-Petersen, & Leirs, 2008). Often, investigation into the population genetics of rodents reports high genetic diversity, such as in South American rodents (Ojeda, 2010), Chinese subterranean rodents (Lin, Cai, Zhang, Su, & Thirgood, 2008), Southern plains woodrats (Méndez-Harclerode et al., 2005), and Patagonian mice (Kim et al., 1998). Despite being relatively uncommon, low genetic diversity in rodent populations has been previously reported, and generally attributed to founder effects or severe bottlenecks, such as in the case of Eurasian red squirrels (Madsen et al., 2015). A study on another commensal and cosmopolitan species (like the house mouse) found a similar result, where rock pigeons (*Columba livia*) living in Singapore formed a single population with low genetic diversity, and the authors attributed this to rapid expansion from a founder population characterised by genetic homogeneity (Tang et al., 2018).

A strong link between mainland Australian *M. m. domesticus* and mice from the British Isles was discovered by Gabriel et al. (2011), which was in agreement with historical human associations between the two countries; however the analysis was limited to a subset of European countries and did not include territories outside of Europe, and thus failed to uncover the genetic similarities between Australian mice and those of Iran, which was highlighted in the present analysis. The network of this study suggests a central position for Iranian mice, whose genetic divergence indicates an old (or ancestral) population (Kho et al., 2010). This could indicate a centre of origin for the species, which concurs with the assumed origin of the commensal relationship between mice and humans that was established in the Middle East

around 10,000 BC when humans first formed large settlements in the Fertile Crescent (Lippens et al., 2017). The expansion of *M. m. domesticus* would have been aided by human transport and trades into the Mediterranean Sea during the Iron Age, and then to colonies by European settlers (Cucchi, Auffray, & Vigne, 2012; Jones, Eager, Gabriel, Jóhannesdóttir, & Searle, 2013; Searle et al., 2009).

The previous phylogeographic study of *M. musculus* in Australia by Gabriel et al. (2011) documented the presence of *domesticus* as the predominant (and based on their data only) subspecies of house mouse in Australia, however mitochondrial D-loop data from mice of Browse Island uncovered that the *castaneus* subspecies of house mouse is present. Anecdotally, this south-east Asian house mouse was known to reside on the island, and it was confirmed that this population is *M. m. castaneus* recently by genetic screening (Moro et al., 2018). The study by Gabriel et al. (2011) excluded the possibility of invasion from south-east Asia and identified the house mice on the Australian mainland as originating from western Europe, specifically coming with Dutch or British ships. However, the haplotype network of *M. m. castaneus* suggests that the origin of these island mice may be from China and Indonesia, the latter of which is in agreement with previous studies attributing the origin of Australian *M. m. castaneus* to the Indonesian islands of Rote and Timor (Moro et al., 2018).

4.4 Pest management

When planning pest eradications or population control, it is vital to gather information on the resistance of the population in order to choose a control method that can successfully eliminate most of the population. It has been suggested that anticoagulant rodenticides should be used when the dose kills 99% of the population (Fisher, 2005), however eradication attempts and rodent control programs in Western Australia have been carried out with no knowledge of the level of resistance to the chemicals. In Europe, the spread of resistance has been addressed with the commercialisation of alternative non-anticoagulant rodenticides, however the range

of alternative options is still scarce, and ARs are the preferred option for many eradication plans (Zhelev et al., 2019). Thus, it is important to evaluate which anticoagulant rodenticide to employ in order to avoid building resistance in the pest and to achieve the management goal.

Since the level of AR resistance in Western Australia has not been researched before, the aim of this study was to provide data on the *Vkorc1* gene of house mouse of this region, and to suggest pest management measures based on this evidence. In the Perth metropolitan area and on Browse Island, resistance-conferring *Vkorc1* mutations were not detected. The house mouse populations in these areas can be managed with the use of FGARs that have less negative impacts on non-target species or less strong SGARs when the natural tolerance exhibited by mice poses restrictions on the use of first generation compounds, however mice should be regularly tested to identify any developing resistance and, if resistance is found, management should incorporate a SGAR to eliminate such individuals (Billing, 2000). Alternatively, if this happens a non-anticoagulant rodenticide such as cholecalciferol, may be applied to reduce resistant mice and decrease the risk of bioaccumulation and secondary toxicity (Baldwin, Meinerz, & Witmer, 2016; Lohr & Davis, 2018).

Rodenticide resistance in house mouse of Western Australia cannot be ruled out entirely as it is known that resistance can arise through other mechanisms, and professionals have anecdotally observed resistance in the house mice population of Perth (Piggott D 2019, written communication, 21st February 2019). It is recommended to test the rodent population through feeding trials or blood clotting response tests in order to select the most suitable control method. *Mus musculus* exhibits a natural tolerance to anticoagulant rodenticides, thus other acute toxins may need to be used, such as cholecalciferol and fluoroacetate, either by themselves or in combination with the readily available ARs (Cowan et al., 2017).

The use of combination baits has been tested on rodents and found to successfully eliminate individuals in resistant populations, as well as reduce the time to death (Baldwin et al., 2016). For example, cholecalciferol plus anticoagulant baits achieve death in five days, thus they are highly effective, and the concentration of active ingredients is lower than baits containing a single active ingredient (Baldwin et al., 2016). Combination baits pose less risk to non-target predators and scavenging species due to the lower concentrations of active ingredients, which also renders the baits more palatable to the rodents compared to cholecalciferol alone (Baldwin et al., 2016). Therefore, combination baits are a viable option to deal with rodents where the risk of secondary poisoning to non-target species is high, while accomplishing pest mortality without inducing bait shyness.

The mitochondrial D-loop data highlighted the genetic homogeneity of western house mice populating the Perth metropolitan area. The commensal house mouse is characterised by lower rates of dispersal compared to feral populations located in agricultural settings (Pocock et al., 2005), therefore the panmictic population discovered in Perth is unusual. As Perth is close to a major commercial port (Department of Treasury and Finance, 2004), the risk of new mice invading the area is high if transport of mice by humans from other areas into Perth is not prevented. Despite a reported genetic resilience of established mouse populations to new migrants (Mora et al., 2013), in the event that resistant mice reach Perth these new individuals would be at a highly advantageous position due to the intense use of ARs, and resistance would be selected for, thus providing conditions that would favour the invader (Ishizuka et al., 2008).

Preventing the arrival of pests such as house mouse is managed by the Agriculture and Food division of the Department of Primary Industries and Regional Development (DPIRD), whose work is directed to the safeguard of the state's resources from biological threats (Department of Primary Industries and Regional Development, n.d.). A biosecurity and quarantine system has been developed to

defend the state against incursions of pests, and checkpoints are in place at the state's border and at all airports (Department of Primary Industries and Regional Development, 2015, 2017). To minimise the introduction of new immigrant mice by shipping, stringent biosecurity protocols should be established for all wharf facilities and staff should ensure that ships comply with these measures (Tasmania Parks and Wildlife Service, 2009). Vessels should be certified free of rodents and provide evidence that no re-infestation occurred since the certificate was issued (Tasmania Parks and Wildlife Service, 2009).

The absence of resistance-related mutations in house mice of Perth does not suggest that rats inhabiting the same area do not carry mutations. An example of the potential for the two rodents to differ comes from Norway rats of Somerset in the United Kingdom that were found to be homozygous for *Vkorc1* mutations related to resistance, while *M. musculus* of the same area were susceptible to rodenticides (Prescott et al., 2018) In other cases the opposite was true, as house mice from Lord Howe Island (New South Wales) displayed resistance to warfarin and brodifacoum, while rats were susceptible even after a decade of intensive use of ARs (Billing, 2000; Wheeler et al., 2018). As resistance is not always concomitant in rat and mice species, rats of the Perth area should be tested to identify if resistance is present. In terms of management, should rats and mice of Perth exhibit different levels of resistance, the control program should include an alternative rodenticide to substitute the AR in order to control any developing resistance, and maintain low numbers in a resistant population (Billing, 2000).

On Browse Island, mouse densities are very high and this might explain the abandonment of the island by breeding seabird populations (Moro et al., 2018), as the predatory behaviour of *M. musculus* has been observed on many islands (Australian Centre for International Agricultural Research, 2003). Bird activity has been recorded as disappointingly low since 1949, despite Browse Island being located between Ashmore Reef and Adele Island and therefore constituting a

valuable connection between the breeding colonies visiting the two locations (Moro et al., 2018). Furthermore, the presence of a mouse subspecies different from *domesticus* on the island poses a biosecurity risk to mainland Western Australia, as the quarantine legislation is operated to prevent any new pest introduction to the state (Department of Primary Industries and Regional Development, n.d.).

The lack of data on the *Vkorc1* gene of *M. m. castaneus* from other locations does not allow for the investigation of whether the silent mutations expressed by the Browse Island population constitute a signature of the subspecies or if it has arisen independently and spread in the island population. Additionally, the introgression by *Mus spretus* that has been detected in the *Vkorc1* gene of the *castaneus* subspecies cannot be compared to other data to identify the origin of the introgression event nor date it. Further data on the *Vkorc1* gene of *M. m. castaneus* will aid in distinguishing between introgression and incomplete lineage sorting, and will guide the evaluation and dating of such events. Fortunately, the introgression between Browse Island mice and *M. spretus* was limited to intronic sequences, and resistance-related mutations were not carried by any individual from Browse Island that has been sequenced.

