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Population Genetics and Genomics of a Marsupial Species: Analysis of Native and Invasive Brushtail Possum Populations (*Trichosurus vulpecula*)

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in

Conservation Biology

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Abstract

One of the leading causes of global biodiversity decline is the introduction of invasive pest species that destroy native flora and compete with native fauna for food and other resources. Aotearoa New Zealand is one of the foremost countries in the world that has focussed on eradicating pest species and in particular exotic mammals from the archipelago, which lacks native, terrestrial mammals. The New Zealand Government recently set in train the ambitious task of removing all mustelids, rats, and possums from the terrestrial landscape by the year 2050. Brushtail possums (*Trichosurus vulpecula*) were introduced to Aotearoa New Zealand from Australia in the mid-1800s, after which they were translocated across the country and have become widespread, destroying indigenous habitat, eating native birds and invertebrates, and spreading bovine TB.

Control efforts have seen possum numbers decline in the last two decades from close to 75 million in 2002 to 40 million in 2020. There is, however, a gap in the scientific understanding of possum populations with respect to their genetic composition and population structure across the country, and this knowledge could help us develop effective and dynamic management strategies to eradicate possums on a nationwide scale.

In this thesis, I focus on three aspects of population structure and diversity of brushtail possums. First, I investigated a small geographical study area - The Kenepuru Peninsula - where I sought evidence of genetic correlations with geography, time and fur colour. I used two types of genetic markers that target the nuclear and mitochondrial regions of possum DNA with large population samples. In every case, it was determined that the possums comprised one freely interbreeding population at this scale. In particular I demonstrated that colour morphs associated with distinct subspecies in Australia, freely interbreed in New Zealand. I then increased the scale of sampling to include representation of populations across New Zealand and Australia, with the same genetic markers. This threw light on the heterogenous nature of possum diversity in New Zealand, and showed that even after ~110 generations, possums retained genetic separation among spatial groups. Additionally, the data showed evidence of multiple possum lineages across New Zealand that are derived from several Australian populations. High haplotype diversity in New Zealand suggests that the rapidly expanding population has retained novel haplotypes and the data thus far indicated a non-homogenous (metapopulation) distribution of possums without geographical concordance. As the project progressed, I was able to apply high-throughput genotyping-by-sequencing to generate a large genomic dataset. This dataset provided much more detail of the genotypic distribution of possums in Australia and among invasive metapopulations in New

Zealand, as well as informing us of the relationship between them. This large, robust database of possum population structure and genetic diversity throughout Aotearoa New Zealand will support future studies in providing informed management decisions to eradicate brushtail possums.

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Declaration

This thesis is based on publications, and the formatting style of each chapter follows the guidelines for the journal to which it will be submitted or in which it has been published or accepted for publication. Hence, there are inconsistencies in writing style and some repetition of methods and contents between the chapters presented in this thesis.

“Ain't no mountain high enough

Ain't no valley low enough

Ain't no river wide enough

To keep me from getting to you”

- Nickolas Ashford & Valerie Simpson

Chapter One

General Introduction

Origin and background history

Aotearoa New Zealand welcomed the brushtail possum (Trichosurus vulpecula), a generalist marsupial species from Australia as early as 1837 during attempts to establish a fur industry (Pracy 1962). In Australia, brushtail possums occur widely across the vast landscape occupying a range of habitats from dense sclerophyll forests to arid woodlands (How 1983). Historically, they have been identified as part of Aboriginal peoples' existence as sources of food amd tools, and bearing cultural significance before being recorded by early European settlers as occurring abundantly, especially among eucalypt forests (Kerle 1992). Declining possum numbers in Australia have been associated with post European settlement activities including habitat invasion and destruction, increased hunting, predation, and competition that led to local extirpation and a mosaic of small geographically isolated populations (Kerle 1992).

Currently under the umbrella of Trichosurus vulpecula brushtail possums have been classified into six subspecies based on their geographic location and morphology (coat colour, size): Northern (Trichosurus vulpecula arnhemensis), Cape York (T. v. eburacensis), copper-coloured (T. v. johnsonnii), Tasmanian (T. v. fulginosus), south-western (T. v. hypoleucus) and south-eastern (T. v. vulpecula). Possums were brought into Aotearoa mainly from mainland southeastern Australia and the offshore island Tasmania (Pracy 1962), and therefore were classified as mainly "grey" and "black" morphs, respectively (Figure 1.1). Although recorded as two-colour morphs, imported possums included a variety of coat colours such as brown, red-brown, grey-brown, and silver-grey (Triggs & Green 1989).