Since the south-east Asian house mouse population on Browse Island carries silent *Vkorc1* mutations that do not impair rodenticide activity, the use of ARs to achieve pest eradication is possible, and it can be hoped that the eradication of *M. m. castaneus* from the island will promote breeding of indigenous species by removing the risk of egg predation of both seabirds and green turtles that nest on the island. Landing on Browse island is prohibited due to the biosecurity risk posed by tourism vessels and Indonesian boats, however, Indonesian fishermen are known to have disembarked on the island (Limpus, 2002). For this reason, recolonisation of the island by house mice transported on Indonesian boats is possible. Even regular sea and air patrols might not be sufficient to prevent recolonisation, but they can assess the pest population and organise its control in the early stages of establishment.

The use of anticoagulant rodenticides on the island should be carefully planned to reduce the impact of toxins to the fauna inhabiting the island and coastal waters, since broadcast applications of rodenticides on islands are linked to exposure of non-target wildlife in coastal environments (Masuda, Fisher, & Beaven, 2015). Anticoagulant rodenticides can enter the aquatic environment through various ways, including by aerial broadcast on islands with high tides, which wash the pellets into the coastal area (Kotthoff et al., 2018; Masuda et al., 2015). In coastal environments, brodifacoum residues have been detected at sub-lethal concentrations in mussels, limpets and blue cod following island rodent eradication (Masuda et al., 2015). The waters around Browse Island are part of the Australian Fishing Zone, and are often visited by Indonesian fishermen who harvest marine organisms for consumption; as this biota might be exposed to ARs, the use of toxins threatens human health through possible secondary poisoning (Masuda et al., 2015; Stacey, 2007).

To reduce the risk of primary and secondary poisoning of non-target species, various mitigation techniques have been used during eradication campaigns, including trapping and temporary holding of vertebrates, establishing a refuge area that is free of rodenticides, and developing bait boxes that exclude non-target species (Howald et al., 2007). These mitigation procedures are vital to ensure the future of viable populations of native species, however, the many examples of detection of ARs in non-target species shows that the use of ARs is still problematic.

The aim of this study was to measure the resistance levels of two contrasting house mouse populations, and to make suggestions for conservation and biosecurity based on the evidence gathered. The absence of resistance-conferring *Vkorc1* mutations in both Perth metropolitan area and Browse Island house mouse populations indicates that ARs can be employed to control and/or eradicate the pest, and biosecurity procedures should be in place to avoid the introduction of potential resistant mice in Western Australia.

5 Conclusion

Since the first human settlements created an altered environment, certain plant and animal populations have been able to exploit the resources provided, and the establishment of commensal populations led to conflict between humans and pests (Horvitz et al., 2017; Riyahi et al., 2013; Saraswat et al., 2015). Human-mediated dispersal of species led to the expansion of pests distributions, which can result in genetic homogeneity (Riyahi et al., 2013), as noted in the close relatedness of house mice worldwide. Anticoagulant rodenticides have been used since the 1940s to control pest populations of rodents, but this has selected for resistance in the rodents and negatively impacting non-target species (Capizzi et al., 2014; Kotthoff et al., 2018; Lohr & Davis, 2018; López-Perea et al., 2019; Rattner et al., 2014a; Ruiz-Suárez et al., 2014).

Vkorc1 screening in Europe has discovered high frequencies of mutations that infer resistance to ARs in multiple countries (Goulois et al., 2016; Grandemange et al., 2010; Mayumi Ishizuka et al., 2007; Pelz et al., 2012). In Australia, strong ARs can be purchased from the supermarket and retail outlets, and are routinely and intensively used in residential and commercial settings (Lohr & Davis, 2018). These factors, along with anecdotal evidence, led to the hypothesis that *Mus musculus* of Perth had a high probability of carrying resistance-related mutations in the *Vkorc1* gene. The absence of *Vkorc1* mutations in Western Australian mice as found in this study does not rule out the presence of resistance *per se*, however, it is crucial to prevent the introduction of such mutations in Perth mouse populations as these could be selected for due to the intense use of ARs.

The existence of a different subspecies of house mouse on Browse Island poses a biosecurity threat to mainland Australia, and the high density of *Mus musculus castaneus* could be responsible for the discontinued use of the island as a breeding site by seabirds (Moro et al., 2018). Eradication of the south-east Asian house mouse from Browse Island will promote increased biodiversity on the island and remove a

biosecurity threat for mainland Australia. As Indonesian fishermen visit the area, reintroduction of the pest on the island is possible, and regular patrols should identify establishing populations to immediately manage them.

A general lack of data regarding the levels of AR resistance in commensal rodents in Australia and the impact of rodenticides on non-target biota should motivate future research to investigate resistance in mouse and rat populations throughout Australia. *Vkorc1* screening and laboratory feeding trials are warranted in rodent populations that are exposed to widespread AR use and should be repeated periodically in case of introduction and/or independently arising mutations. Currently, anticoagulant rodenticides are likely to continue to be effective in controlling mouse populations (at least in the short-term) and there should be a focus on: preventing the introduction of potentially resistant house mice to Western Australia through the port of Fremantle and other routes; undertaking an eradication program on Browse Island that minimises the risk of coastal water contamination; and, generally lessening the threat of secondary poisoning to non-target species in both environments by careful planning and application of ARs.

References

Alomar, H., Chabert, A., Coeurdassier, M., Vey, D., & Berny, P. (2018). Accumulation of anticoagulant rodenticides (chlorophacinone, bromadiolone and brodifacoum) in a non-target invertebrate, the slug, *Deroceras reticulatum*. *Science of the Total Environment*, *610–611*, 576–582. https://doi.org/10.1016/j.scitotenv.2017.08.117

Australian Bureau of Statistics. (2018). Australian Demographic Statistics. Retrieved May 24, 2019, from https://www.abs.gov.au/AUSSTATS/abs@.nsf/mf/3101.0

Australian Centre for International Agricultural Research. (2003). Rats, mice and people: rodent biology and management. In G. R. Singleton, L. A. Hinds, C. J. Krebs, & D. M. Spratt (Eds.), *Rats, mice and people: rodent biology and management*. Retrieved from www.aciar.gov.au

Baert, K., Stuyck, J., Breyne, P., Maes, D., & Casaer, J. (2012). Distribution of anticoagulant resistance in the brown rat in Belgium. *Belgian Journal of Zoology*, *142*(1).

Baldwin, R. A., Meinerz, R., & Witmer, G. W. (2016). Cholecalciferol plus diphacinone baits for vole control: a novel approach to a historic problem. *Journal of Pest Science*, *89*(1), 129–135. https://doi.org/10.1007/s10340-015-0653-3

Bandelt, H.-J., Forster, P., & Rohl, A. (1994). Median-joining networks for inferring intraspecic phylogenies. *Molecular Biology*, *16*(1), 37–48. https://doi.org/10.1093/oxfordjournals.molbev.a026036

Berney, P., Esther, A., Jacob, J., & Prescott, C. (2014). *Risk mitigation measures for anticoagulant rodenticides as biocidal products*. European Commission. https://doi.org/10.2779/241180

Billing, J. (2000). The control of introduced *Rattus rattus* L. on Lord Howe Island. II. The status of warfarin resistance in rats and mice. *Wildlife Research*, *27*. https://doi.org/10.1071/WR99013

Bonhomme, F., Orth, A., Cucchi, T., Rajabi-Maham, H., Catalan, J., Boursot, P., … Britton-Davidian, J. (2011). Genetic differentiation of the house mouse around the Mediterranean basin: Matrilineal footprints of early and late colonization. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1708), 1034–1043. https://doi.org/10.1098/rspb.2010.1228

Bozikova, E., Munclinger, P., Teeter, K. C., Tucker, P. K., Macholan, M., & J, P. (2005). Mitochondrial DNA in the hybrid zone between *Mus musculus musculus* and *Mus musculus domesticus*: a comparison of two transects. *Biological Journal of the Linnean Society*, *84*, 363–378.

Bradfield, A. A. G., & Gill, J. E. (1984). Laboratory trials of five rodenticides for the control of *Mesocricetus auratus* Waterhouse. *Journal of Hygiene*, *93*(2), 389–394. https://doi.org/10.1017/S0022172400064950

Brouat, C., Tollenaere, C., Estoup, A., Loiseau, A., Sommer, S., Soanandrasana, R., … Duplantier, J. M. (2014). Invasion genetics of a human commensal rodent: The black rat *Rattus rattus* in Madagascar. *Molecular Ecology*, *23*(16), 4153–4167. https://doi.org/10.1111/mec.12848

Capizzi, D., Bertolino, S., & Mortelliti, A. (2014). Rating the rat: Global patterns and research priorities in impacts and management of rodent pests. *Mammal Review*, *44*(2), 148–162. https://doi.org/10.1111/mam.12019

Christensen, P., & Burrows, N. (1994). Project desert dreaming: experimental reintroduction of mammals to the Gibson Desert, Western Australia. In M. Serena (Ed.), *Reintroduction Biology of Australian and New Zealand Fauna* (pp. 199–209). Chipping Norton: Surrey Beatty & Sons.

Clarke, R. H. (2010). *The status of seabirds and shorebirds at Ashmore Reef and Carter and Browse Islands: Monitoring program for the Montara well release*. Clayton, Victoria: Australian Centre for Biodiversity.

Clout, M. N., & Russell, J. C. (2006). The eradication of mammals from New Zealand islands. *Assessment and Control of Biological Invasion*, 127–141. Retrieved from https://www.stat.auckland.ac.nz/~jrussell/files/papers/CloutRussell2006.pdf

Cory, F., Wilson, A., Priddel, D., Carlile, N., & Klomp, N. (2011). Eradication of the House Mouse (*Mus musculus*) from Montague Island, New South Wales, Australia. *Ecological Management & Restoration*, *12*(2). https://doi.org/10.1111/j.1442-8903.2011.00583.x

Cowan, P. E., Gleeson, D. M., Howitt, R. L. J., Ramón-Laca, A., Esther, A., & Pelz, H. J. (2017). *Vkorc1* sequencing suggests anticoagulant resistance in rats in New Zealand. *Pest Management Science*, *73*. https://doi.org/10.1002/ps.4304

Craik, W., Palmer, D., & Sheldrake, R. (2017). *Priorities for Australia's biosecurity system*. Canberra: Department of Agriculture and Water Resources.

Cucchi, T., Auffray, J.-C., & Vigne, J.-D. (2012). On the origin of the house mouse synanthropy and dispersal in the Near East and Europe: zooarcheological review and perspectives. In M. Macholan, S. J. E. Baird, P. Muclinger, & J. Pialek (Eds.), *Evolution in Our Neighbourhood: The House Mouse as a Model in Evolutionary Research.* (pp. 65–93). Cambridge: Cambridge University Press. https://doi.org/10.1017/cbo9781139044547.005

Cuthbert, R. J., Visser, P., Louw, H., & Ryan, P. G. (2011). Palatability and efficacy of rodent baits for eradicating house mice (Mus musculus) from Gough Island, Tristan da Cunha. *Wildlife Research*, *38*(3), 196. https://doi.org/10.1071/wr11016

Davies, D., Dilley, B. J., Bond, A. L., Cuthbert, R. J., & Ryan, P. G. (2015). Trends and tactics of mouse predation on Tristan Albatross *Diomedea dabbenena* chicks at Gough Island, South Atlantic Ocean. *Avian Conservation and Ecology*, *10*(1). https://doi.org/10.5751/ace-00738-100105

Dejager, L., Libert, C., & Montagutelli, X. (2009). Thirty years of *Mus spretus*: a promising future. *Trends in Genetics*, *25*(5), 234–241. https://doi.org/10.1016/j.tig.2009.03.007

Department of Primary Industries and Regional Development. (n.d.). Biosecurity & quarantine. Retrieved May 24, 2019, from https://www.agric.wa.gov.au/biosecurity-quarantine

Department of Primary Industries and Regional Development. (2015). WA State barrier animal inspections. Retrieved May 24, 2019, from https://www.agric.wa.gov.au/quarantine/wa-state-barrier-animal-inspections

Department of Primary Industries and Regional Development. (2017). Quarantine WA border checkpoints. Retrieved May 24, 2019, from https://www.agric.wa.gov.au/importing-animals/quarantine-wa-border-checkpoints

Department of Treasury and Finance. (2004). *An Economic History of Western Australia Since Colonial Settlement*. Perth: Department of Treasury and Finance.

Desvars-Larrive, A., Hammed, A., Hodroge, A., Berny, P., Benoît, E., Lattard, V., & Cosson, J. F. (2018). Population genetics and genotyping as tools for planning rat management programmes. *Journal of Pest Science*. https://doi.org/10.1007/s10340-018-1043-4

Fisher, P. (2005). Review of house mouse (*Mus musculus*) susceptibility to anticoagulant poisons. *DOC Science Internal Series*, *198*, 1–18.

Forster, D. W., Gunduz, I., Nunes, A. C., Gabriel, S., Ramalhinho, M. G., Mathias, M. L., … Searle, J. B. (2009). Molecular insights into the colonization and chromosomal diversification of Madeiran house mice. *Molecular Ecology*, *18*, 4477–4494. https://doi.org/10.1111/j.1365-294X.2009.04344.x

Gabriel, S. I., Stevens, M. I., da Luz Mathias, M., & Searle, J. B. (2011). Of mice and "Convicts": Origin of the Australian house mouse, *Mus musculus*. *PLoS ONE*, *6*. https://doi.org/10.1371/journal.pone.0028622

García-Rodríguez, O., Andreou, D., Herman, J. S., Mitsainas, G. P., Searle, J. B., Bonhomme, F., … Hardouin, E. A. (2018). Cyprus as an ancient hub for house mice and humans. *Journal of Biogeography*, *45*, 2619–2630. https://doi.org/10.1111/jbi.13458

Geraldes, A., Basset, P., Gibson, B., Smith, K. L., Harr, B., Yu, H., … Nachman, M. W. (2008). Inferring the history of speciation in house mice from autosomal, X-linked, Y-linked and mitochondrial genes. *Molecular Ecology*, *17*(24), 5349–5363. https://doi.org/10.1111/j.1365-294X.2008.04005.x.Inferring

Goulois, J., Hascoët, C., Dorani, K., Besse, S., Legros, L., Benoit, E., & Lattard, V. (2017). Study of the efficiency of anticoagulant rodenticides to control *Mus musculus domesticus* introgressed with *Mus spretus Vkorc1*. *Pest Management Science*, *73*(2), 325–331. https://doi.org/10.1002/ps.4319

Goulois, J., Lambert, V., Legros, L., Benoit, E., & Lattard, V. (2016). Adaptative evolution of the *Vkorc1* gene in *Mus musculus domesticus* is influenced by the selective pressure of anticoagulant rodenticides. *Ecology and Evolution*, *7*. https://doi.org/10.1002/ece3.2829

Grandemange, A., Lasseur, R., Longin-Sauvageon, C., Benoit, E., & Berny, P. (2010). Distribution of *Vkorc1* single nucleotide polymorphism in wild *Rattus norvegicus* in France. *Pest Management Science*, *66*. https://doi.org/10.1002/ps.1869

Grund, R. (1996). A butterfly record from Browse Island, North West Shelf, Australia. *Australian Entomologist*, *23*(3), 86.

Gryseels, S., Leirs, H., Makundi, R., & Gouy De Bellocq, J. (2015). Polymorphism in *Vkorc1* Gene of Natal Multimammate Mice, *Mastomys natalensis*, in Tanzania. *Journal of Heredity*, *106*. https://doi.org/10.1093/jhered/esv054

Gündüz, I., Tez, C., Malikov, V., Vaziri, A., Polyakov, A. V., & Searle, J. B. (2000). Mitochondrial DNA and chromosomal studies of wild mice (*Mus*) from Turkey and Iran. *Heredity*. https://doi.org/10.1046/j.1365-2540.2000.00694.x

Hardouin, E. A., Chapuis, J. L., Stevens, M. I., Van Vuuren, J. B., Quillfeldt, P., Scavetta, R. J., … Tautz, D. (2010). House mouse colonization patterns on the sub-Antarctic Kerguelen Archipelago suggest singular primary invasions and resilience against re-invasion. *BMC Evolutionary Biology*, *10*(1). https://doi.org/10.1186/1471-2148-10-325

Harris, D. B. (2009). Review of negative effects of introduced rodents on small mammals on islands. *Biological Invasions*, *11*. https://doi.org/10.1007/s10530-008-9393-0

Hedrick, P. W. (2013). Adaptive introgression in animals: Examples and comparison to new mutation and standing variation as sources of adaptive variation. *Molecular Ecology*, *22*(18), 4606–4618. https://doi.org/10.1111/mec.12415

Heiberg, A. C., Leirs, H., & Siegismund, H. R. (2006). Reproductive success of bromadiolone-resistant rats in absence of anticoagulant pressure. *Pest Management Science*, *62*. https://doi.org/10.1002/ps.1249

Hindmarch, S., Elliott, J. E., Mccann, S., & Levesque, P. (2017). Habitat use by barn owls across a rural to urban gradient and an assessment of stressors including, habitat loss, rodenticide exposure and road mortality. *Landscape and Urban Planning*, *164*, 132–143. https://doi.org/10.1016/j.landurbplan.2017.04.003

Horak, K., Fisher, P., & Hopkins, B. (2018). Pharmacokinetics of Anticoagulant Rodenticides in Target and Non-Target Organisms. In N. W. van den Brink et al. (Eds.) *Anticoagulant Rodenticides and Wildlife.* Lincoln: University of Nebraska.