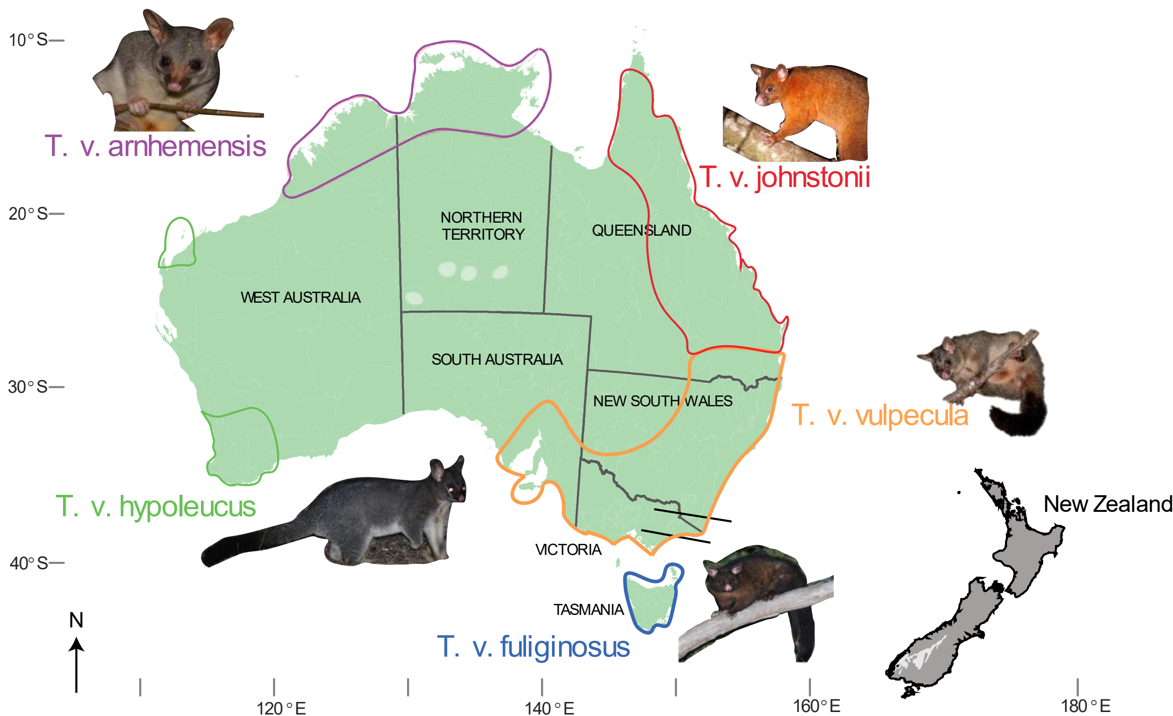

Figure 1.1 Putative geographic distribution of five subspecies of *Trichosurus vulpecula* in Australia. Clockwise: *T. v. johnstonii* (photo by Ian Montgomery) mostly found in the northeastern part of Australia, *T.v.vulpecula* (photo by Tony Jewell) found in southeastern mainland Australia, *T. v. fuliginosus* (photo by Craig Greer) found in Tasmania, *T.v. hypoleucus* (photo by snakesrcool) found in western Australia, and *T.v.arnhemensis* (photo by Nick Lambert) found in northern Australia.

Initially, for the fur industry, there was more interest in dark coat colours between 1885–1915 followed by an escalation in demand for grey-furred possums, affecting introduction patterns of brushtail possums to Aotearoa New Zealand (Pracy 1962) (Figure 1.2). These multiple introductions from Australia occurred from the late 1850s to 1925, after which New Zealand intra-island translocations occurred till the 1940s. During this time, especially the early 1920s, some biologists claimed that possums had a negligible impact on native biodiversity compared to the economic revenue gained and therefore should not be considered a threat to New Zealand (Pracy 1962). By the 1940s, however, the capacity of possums to occupy most habitat types in New Zealand, consume up to 70 species of native flora (e.g. northern rātā Metrosideros robusta, southern rātā Metrosideros umbellate, and tōtara Podocarpus totara), native birds (e.g. Kōkako, kiwi, kererū) and their eggs, and native invertebrates including wētā and snails was known (Nugent et al. 2000). Furthermore, by 1964, brushtail possums were recognised as the largest carriers of Mycobacterium bovis that bacterium that causes Bovine tuberculosis (TB) in cattle. Possums infected with the disease can live up to 6 months developing lesions, which release lime-green pus

to various surroundings they encounter (Cowan 2005). Cattle can be infected by the bacteria either by direct transmission from possums or when they come in contact with infected feed and other surfaces (Livingstone 2015).