Horvitz, N., Wang, R., Wan, F. H., & Nathan, R. (2017). Pervasive human-mediated large-scale invasion: analysis of spread patterns and their underlying mechanisms in 17 of China's worst invasive plants. *Journal of Ecology*, *105*(1), 85–94. https://doi.org/10.1111/1365-2745.12692

Howald, G., Donlan, C. J., Galván, J. P., Russell, J. C., Parkes, J., Samaniego, A., … Tershy, B. (2007). Invasive rodent eradication on islands. *Conservation Biology*, *21*(5), 1258–1268. https://doi.org/10.1111/j.1523-1739.2007.00755.x

Ishizuka, M., Tanaka, K. D., Heewon, M., Okajima, F., Sakamoto, K. Q., & Fujita, S. (2008). Pesticide resistance in wild mammals – Mechanisms of anticoagulant resistance in wild rodents. *The Journal of Toxicological Sciences*, *33*(3).

Ishizuka, M., Okajima, F., Tanikawa, T., Min, H., Tanaka, K. D., Sakamoto, K. Q., & Fujita, S. (2007). Elevated warfarin metabolism in warfarin-resistant roof rats (*Rattus rattus*) in Tokyo. *Drug Metabolism and Disposition*, *35*(1). https://doi.org/10.1124/dmd.106.011775

Jackson, T. P., & Van Aarde, R. J. (2003). Advances in vertebrate pest control: Implications for the control of feral house mice on Marion Island. *South African Journal of Science*, *99*(3–4), 130–136.

Jacob, J., Endepols, S., Pelz, H. J., Kampling, E., Cooper, T. G., Yeung, G. H., … Schlatt, S. (2012). Vitamin K requirement and reproduction in bromadiolone resistant Norway rats. *Pest Management Science*, *68*.

Jing, M., Yu, H. T., Bi, X., Lai, Y. C., Jiang, W., & Huang, L. (2014). Phylogeography of Chinese house mice (*Mus musculus musculus/castaneus*): Distribution, routes of colonization and geographic regions of hybridization. *Molecular Ecology*, *23*(17), 4387–4405. https://doi.org/10.1111/mec.12873

Jones, E. P., Eager, H. M., Gabriel, S. I., Jóhannesdóttir, F., & Searle, J. B. (2013). Genetic tracking of mice and other bioproxies to infer human history. *Trends in Genetics*, *29*(5), 298–308. https://doi.org/10.1016/j.tig.2012.11.011

Jones, M. G. W., & Ryan, P. G. (2009). Evidence of mouse attacks on albatross chicks on sub-Antarctic Marion Island. *Antarctic Science*, *22*(1), 39–42. https://doi.org/10.1017/S0954102009990459

Kappes, P. J., Bond, A. L., Russell, J. C., & Wanless, R. M. (2019). Diagnosing and responding to causes of failure to eradicate invasive rodents. *Biological Invasions*, *21*(7), 2247–2254. https://doi.org/10.1007/s10530-019-01976-0

Keane, T. M., Goodstadt, L., Danecek, P., White, M. A., Wong, K., Yalcin, B., … Adams, D. J. (2011). Mouse genomic variation and its effect on phenotypes and gene regulation. *Nature*, *477*(7364), 289–294. https://doi.org/10.1038/nature10413

Kho, W.-G., Kano, S., Iwagami, M., Tanabe, K., Hayakawa, T., Kim, S.-H., … Hwang, S.-Y. (2010). Geographical origin of Plasmodium vivax in the Republic of Korea: haplotype network analysis based on the parasite's mitochondrial genome. *Malaria Journal*, *9*(1), 184. https://doi.org/10.1186/1475-2875-9-184

Kim, I., Phillips, C. J., Monjeau, J. A., Birney, E. C., Noack, K., Pumo, D. E., … Dole, J. A. (1998). Habitat islands, genetic diversity, and gene flow in a Patagonian rodent. *Molecular Ecology*, *7*(6), 667–678. https://doi.org/10.1046/j.1365-294x.1998.00369.x

King, C. M. (1983). The Relationships between Beech (*Nothofagus* Sp.) seedfall and populations of mice (*Mus musculus*), and the demographic and dietary responses of stoats (*Mustela erminea*), in three New Zealand forests. *The Journal of Animal Ecology*, *52*(1), 141. https://doi.org/10.2307/4593

Kotthoff, M., Rüdel, H., Jürling, H., Severin, K., Hennecke, S., Friesen, A., & Koschorreck, J. (2018). First evidence of anticoagulant rodenticides in fish and suspended particulate matter: spatial and temporal distribution in German freshwater aquatic systems. *Environmental Science and Pollution Research*, 1–11. https://doi.org/10.1007/s11356-018-1385-8

Leigh, J. W., & Bryant, D. (2015). POPART: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, *6*(9), 1110–1116. https://doi.org/10.1111/2041-210X.12410

Limdi, N. A., & Veenstra, D. L. (2008). Warfarin pharmacogenetics. *Pharmacotherapy*, *28*(9), 1084–1097. https://doi.org/10.1016/j.tcm.2014.09.001

Limpus, C. J. (2002). *Western Australia marine turtle review*. Brisbane: Department of Conservation and Land Management.

Lin, G. H., Cai, Z. Y., Zhang, T. Z., Su, J. P., & Thirgood, S. J. (2008). Genetic diversity of the subterranean Gansu zokor in a semi-natural landscape. *Journal of Zoology*, *275*(2), 153–159. https://doi.org/10.1111/j.1469-7998.2008.00423.x

Lippens, C., Estoup, A., Hima, M. K., Loiseau, A., Tatard, C., Dalecky, A., … Brouat, C. (2017). Genetic structure and invasion history of the house mouse (*Mus musculus domesticus*) in Senegal, West Africa: a legacy of colonial and contemporary times. *Heredity*, 1–12. https://doi.org/10.1038/hdy.2017.18

Liu, K. J., Steinberg, E., Yozzo, A., Song, Y., Kohn, M. H., & Nakhleh, L. (2015). Interspecific introgressive origin of genomic diversity in the house mouse. *Proceedings of the National Academy of Sciences*, *112*(1). https://doi.org/10.1073/pnas.1406298111

Lohr, C., Van Dongen, R., Huntley, B., Gibson, L., & Morris, K. (2014). Remotely monitoring change in vegetation cover on the Montebello Islands, Western Australia, in response to introduced rodent eradication. *PLoS ONE*, *9*(12). https://doi.org/10.1371/journal.pone.0114095

Lohr, M. T. (2018). Anticoagulant rodenticide exposure in an Australian predatory bird increases with proximity to developed habitat. *Science of the Total Environment*, *643*. https://doi.org/10.1016/j.scitotenv.2018.06.207

Lohr, M. T., & Davis, R. A. (2018). Anticoagulant rodenticide use, non-target impacts and regulation: A case study from Australia. *Science of the Total Environment*, *634*. https://doi.org/10.1016/j.scitotenv.2018.04.069

López-Perea, J. J., Camarero, P. R., Sánchez-Barbudo, I. S., & Mateo, R. (2019). Urbanization and cattle density are determinants in the exposure to anticoagulant rodenticides of non-target wildlife. *Environmental Pollution*, *244*, 801–808. https://doi.org/10.1016/j.envpol.2018.10.101

MacKay, J. W. B., Alexander, A., Hauber, M. E., Murphy, E. C., & Clout, M. N. (2013). Does genetic variation among invasive house mice in New Zealand affect eradication success? *New Zealand Journal of Ecology*, *37*(1).

Madsen, C. L., Vilstrup, J. T., Fernández, R., Marchi, N., Hakansson, B., Krog, M., … Orlando, L. (2015). Mitochondrial genetic diversity of eurasian red squirrels (*Sciurus vulgaris*) from Denmark. *Journal of Heredity*, *106*(6), 719–727. https://doi.org/10.1093/jhered/esv074

Mahmoud, W., & Redfern, R. (1981). The response of the Egyptian spiny mouse (*Acomys cahirinus*) and two other species of commensal rodents to anticoagulant rodenticides. *Journal of Hygiene*, *86*(3), 329–334. https://doi.org/10.1017/S0022172400069072

Markussen, M. D. K., Heiberg, A. C., Nielsen, R., & Leirs, H. (2003). Vitamin K requirement in Danish anticoagulant-resistant Norway rats (*Rattus norvegicus*). *Pest Management Science*, *59*. https://doi.org/10.1002/ps.703

Masuda, B. M., Fisher, P., & Beaven, B. (2015). Residue profiles of brodifacoum in coastal marine species following an island rodent eradication. *Ecotoxicology and Environmental Safety*, *113*, 1–8. https://doi.org/10.1016/j.ecoenv.2014.11.013

Masuda, B. M., Fisher, P., & Jamieson, I. G. (2014). Anticoagulant rodenticide brodifacoum detected in dead nestlings of an insectivorous passerine. *New Zealand Journal of Ecology*, *38*(1), 110–115.