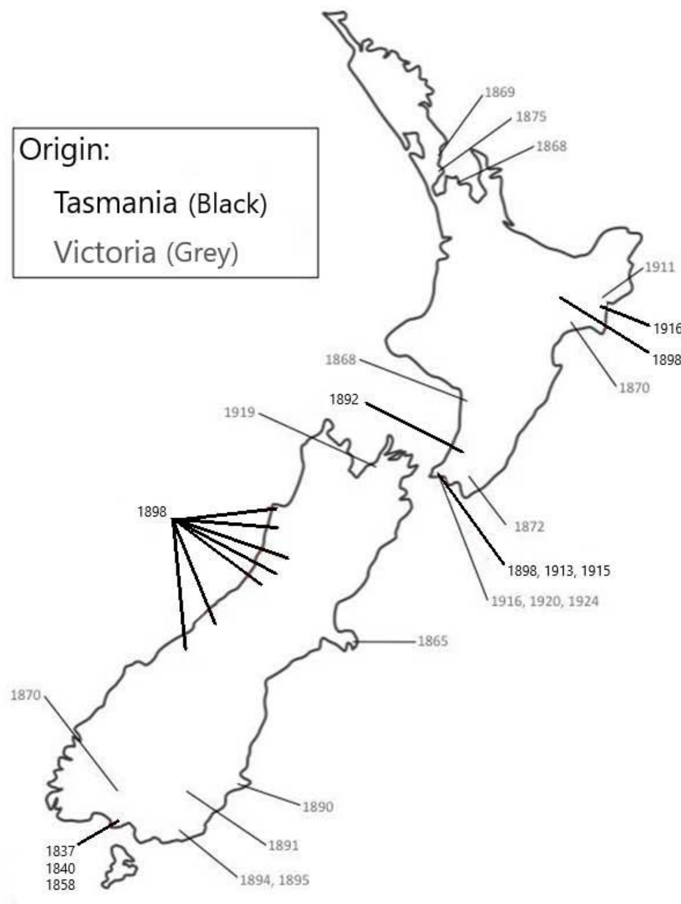

Figure 1.2 The initial history of recorded introductions of brushtail possums (*Trichosurus vulpecula*) to Aotearoa New Zealand from Australia (Pracy 1962).

Biology and dispersal of possums in Aotearoa New Zealand

Brushtail possums in Aotearoa New Zealand are known to inhabit habitats from pastures and urban areas to exotic tree plantation and native forests with the exception of mountainous regions like western Fiordland and Ruapehu due to the cold conditions at high elevation (Cowan 2005). Many hundreds of introduction events across the two main islands as well as offshore islands favoured their expansion and flourishment. Reproductive behaviour tends to polygamy, with females reaching maturity between one and two years old, breeding once a year (twice if body and environmental conditions are good), until they are six to even eight years old. (Kerle 1984). Like other marsupials brushtail possums' gestation is short, only 18 days, with joeys being born

with fully developed internal organs but not limbs, and then staying in the mother's pouch for another 120 days to further develop (Fletcher & Selwood 2000).

Both reproduction and long-distance dispersal are male biased (Cowan 2005), with ecological studies finding males (especially juveniles) disperse as much as 10 kms/week (Cowan et al. 1997) whereas females often take over their mother's dens and exhibit strong overlap in home ranges (Cowan & Clout 2000). A recent genetic study of possums across the Taranaki ring-plain and Egmont National Park estimated dispersal to only be about 3–3.4 kms for both sexes and home ranges for males (440 m) and females (350 m) were found to overlap extensively (Veale & Etherington 2023). This pattern probably reflects a high population density in the region.