Matisoo-Smith, E., & Robins, J. H. (2004). Origins and dispersals of Pacific peoples: Evidence from mtDNA phylogenies of the Pacific rat. *Proceedings of the National Academy of Sciences*, *101*(24), 9167–9172. https://doi.org/10.1073/pnas.0403120101

Méndez-Harclerode, F. M., Hanson, J. D., Fulhorst, C. F., Milazzo, M. L., Ruthven, D. C., & Bradley, R. D. (2005). Genetic diversity within the southern plains woodrat (*Neotoma Micropus*) in southern Texas. *Journal of Mammalogy*, *86*(1), 180–190. https://doi.org/10.1644/1545-1542(2005)086<0180:gdwtsp>2.0.co;2

Mooney, J., Lynch, M. R., Prescott, C. V., Clegg, T., Loughlin, M., Hannon, B., … Faulkner, R. (2018). *Vkorc1* sequence variants associated with resistance to anticoagulant rodenticides in Irish populations of *Rattus norvegicus* and *Mus musculus domesticus*. *Scientific Reports*, *8*. https://doi.org/10.1038/s41598-018-22815-7

Mora, M. S., Cutrera, A. P., Lessa, E. P., Vassallo, A. I., D'Anatro, A., & Mapelli, F. J. (2013). Phylogeography and population genetic structure of the Talas tuco-tuco (*Ctenomys talarum*): integrating demographic and habitat histories. *Journal of Mammalogy*, *94*(2), 459–476. https://doi.org/10.1644/11-mamm-a-242.1

Morando, M., Avila, L. J., Baker, J., & Sites, J. W. (2004). Phylogeny and phylogeography of the *Liolaemus Darwinii* complex (Squamata: Liolaemidae): Evidence for introgression and incomplete lineage sorting. *Evolution*, *58*(4), 842–859. https://doi.org/10.1554/03-009

Moro, D., Palmer, R., Greatwich, B., Dickinson, R., & Anderson, H. (2018). Browse Island. A journey to Western Australia's most remote nature reserve. *Landscope*, (December), 22–27.

Newman, D. G. (1994). Effects of a mouse, *Mus musculus*, eradication programme and habitat change on lizard populations on Mana Island, New Zealand, with special reference to McGregor's Skink, *Cyclodina macgregori*. *New Zealand Journal of Zoology*, *21*(4).

Ojeda, A. A. (2010). Phylogeography and genetic variation in the South American rodent *Tympanoctomys barrerae* (Rodentia : Octodontidae). *Journal of Mammalogy*, *91*(2), 302–313. https://doi.org/10.1644/09-MAMM-A-177.1.Key

Pauli, N., & Boruff, B. (2016). Natural environments, ecosystem services and green infrastructure: Planning for Perth's "green" matrix. In S. Biermann, D. Olaru, & V. Paul (Eds.), *Planning Boomtown and Beyond* (pp. 234–276). University of Western Australia Publishing. Retrieved from http://www.patrec.uwa.edu.au/__data/assets/pdf_file/0011/3088037/10-Pauli,-Boruff.pdf

Pelz, H. J., Rost, S., Hünerberg, M., Fregin, A., Heiberg, A. C., Baert, K., … Müller, C. R. (2005). The genetic basis of resistance to anticoagulants in rodents. *Genetics*, *170*. https://doi.org/10.1534/genetics.104.040360

Pelz, H. J., Rost, S., Müller, E., Esther, A., Ulrich, R. G., & Müller, C. R. (2012). Distribution and frequency of *Vkorc1* sequence variants conferring resistance to anticoagulants in *Mus musculus*. *Pest Management Science*, *68*. https://doi.org/10.1002/ps.2254

Pocock, M. J. O., Hauffe, H. C., & Searle, J. B. (2005). Dispersal in house mice. *Biological Journal of Linnaean Society*, *84*, 565–583.

Prescott, C., Coan, E., Jones, C., Baxter, M., Rymer, D., & Buckle, A. (2018). *Anticoagulant Resistance in Rats and Mice in the UK - Current Status in 2018*. *Campaign for Responsible Rodenticide Use UK*. Retrieved from http://www.pestmagazine.co.uk/_attachments/Resources/624_S4.pdf

Rajabi-Maham, H, Orth, A., Boursot, P., Siahsarvi, R., & Bonhomme, F. (2011). The south-eastern house mouse *Mus musculus castaneus* is a polyphyletic subspecies. *Submitted*, 295–306.

Rajabi-Maham, Hassan, Orth, A., & Bonhomme, F. (2008). Phylogeography and postglacial expansion of *Mus musculus domesticus* inferred from mitochondrial DNA coalescent, from Iran to Europe. *Molecular Ecology*, *17*(2), 627–641. https://doi.org/10.1111/j.1365-294X.2007.03601.x

Rattner, B. A., Lazarus, R. S., Elliott, J. E., Shore, R. F., & Van Den Brink, N. (2014a). Adverse outcome pathway and risks of anticoagulant rodenticides to predatory wildlife. *Environmental Science and Technology*, *48*(15), 8433–8445. https://doi.org/10.1021/es501740n

Rattner, B. A., Lazarus, R. S., Elliott, J. E., Shore, R. F., & Van Den Brink, N. (2014b). Adverse outcome pathway and risks of anticoagulant rodenticides to predatory wildlife. *Environmental Science and Technology*. https://doi.org/10.1021/es501740n

Rheindt, F. E., Christidis, L., & Norman, J. A. (2009). Genetic introgression, incomplete lineage sorting and faulty taxonomy create multiple cases of polyphyly in a montane clade of tyrant-flycatchers (Elaenia, Tyrannidae). *Zoologica Scripta*, *38*(2), 143–153. https://doi.org/10.1111/j.1463-6409.2008.00369.x

Riyahi, S., Hammer, Ø., Arbabi, T., Sánchez, A., Roselaar, C. S., Aliabadian, M., & Sætre, G. P. (2013). Beak and skull shapes of human commensal and non-commensal house sparrows *Passer domesticus*. *BMC Evolutionary Biology*, *13*(1). https://doi.org/10.1186/1471-2148-13-200

Ruiz-Suárez, N., Henríquez-Hernández, L. A., Valerón, P. F., Boada, L. D., Zumbado, M., Camacho, M., … Luzardo, O. P. (2014). Assessment of anticoagulant rodenticide exposure in six raptor species from the Canary Islands (Spain). *Science of the Total Environment*, *485–486*(1), 371–376. https://doi.org/10.1016/j.scitotenv.2014.03.094

Russell, J. C., Abrahão, C. R., Silva, J. C. R., & Dias, R. A. (2018). Management of cats and rodents on inhabited islands: An overview and case study of Fernando de Noronha, Brazil. *Perspectives in Ecology and Conservation*, *16*(4), 193–200. https://doi.org/10.1016/j.pecon.2018.10.005

Saraswat, R., Sinha, A., & Radhakrishna, S. (2015). A god becomes a pest? Human-rhesus macaque interactions in Himachal Pradesh, northern India. *European Journal of Wildlife Research*, *61*(3), 435–443. https://doi.org/10.1007/s10344-015-0913-9

Saunders, G. R. (1978). Resistance to warfarin in the Roof rat in Sydney, NSW. *Search*, *9*(1–2), 39–40.

Searle, J. B., Jones, C. S., Scascitelli, M., Jones, E. P., Herman, J. S., Rambau, R. V., … Gime, M. D. (2009). Of mice and (Viking?) men: phylogeography of British and Irish house mice. *Proceedings of the Royal Society*, *276*, 201–207. https://doi.org/10.1098/rspb.2008.0958

Singleton, G. (2003). Impacts of rodents on rice production in Asia. *IRRI Discussion Paper Series*, *45*. Retrieved from http://books.irri.org/971220183X_content.pdf

Smith, A., Singleton, G., Hansen, G., & Shellam, G. (1993). A serologic survey for viruses and *Mycoplasma pulmonis* among wild house mice (*Mus domesticus*) in southeastern Australia. *Journal of Wildlife Diseases*, *29*(2), 219–229. https://doi.org/10.7589/0090-3558-29.2.219

Song, Y., Endepols, S., Klemann, N., Richter, D., Matuschka, F. R., Shih, C. H., … Kohn, M. H. (2011a). Adaptive introgression of anticoagulant rodent poison resistance by hybridization between old world mice. *Current Biology*, *21*(15), 1296–1301. https://doi.org/10.1016/j.cub.2011.06.043

Song, Y., Endepols, S., Klemann, N., Richter, D., Matuschka, F. R., Shih, C. H., … Kohn, M. H. (2011b). Adaptive introgressive hybridization with the Algerian mouse (*Mus spretus*) promoted the evolution of anticoagulant rodenticide resistance in European house mice (*M. musculus domesticus*). In *European Vertebrate Pest Management conference* (pp. 67–69). https://doi.org/10.5073/jka.2011.432.034

Song, Y., Lan, Z., & Kohn, M. H. (2014). Mitochondrial DNA phylogeography of the Norway rat. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0088425

Sorek, R., & Ast, G. (2003). Intronic sequences flanking alternatively spliced exons are conserved between human and mouse. *Genome Research*, *13*(7), 1631–1637. https://doi.org/10.1101/gr.1208803

St Clair, J. J. H. (2011). The impacts of invasive rodents on island invertebrates. *Biological Conservation*, *144*, 68–81. https://doi.org/10.1016/j.biocon.2010.10.006

Stacey, N. (2007). *Boats to Burn: Bajo Fishing Activity in the Australian Zone*. Canberra: Australian National University Press.

Stannage, T. (2015). The People of Perth. *Studies in Western Australian History*, *29*, 95–97. https://doi.org/10.2307/27508417

Staubach, F., Lorenc, A., Messer, P. W., Tang, K., Petrov, D. A., & Tautz, D. (2012). Genome patterns of selection and introgression of haplotypes in natural populations of the house mouse (*Mus musculus*). *PLoS Genetics*, *8*(8). https://doi.org/10.1371/journal.pgen.1002891

Suzuki, H., Nunome, M., Kinoshita, G., Aplin, K. P., Vogel, P., Kryukov, A. P., … Moriwaki, K. (2013). Evolutionary and dispersal history of Eurasian house mice *Mus musculus* clarified by more extensive geographic sampling of mitochondrial DNA. *Heredity*, *111*(5), 375–390. https://doi.org/10.1038/hdy.2013.60

Tang, Q., Low, G. W., Lim, J. Y., Gwee, C. Y., & Rheindt, F. E. (2018). Human activities and landscape features interact to closely define the distribution and dispersal of an urban commensal. *Evolutionary Applications*, *11*(9), 1598–1608. https://doi.org/10.1111/eva.12650

Tasmania Parks and Wildlife Service. (2009). *Macquarie Island Pest Eradication Plan*. Tasmania: Department of Primary Industries, Parks, Water and Environment.

The UniProt Consortium. (2018). UniProt: a worldwide hub of protein knowledge. *Nucleic Acids Research*, *47*, D506–D515. https://doi.org/10.1093/nar/gky1049

Ullrich, K. K., Linnenbrink, M., & Tautz, D. (2017). Introgression patterns between house mouse subspecies and species reveal genomic windows of frequent exchange. *Doi.Org*, *1*(Ld), 168328. https://doi.org/10.1101/168328

United States Environmental Protection Agency. (2017). Restrictions on Rodenticide Products. Retrieved May 30, 2019, from https://www.epa.gov/rodenticides/restrictions-rodenticide-products

Van Hooft, P., Cosson, J. F., Vibe-Petersen, S., & Leirs, H. (2008). Dispersal in *Mastomys natalensis* mice: Use of fine-scale genetic analyses for pest management. *Hereditas*, *145*(6), 262–273. https://doi.org/10.1111/j.1601-5223.2008.02089.x

Wanless, R. M., Angel, A., Cuthbert, R. J., Hilton, G. M., & Ryan, P. G. (2007). Can predation by invasive mice drive seabird extinctions? *Biology Letters*, *3*. https://doi.org/10.1098/rsbl.2007.0120

Wheeler, R., Priddel, D., Dwyer, T. O., Carlile, N., Portelli, D., Wilkinson, I., & Portelli, N. C. Á. D. (2018). Evaluating the susceptibility of invasive black rats (*Rattus rattus*) and house mice (*Mus musculus*) to brodifacoum as a prelude to rodent eradication on Lord Howe Island. *Biological Invasions*, *4*. https://doi.org/10.1007/s10530-018-1863-4

Wyatt, K. B., Campos, P. F., Gilbert, M. T. P., Kolokotronis, S. O., Hynes, W. H., DeSalle, R., … Greenwood, A. D. (2008). Historical mammal extinction on Christmas Island (Indian Ocean) correlates with introduced infectious disease. *PLoS ONE*, *3*(11). https://doi.org/10.1371/journal.pone.0003602

Yang, H., Wang, J. R., Didion, J. P., Buus, R. J., Bell, T. A., Welsh, C. E., … De Villena, F. P. M. (2011). Subspecific origin and haplotype diversity in the laboratory mouse. *Nature Genetics*, *43*(7), 648–655. https://doi.org/10.1038/ng.847

Yin, J. X., Geater, A., Chongsuvivatwong, V., Dong, X. Q., Du, C. H., Zhong, Y. H., & McNeil, E. (2008). Predictors for presence and abundance of small mammals in households of villages endemic for commensal rodent plague in Yunnan Province, China. *BMC Ecology*, *8*, 1–11. https://doi.org/10.1186/1472-6785-8-8

Zhelev, G. G., Koev, K. P., Dimitrov, V. D., & Petrov, V. S. (2019). Anticoagulant Resistance in Synanthropic Rodents in the Stara Zagora Region, Bulgaria. *Macedonian Veterinary Review*, *42*(1). https://doi.org/10.2478/macvetrev-2019-0010

Appendices

Appendix 1 Mus musculus samples collected and retrieved from GenBank, recording the species, subspecies, sex, location of origin, and the gene sequenced.

Name/Accession number	Species	Sex	Location	Gene	Reference
T5260	*Mus musculus castaneus*	Female	Browse Island	D-loop, entire *Vkorc1*	This study
T5261	*M. m. castaneus*	Female	Browse Island	D-loop, entire *Vkorc1*	This study
T5266	*M. m. castaneus*	Male	Browse Island	D-loop, entire *Vkorc1*	This study
T5269	*M. m. castaneus*	Male	Browse Island	D-loop, entire *Vkorc1*	This study
T5271	*M. m. castaneus*	Male	Browse Island	D-loop, entire *Vkorc1*	This study
T5244	*M. m. castaneus*	Male	Browse Island	D-loop, entire *Vkorc1*	This study
T5251	*M. m. castaneus*	Male	Browse Island	D-loop, entire *Vkorc1*	This study
T5252	*M. m. castaneus*	Male	Browse Island	D-loop, entire *Vkorc1*	This study
T5255	*M. m. castaneus*	Male	Browse Island	D-loop, entire *Vkorc1*	This study
T5257	*M. m. castaneus*	Male	Browse Island	D-loop, entire *Vkorc1*	This study
T5253	*M. m. castaneus*	Female	Browse Island	D-loop, entire *Vkorc1*	This study

Name/Accession number	Species	Sex	Location	Gene	Reference
T5254	*M. m. castaneus*	Female	Browse Island	D-loop, entire *Vkorc1*	This study
T5256	*M. m. castaneus*	Female	Browse Island	D-loop, *Vkorc1* exon 1, partial exon 2, exon 3	This study
T5258	*M. m. castaneus*	Female	Browse Island	D-loop, *Vkorc1* exon 3	This study
T5259	*M. m. castaneus*	Female	Browse Island	D-loop, entire *Vkorc1*	This study
MM008	*Mus musculus domesticus*	Female	Kingsley, WA	D-loop, *Vkorc1* exon 1, partial exon 2	This study
MM007	*M. m. domesticus*	Male	Kingsley, WA	D-loop, *Vkorc1* exon 1, partial exon 2	This study
MR011	*M. m. domesticus*	Female	Wellard, WA	D-loop, *Vkorc1* exon 1, partial exon 2	This study
MR010	*M. m. domesticus*	Female	Wellard, WA	D-loop, *Vkorc1* exon 1, partial exon 2	This study
MR009	*M. m. domesticus*	Male	Wellard, WA	D-loop, *Vkorc1* exon 1, exon 2	This study
MR008	*M. m. domesticus*	Male	Wellard, WA	D-loop, *Vkorc1* exon 1, exon 2	This study
MM005	*M. m. domesticus*	Male	Wanneroo, WA	D-loop, *Vkorc1* exon 1, exon 2	This study
MM004	*M. m. domesticus*	Male	Wanneroo, WA	D-loop, *Vkorc1* exon 1, exon 2	This study

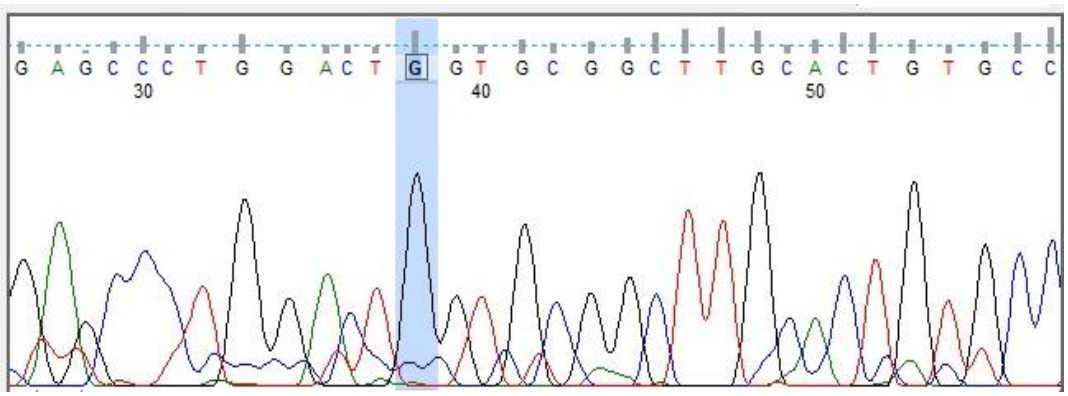
Name/Accession number	Species	Sex	Location	Gene	Reference
MM003	*M. m. domesticus*	Female	Wanneroo, WA	D-loop, entire *Vkorc1*	This study
MM002	*M. m. domesticus*	Male	Wanneroo, WA	D-loop, *Vkorc1* exon 1, partial exon 2, partial exon 3	This study
MM001	*M. m. domesticus*	Male	Wanneroo, WA	D-loop, *Vkorc1* exon 1, partial exon 2	This study
MR002	*M. m. domesticus*	Male	Stoneville, WA	D-loop, *Vkorc1* exon 1, partial exon 2, exon 3	This study
MR001	*M. m. domesticus*	Female	Stoneville, WA	D-loop, *Vkorc1* exon 1, exon 2	This study
MM009	*M. m. domesticus*	Female	High Wycombe, WA	D-loop	This study
MM010	*M. m. domesticus*	Male	Beldon, WA	D-loop, entire *Vkorc1*	This study
MM006	*M. m. domesticus*	Male	Beldon, WA	D-loop, *Vkorc1* exon 1, partial exon 2	This study
MM012	*M. m. domesticus*	Female	Mandurah, WA	D-loop, entire *Vkorc1*	This study
MM013	*M. m. domesticus*	Juvenile	Victoria Park, WA	D-loop, *Vkorc1* exon 1, partial exon 2, exon 3	This study
MM017	*M. m. domesticus*	Female	Wanneroo, WA	D-loop, entire *Vkorc1*	This study
MM018	*M. m. domesticus*	Female	Wanneroo, WA	D-loop, *Vkorc1* exon 1, exon 2	This study