Eradication efforts

The Aotearoa New Zealand government has undertaken eradication of invasive pests from various offshore islands starting as early as 1964 with the clearing of rats from Ruapuke/Maria Island (Russell & Broome 2016). Planned eradication campaigns began in the 1980s (Clout & Russell 2006) becoming more ambitious in the following decades, resulting in possum eradications from Rangitoto and Motutapu Islands in 1992 (Mowbray 2002) off the coast of Auckland to Kāpiti Island (Cowan 1992) off the coast of the Wellington region, and invasive mammals being completely eradicated from 134 islands (Parkes et al. 2017). These successful programs provide safe havens for native endangered fauna; however, it is more difficult to apply these efforts on the mainland. While technological advances to tackle this issue are being tested (Bell et al. 2019; Murphy et al. 2019), the most commonly used method of control is currently aerial and ground bait with of sodium fluoroacetate (1080) poison that is part of the toolbox being evaluated for ways to progress towards predator-free 2050 goal (Tompkins 2018) (Figure 1.3). Beyond implications for biodiversity loss, mainland eradication efforts of possums have been focussed on farmland and surrounding areas spanning about eight million hectares of the New Zealand (Livingstone 2015). These efforts have resulted in over 95% reduction in TB occurrence in cattle since 1994 and these efforts are speculated to cease if TB is eradicated by 2040 (Parkes et al. 2017).

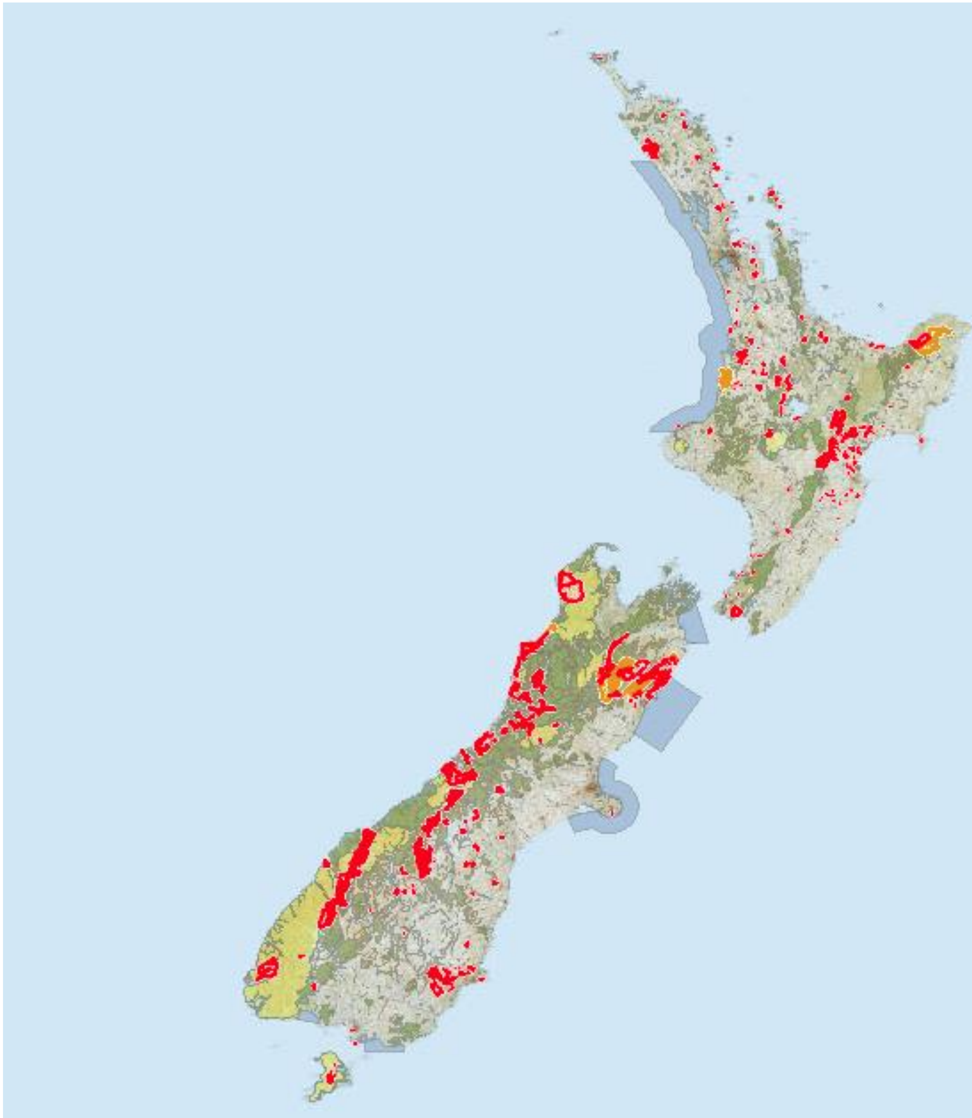

Figure 1.3 A map of Aotearoa New Zealand indicating sites where pesticides (including 1080 operations) have been laid (in red) and will be laid (in orange). Blue-shaded areas represent marine reserves, yellow-shaded areas represent National Parks and green-shaded areas represent Public Conservation land. Map provided by Department of Conservation (2023).

Why this study?

Aotearoa New Zealand is one of the leaders for restoring islands from the detrimental effects of invasive pests (Bell et al. 2019). This effort, however, stems from a necessity as the landscape has lost 40% of all native terrestrial birds since human settlement in the 13th century, and the same percentage of the remaining avifauna is currently classified as 'threatened' (Clout 2001). Similar patterns of extinction and population reduction are apparent in other, less visible, biota (Dean 2001; Towns et al. 2012; Department of Conservation 2020). The leading cause of these declines are biological invasions (Doherty et al. 2016). While past research has clearly highlighted the impacts of mammalian introductions to small island systems (Parkes et al. 1996; Crouchamp et al.

2002; Blackie et al. 2013; Towns et al. 2013; Burgess et al. 2021) and the specific impacts of possums in Aotearoa New Zealand are well recognised, gaps remain in our understanding of invasive species population biology. In particular, the response of invasive species to control measures has received little attention. While impressive reductions in possum numbers are routinely achieved (Brown & Sherley 1992; Warburton et al. 2009; Cook & Mulgan 2022), these are within relatively small patches of habitat and pest numbers increase rapidly over a few years after an eradication attempt. Population recovery is likely to be partly an effect of dispersal into low density areas, which would result in gene flow and potentially an increased risk of disease transfer. Understanding the pattern and scale of dispersal provides direct information about the pest invasiveness (Travis & Dytham 2002; Wilson et al. 2009; Capellini et al. 2015; Ochocki & Miller 2017).

How did I attempt to do that?

The method of using genetics to recognise establishment and evolution of invasive species was emphasised in the 1960s publication by Baker and Stebbins (1965) as invasive genetics. The establishment and spread of a species post-introduction, including factors like number of introductions and scale of import, could have consequences that are reflected in the genetics of the species (e.g. evolutionary potential and adaptive responses to control; Duglosch & Parker 2008; Searle 2008; Barrett 2015). Population genetic tools can address pertinent aspects that impact pest management by identifying origin and invasive pathways of non-native species which helps us determine the scale of introductions, the breadth/extent of genetic diversity (that may increase their ability to establish populations and spread), proportion of diversity that exists in founder populations (Sakai et al. 2001; Frantz et al. 2013; Tepolt & Palumbi 2015; Iannella et al. 2019). Population genetic tools also help identify population structure of an invasive species i.e., how genetic diversity is distributed, the extent of intermixing between and within populations that will help determine management units for targeted control and eventually eradication (Robertson & Gemmell 2004; Hampton et al. 2004; Atterby et al. 2015; Porretta et al. 2008; Combs et al. 2019).

What I used- briefly

Three main types of genetic markers used in this study to understand historical and current state of brushtail possums in Aotearoa New Zealand and their relationship to their source populations in Australia: The maternally inherited mitochondrial genome (mtDNA) is the most used marker in phylogenetic studies (Patwardhan et al. 2014). Characteristics such as non-recombination, high mutation rates and high polymorphism help determine an invasive species' history (Janke et al. 1994), especially for attempts to link invasive and source populations (Rollins et al. 2011; Jeffery et al. 2017; Acosta et al. 2022; Sato et al. 2023). For this project, the control region of the possum mitochondrion (~17kbp) was used to identify the relationship among possums in Aotearoa New Zealand and to their counterparts in their native range of Australia. mtDNA markers are normally used alongside nuclear microsatellite markers to trace population history based on genetic differences between populations (allocation of populations to various genetic clusters) and to gauge spatial population structure by analysing many populations that exist within New Zealand (Eggert et al. 2002; Fraser et al 2013; Adams et al 2014; Larroque et al. 2023). While microsatellite and mitochondrial markers provide an established genetic database of brushtail possums in New Zealand (Taylor et al. 2000; Clinchy et al. 2004; Taylor et al. 2004; Blyton 2012; Adams 2013; DeGabriel et al. 2014; Duenas et al. 2014; Sarre et al. 2014; Pattabiraman et al. 2022; Carmalet-Rescan et al. 2022), we proceeded to obtain detailed insights into the genetic variation of possums by generating genome-wide SNP data using genotyping-by-sequencing (GBS) (Elshire et al 2011; Andrews et al. 2016). By aligning GBS sequences against the nuclear reference genome for brushtail possums, we could identify population structure and allele frequencies in much higher resolution than traditional sequencing methods (Wright et al. 2019). By using these genomic markers, we maximise our sample utilisation, evaluate and compare our results with previous research and consequently, reduce sampling effort and costs.