Name/Accession number	Species	Sex	Location	Gene	Reference
MM019	*M. m. domesticus*	Male	Victoria Park, WA	D-loop	This study
MM020	*M. m. domesticus*	Female	Wanneroo, WA	D-loop, *Vkorc1* exon 1, partial exon 2, exon 3	This study
MM021	*M. m. domesticus*	Male	Wanneroo, WA	D-loop, *Vkorc1* exon 1, partial exon 2	This study
MM022	*M. m. domesticus*	Female	Wanneroo, WA	D-loop, entire *Vkorc1*	This study
MM023	*M. m. domesticus*	Male	Wanneroo, WA	D-loop, *Vkorc1* exon 1, exon 2	This study
MM024	*M. m. domesticus*	Male	Quinns Rocks, WA	D-loop, *Vkorc1* exon 1, partial exon 2	This study
MM025	*M. m. domesticus*	Male	Clarkson, WA	D-loop, *Vkorc1* exon 1, exon 2	This study
MM026	*M. m. domesticus*	Female	Clarkson, WA	D-loop, *Vkorc1* exon 1, partial exon 2	This study
MM027	*M. m. domesticus*	Juvenile	Clarkson, WA	D-loop, *Vkorc1* exon 1, exon 2	This study
MM028	*M. m. domesticus*	Juvenile	Clarkson, WA	D-loop, entire *Vkorc1*	This study
MM029	*M. m. domesticus*	Juvenile	Clarkson, WA	D-loop, *Vkorc1* exon 1, exon 2	This study
MM030	*M. m. domesticus*	Juvenile	Clarkson, WA	D-loop, entire *Vkorc1*	This study

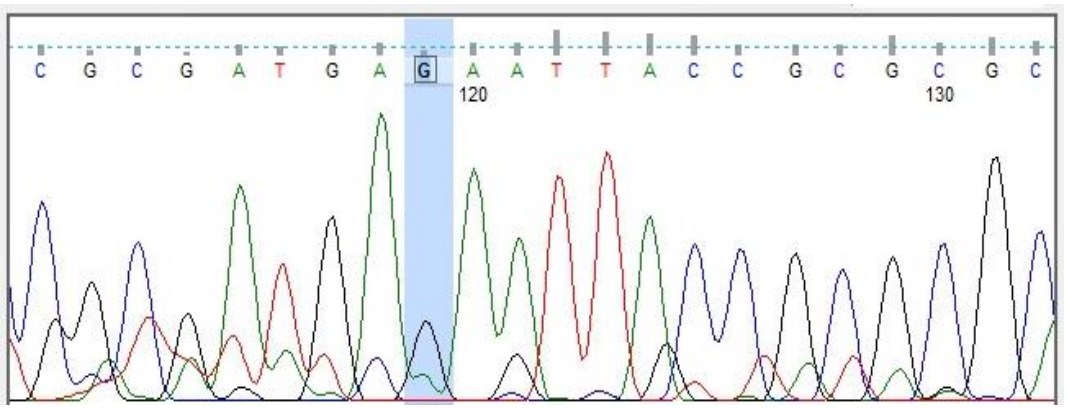
Name/Accession number	Species	Sex	Location	Gene	Reference
MM032	*M. m. domesticus*	Female	Clarkson, WA	D-loop, entire *Vkorc1*	This study
MM033	*M. m. domesticus*	Male	Clarkson, WA	D-loop, *Vkorc1* exon 1, partial exon 2	This study
FM211642-44	*M. m. castaneus*	N/A	New Zealand	D-loop	(MacKay, Alexander, Hauber, Murphy, & Clout, 2013)
JN416649-50	*M. m. castaneus*	N/A	Afghanistan	D-loop	(Rajabi-Maham et al., 2011)
JN416651-54, JN416657-63, JN416665, EU939063-95	*M. m. castaneus*	N/A	India	D-loop	(Geraldes et al., 2008; Jing et al., 2014; Rajabi-Maham et al., 2011)
JN416656, JN416743-52	*M. m. castaneus*	N/A	Kenya	D-loop	(Rajabi-Maham et al., 2011)
JN416664, JN416753-63	*M. m. castaneus*	N/A	Pakistan	D-loop	(Rajabi-Maham et al., 2011)
JN416666-742, AB649614, AJ286322-24	*M. m. castaneus*	N/A	Iran	D-loop	(Gündüz et al., 2000; Rajabi-Maham et al., 2011)
JN416764-65	*M. m. castaneus*	N/A	Thailand	D-loop	(Rajabi-Maham et al., 2011)
EU939125, AB649685	*M. m. castaneus*	N/A	Taiwan	D-loop	(Jing et al., 2014; Suzuki et al., 2013)
KM114686-KM114728, AB649672-84, AB649743, AB820937, AB820941	*M. m. castaneus*	N/A	China	D-loop	(Jing et al., 2014; Suzuki et al., 2013)
AB649647-51, AB820924	*M. m. castaneus*	N/A	Indonesia	D-loop	(Suzuki et al., 2013)

Name/Accession number	Species	Sex	Location	Gene	Reference
AB649652-53	*M. m. castaneus*	N/A	Philippines	D-loop	(Suzuki et al., 2013)
AB820922	*M. m. castaneus*	N/A	Sri Lanka	D-loop	(Suzuki et al., 2013)
AB819902-03	*M. m. castaneus*	N/A	Vietnam	D-loop	(Suzuki et al., 2013)
AB649654, AB649655-58	*M. m. castaneus*	N/A	Russia	D-loop	(Suzuki et al., 2013)
AB649659-71	*M. m. castaneus*	N/A	Japan	D-loop	(Suzuki et al., 2013)
AB649641-43	*M. m. castaneus*	N/A	Bangladesh	D-loop	(Suzuki et al., 2013)
AB649644-46	*M. m. castaneus*	N/A	Myanmar	D-loop	(Suzuki et al., 2013)
FM211596-631	*M. m. domesticus*	N/A	British Isles	D-loop	(Searle et al., 2009)
FM211632-39	*M. m. domesticus*	N/A	New Zealand	D-loop	(MacKay et al., 2013)
GQ241989-GQ242005	*M. m. domesticus*	N/A	Madeira	D-loop	(Forster et al., 2009)
GQ242006-20	*M. m. domesticus*	N/A	Portugal	D-loop	(Forster et al., 2009)
JF277281-93	*M. m. domesticus*	N/A	Australia	D-loop	(Gabriel et al., 2011)
JF277294-300	*M. m. domesticus*	N/A	Netherlands	D-loop	(Gabriel et al., 2011)
JN416766-68, AB649593, EU194609-47	*M. m. domesticus*	N/A	Iran	D-loop	(Rajabi-Maham et al., 2011; Rajabi-Maham, Orth, & Bonhomme, 2008)
KY686322-99, AB649608	*M. m. domesticus*	N/A	Senegal	D-loop	(Lippens et al., 2017)
AJ843821-71	*M. m. domesticus*	N/A	Turkey	D-loop	(Jing et al., 2014)
EU938914-24	*M. m. domesticus*	N/A	Israel	D-loop	(Jing et al., 2014)
AB649590-92, HQ185282	*M. m. domesticus*	N/A	France	D-loop	(Hardouin et al., 2010; Suzuki et al., 2013)
AB649594-95	*M. m. domesticus*	N/A	Indonesia	D-loop	(Suzuki et al., 2013)
AB649601-04, AB649597	*M. m. domesticus*	N/A	Russia	D-loop	(Suzuki et al., 2013)
AB649609	*M. m. domesticus*	N/A	Somalia	D-loop	(Suzuki et al., 2013)
AB649598	*M. m. domesticus*	N/A	Philippines	D-loop	(Suzuki et al., 2013)
AB820930	*M. m. domesticus*	N/A	Georgia	D-loop	(Suzuki et al., 2013)