Thesis Structure

Chapter Two: Unrestricted gene flow between two subspecies of translocated brushtail possums (*Trichosurus vulpecula*) in Aotearoa New Zealand

In order to enhance the population modelling that will be necessary for the proposed eradication of brushtail possums in Aotearoa New Zealand, we tested a recently proposed hypothesis of reproductive segregation. Fur colour phenotypes among New Zealand brushtail possums correspond to distinct Australian subspecies, and any reduction in gene flow among these lineages could alter population responses to control efforts and disease transfer. We applied a population genetic approach to a spatially partitioned population, investigating variance among fur colour phenotypes along with spatial and temporal effects on the segregation of possum lineages confined to the Kenepuru Peninsula study area. The chapter was published as: Pattabiraman, N., Morgan-Richards, M., Powlesland, R., & Trewick, S. A. (2022). Unrestricted gene flow between two subspecies of translocated brushtail possums (Trichosurus vulpecula) in Aotearoa New Zealand. Biological Invasions, 24(1), 247-260.

Chapter Three: Comparative population genetic structure of native and invasive brushtail populations surveyed with mtDNA haplotype and nuclear microsatellite data

I evaluated the contemporary genetic structure of brushtail possums and discuss these patterns in light of their founding histories, contemporary ecology and connectivity and control history. I used the mitochondrial D-loop marker (n=413) and seven microsatellite markers (n=515) on possums from 16 locations across the country. The mtDNA data from this chapter has been published in Carmelet-Rescan et al. 2022 (DOI: 10.1002/ece3.9633). The findings from this study indicate that there is signal of genetic structure within the invasive population, but possum diversity nests within the larger genetic diversity of Australian populations. This leaves us with the potential to dissect population structure with high resolution markers (e.g. GBS) at a large scale of sampling, while including Australian samples. I hope to submit this chapter to a peer-reviewed journal soon.

Chapter Four: Comparison of the genetic structure of source and invasive populations of brushtail possums (*Trichosurus vulpecula*) using genotyping-by-sequencing

In this chapter I address the genetic origins of New Zealand brushtail possums in Australia using SNP data (GBS). Samples from both source and invasive populations (n= 188), after multiple iterative filtering steps, were grouped into three datasets to investigate population structure and genetic diversity among the native (Australian) sample groups, invasive (Aotearoa New Zealand) sample groups and, finally, to determine the relationship between the two using a combined dataset of sample groups from southeast Australia and all of New Zealand. Evidence from extensive analyses reveal well-developed genetic structure in New Zealand, where most possums were closely related to their Tasmanian counterparts, and in very small proportions to mainland sample groups in Victoria. I plan to add more samples from western Australia and across the Cook St in New Zealand to the dataset, update the results and submit it to a peer reviewed journal.

Chapter Five: Thesis discussion

I provide a brief summary and discussion of my results from the three data chapters and explain prospects for adding crucial sample groups and expanding the scope of this research in the future, with the expectation that we can better inform pest management to meet the goals of Predator Free 2050.

References

Abreu-Acosta, N., Pino-Vera, R., Izquierdo-Rodríguez, E., Afonso, O., & Foronda, P. (2023). Zoonotic Bacteria in Anolis sp., an Invasive Species Introduced to the Canary Islands (Spain). *Animals, 13*(3), 414.

Adams, A., Recio, M., Robertson, B., Dickinson, K., & Van Heezik, Y. (2014). Understanding home range behaviour and resource selection of invasive common brushtail possums (*Trichosurus vulpecula*) in urban environments. *Biological Invasions, 16*, 1791-1804.

Adams, A. L. (2013). *Spatial ecology and genetic population structure of the common brushtail possum (Trichosurus vulpecula) within a New Zealand urban environment.* [Doctoral dissertation]. University of Otago.