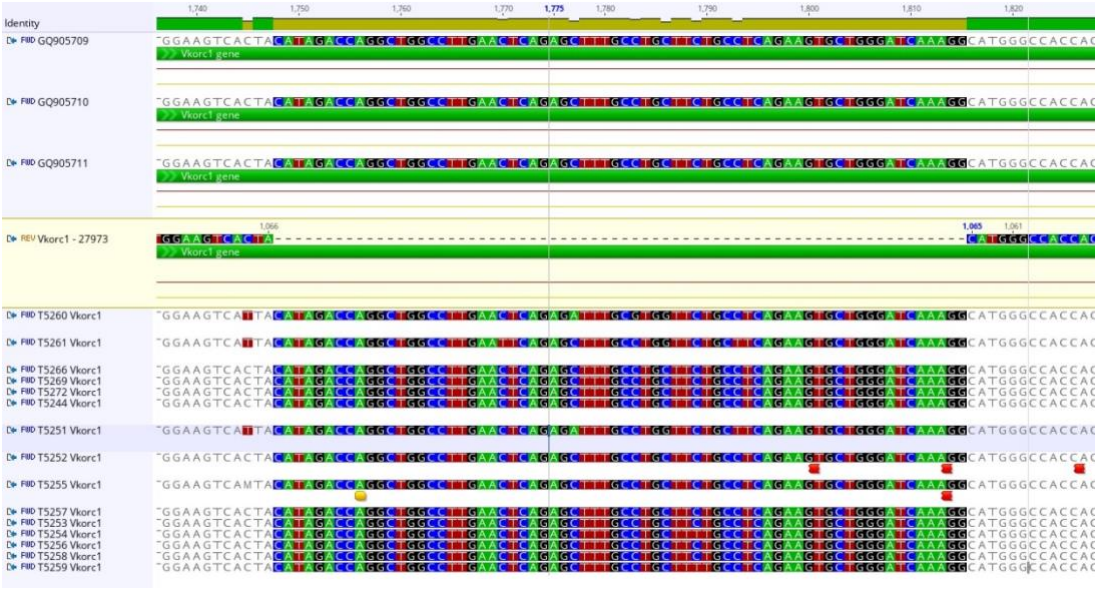
Name/Accession number	Species	Sex	Location	Gene	Reference
AB649586-89, EU194658-76	*M. m. domesticus*	N/A	Italy	D-loop	(Rajabi-Maham et al., 2008; Suzuki et al., 2013)
AB649605, EU194648-57	*M. m. domesticus*	N/A	Bulgaria	D-loop	(Rajabi-Maham et al., 2008; Suzuki et al., 2013)
AB649606	*M. m. domesticus*	N/A	Slovenia	D-loop	(Suzuki et al., 2013)
AB649610-11	*M. m. domesticus*	N/A	Canada	D-loop	(Suzuki et al., 2013)
AB649612-13	*M. m. domesticus*	N/A	Peru	D-loop	(Suzuki et al., 2013)
AB649599-600	*M. m. domesticus*	N/A	China	D-loop	(Suzuki et al., 2013)
AB649607	*M. m. domesticus*	N/A	Tunisia	D-loop	(Suzuki et al., 2013)
HQ185259, HQ185273-81	*M. m. domesticus*	N/A	Kerguelen Archipelago	D-loop	(Hardouin et al., 2010)
HQ185272	*M. m. domesticus*	N/A	South Georgia and the South Sandwich Islands	D-loop	(Hardouin et al., 2010)
HQ185260-71	*M. m. domesticus*	N/A	Falkland Islands	D-loop	(Hardouin et al., 2010)
HQ185258	*M. m. domesticus*	N/A	Amsterdam Island	D-loop	(Hardouin et al., 2010)
MG937349-536	*M. m. domesticus*	N/A	Cyprus	D-loop	(García-Rodríguez et al., 2018)
MG950367-97	*M. m. domesticus*	N/A	Greece	D-loop	(García-Rodríguez et al., 2018)
DQ266070	*Mus spretus*	N/A	N/A	D-loop	
KF510058	*Rattus rattus*	N/A	N/A	D-loop	
GQ905709	*M. m. domesticus*	N/A	N/A	*Vkorc1*	(Song et al., 2011a)
GQ905710	*M. m. domesticus*	N/A	N/A	*Vkorc1*	(Song et al., 2011a)
HM027479	*M. m. domesticus*	N/A	Avinyonet, Spain	*Vkorc1*	(Song et al., 2011a)
27973	*M. musculus*	N/A	N/A	*Vkorc1*	
GQ905711	*M. spretus*	N/A	N/A	*Vkorc1*	(Song et al., 2011a)



Appendix 2 Vkorc1 gene chromatogram of Mus musculus castaneus from Browse Island, Western Australia. Highlighted is the signal representing the SNP at amino acid position 10. The wild type cytosine is replaced by a guanine in this silent mutation resulting in the amino acid leucine being unchanged.

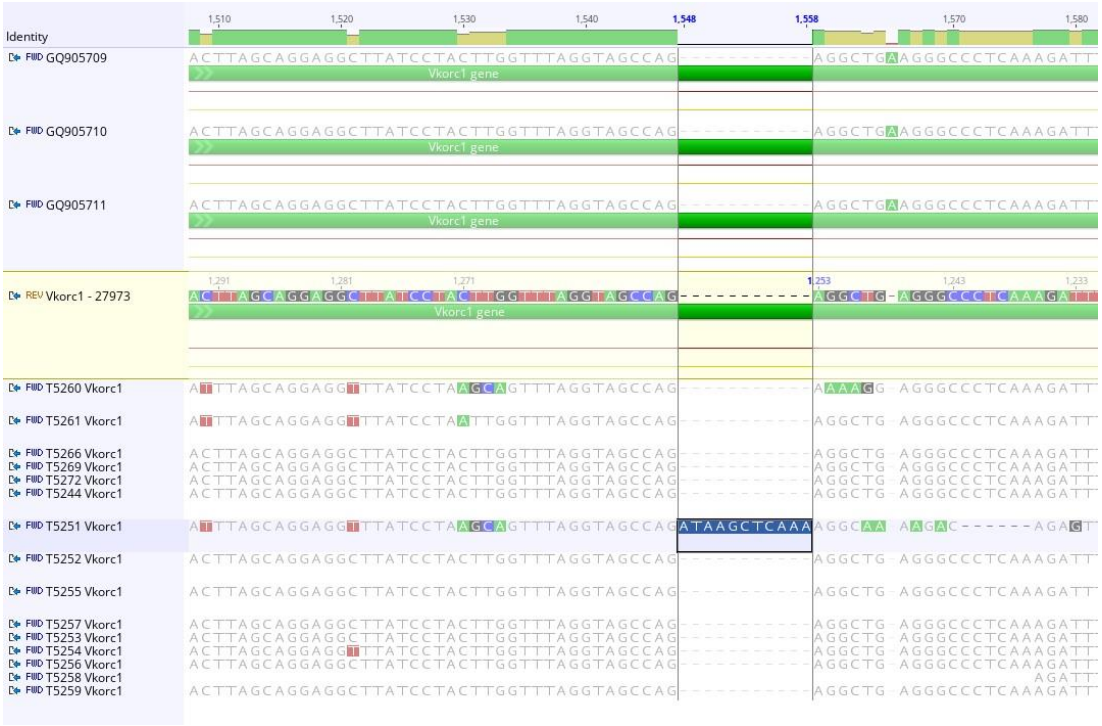


Appendix 3 Vkorc1 gene chromatogram of Mus musculus castaneus from Browse Island, Western Australia. Highlighted is the SNP related to the amino acid in position 37. The wild type adenine is replaced by guanine, resulting in a silent mutation of glutamic acid.



Appendix 4 Alignment of Mus musculus castaneus samples from Browse Island, Western Australia, and reference sequences for the Vkorc1 gene of Mus musculus domesticus and Mus spretus. Highlighted is the 68 base pair insertion in the intron following exon 2, which is exhibited by all Browse Island samples and homologous reference sequences. The insertion is not present in the Vkorc1 'wild' type represented by sequence 27973.

Appendix 5 Alignment of Vkorc1 sequences of Mus musculus castaneus from Browse Island, Western Australia, and reference sequences for the Vkorc1 gene of Mus musculus domesticus and Mus spretus. Highlighted is the 8 base pair deletion in the intron following exon 2. The deletion is exhibited by all samples from Browse Island, by the M. spretus Vkorc1 sequence, and by two M. m. domesticus Vkorc1 sequences. The deletion is not carried by the 'wild' type Vkorc1 sequence 27973.



Appendix 6 Alignment of Vkorc1 sequences of Mus musculus castaneus from Browse Island, Western Australia, and reference sequences for the Vkorc1 gene of Mus musculus domesticus and Mus spretus. Highlighted is an 11 base pair insertion in the intron following exon 2. The insertion is carried by one sample from Browse Island only. The insertion is not displayed by the reference sequences.