Andrews, K. R., Good, J. M., Miller, M. R., Luikart, G., & Hohenlohe, P. A. (2016). Harnessing the power of RADseq for ecological and evolutionary genomics. *Nature Reviews Genetics, 17*(2), 81-92.

Atterby, H., Allnutt, T. R., MacNicoll, A. D., Jones, E. P., & Smith, G. C. (2015). Population genetic structure of the red fox (Vulpes vulpes) in the UK. *Mammal Research, 60*, 9-19.

Avise, J. C. (1995). Mitochondrial DNA polymorphism and a connection between genetics and demography of relevance to conservation. *Conservation Biology, 9*(3), 686-690.

Baker, H. G., & Stebbins, G. L. (1965). *Genetics of colonizing species, proceedings.* Paper presented at the International Union of Biological Sciences Symposia on General Biology 1964: Asilomar, Calif.).

Barrett, S. C. (2016). Foundations of invasion genetics: the Baker and Stebbins legacy. *Invasion genetics: the Baker and Stebbins legacy*, 1-18.

Bell, P., Nathan, H., & Mulgan, N. (2019). Island eradication within large landscapes: the remove and protect model. *Island invasives: scaling up to meet the challenge, 62*, 604.

Blackie, H. M., MacKay, J. W., Allen, W. J., Smith, D. H. V., Barrett, B., Whyte, B. I.,. Ogilvie, S. (2014). Innovative developments for long-term mammalian pest control. *Pest Management Science, 70*(3), 345-351.

Blyton, M. D., Banks, S. C., Peakall, R., & Lindenmayer, D. B. (2012). Using probability modelling and genetic parentage assignment to test the role of local mate availability in mating system variation. *Molecular ecology, 21*(3), 572-586.

Brown, K., & Sherley, G. (2002). The eradication of possums from Kapiti Island, New Zealand. *Turning the tide: the eradication of invasive species*, 46-52.

Burgess, B. T., Irvine, R. L., Howald, G. R., & Russello, M. A. (2021). The promise of genetics and genomics for improving invasive mammal management on islands. *Frontiers in Ecology and Evolution, 9*, 704809.

Campbell, C. D., Cowan, P., Gruber, B., MacDonald, A. J., Holleley, C. E., & Sarre, S. D. (2021). Has the introduction of two subspecies generated dispersal barriers among invasive possums in New Zealand? *Biological Invasions, 23*(12), 3831-3845.

Capellini, I., Baker, J., Allen, W. L., Street, S. E., & Venditti, C. (2015). The role of life history traits in mammalian invasion success. *Ecology Letters, 18*(10), 1099-1107.

Carmelet-Rescan, D., Morgan-Richards, M., Pattabiraman, N., & Trewick, S. A. (2022). Time-calibrated phylogeny and ecological niche models indicate Pliocene aridification drove

intraspecific diversification of brushtail possums in Australia. *Ecology and Evolution, 12*(12), e9633.

Clinchy, M., Taylor, A. C., Zanette, L. Y., Krebs, C. J., & Jarman, P. J. (2004). Body size, age and paternity in common brushtail possums (*Trichosurus vulpecula*). *Molecular ecology, 13*(1), 195-202.

Clout, M. (2001). Where protection is not enough: active conservation in New Zealand. *Trends in ecology & evolution, 16*(8), 415-416.

Clout, M., & Russell, J. (2006). The eradication of mammals from New Zealand islands. *Assessment and control of biological invasion risks*, 127-141.

Combs, M., Byers, K., Himsworth, C., & Munshi-South, J. (2019). Harnessing population genetics for pest management: Theory and application for urban rats. *Human–Wildlife Interactions, 13*(2), 11.

Cook, B., & Mulgan, N. (2022). Targeted Mop up and Robust Response Tools Can Achieve and Maintain Possum Freedom on the Mainland. *Animals, 12*(7), 921.

Courchamp, F., Chapuis, J.-L., & Pascal, M. (2003). Mammal invaders on islands: impact, control and control impact. *Biological reviews, 78*(3), 347-383.

Cowan, P. (1992). The eradication of introduced Australian brushtail possums, *Trichosurus vulpecula*, from Kapiti Island, a New Zealand nature reserve. *Biological Conservation, 61*(3), 217-226.

Cowan, P. (2005). Brushtail possum. In 'The handbook of New Zealand mammals'. 2nd edn.(Ed. CM King.) pp. 56–80. In: Oxford University Press: Melbourne.

Cowan, P., Brockie, R., Smith, R., & Hearfield, M. (1997). Dispersal of juvenile brushtail possums, *Trichosurus vulpecula*, after a control operation. *Wildlife research, 24*(3), 279-288.

Cowan, P., & Clout, M. (2000). Possums on the move: activity patterns, home ranges, and dispersal. In 'The Brushtail Possum: Biology, Impact and Management of an Introduced Marsupial'.(Ed. TL Montague.) pp. 24–34. In: Manaaki Whenua Press: Lincoln, New Zealand.

Dean, T. (2001). *Invasive freshwater fish in New Zealand: DOC's present and future management.* Paper presented at the Managing invasive freshwater fish in New Zealand. Proceedings of a workshop hosted by Department of Conservation.

DeGabriel, J. L., Moore, B. D., Foley, W. J., & Johnson, C. N. (2014). Male-biased predation and its effect on paternity skew and life history in a population of common brushtail possums (*Trichosurus vulpecula*). *PloS one, 9*(11), e111746.

Department of Conservation 2020. Biodiversity in Aotearoa an overview of state, trends and pressures. https://www.doc.govt.nz/globalassets/documents/conservation/biodiversity/anzbs-2020-biodiversity-report.pdf

Department of Conservation 2023. Pesticide Summaries map. https://maps.doc.govt.nz/externalmaps/index.html?viewer=pesticidesummary

Dlugosch, K. M., & Parker, I. M. (2008). Invading populations of an ornamental shrub show rapid life history evolution despite genetic bottlenecks. *Ecology Letters, 11*(7), 701-709.

Doherty, T. S., Glen, A. S., Nimmo, D. G., Ritchie, E. G., & Dickman, C. R. (2016). Invasive predators and global biodiversity loss. *Proceedings of the National Academy of Sciences, 113*(40), 11261-11265.

Dueñas, J. F., Cruickshank, R., & Ross, J. (2015). Optimisation of a microsatellite panel for the individual identification of brushtail possums using low template DNA. *New Zealand Journal of Ecology, 39*(1), 93-102.

Eggert, L. S., Rasner, C. A., & Woodruff, D. S. (2002). The evolution and phylogeography of the African elephant inferred from mitochondrial DNA sequence and nuclear microsatellite markers. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 269*(1504), 1993-2006.

Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., & Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PloS one, 6*(5), e19379.

Fletcher, T., & Selwood, L. (2000). Possum reproduction and development. *The brushtail possum: biology, impact and management of an introduced marsupial, 80*(60), 62.

Frantz, A., Heddergott, M., Lang, J., Schulze, C., Ansorge, H., Runge, M., . . . Hoffmann, L. (2013). Limited mitochondrial DNA diversity is indicative of a small number of founders of the German raccoon (Procyon lotor) population. *European Journal of Wildlife Research, 59*, 665-674.

Hampton, J. O., Spencer, P. B., Alpers, D. L., Twigg, L. E., Woolnough, A. P., Doust, J.,. Pluske, J. (2004). Molecular techniques, wildlife management and the importance of genetic population structure and dispersal: a case study with feral pigs. *Journal of Applied Ecology, 41*(4), 735-743.

Iannella, A., Peacock, D., Cassey, P., & Schwensow, N. (2019). Genetic perspectives on the historical introduction of the European rabbit (Oryctolagus cuniculus) to Australia. *Biological Invasions, 21*(2), 603-614.

Jeffery, N. W., Ellis, E. A., Oakley, T. H., & Gregory, T. R. (2017). The genome sizes of ostracod crustaceans correlate with body size and evolutionary history, but not environment. *Journal of Heredity*

Kerle, J. (1984). Variation in the ecology of Trichosurus: its adaptive significance. *Possums and gliders*, 115-128.

Kerle, J., McKay, G., & Sharman, G. (1991). A systematic analysis of the brushtail possum, Trichosurus-vulpecula (Kerr, 1792)(Marsupialia, Phalangeridae). *Australian Journal of Zoology, 39*(3), 313-331.

Larroque, J., Chevret, P., Berger, J., Ruette, S., Adriaens, T., Van Den Berge, K., . . . Kaerle, C. (2023). Microsatellites and mitochondrial evidence of multiple introductions of the invasive raccoon Procyon lotor in France. *Biological Invasions, 25*(6), 1955-1972.

Lee, C. E., & Gelembiuk, G. W. (2008). Evolutionary origins of invasive populations. *Evolutionary Applications, 1*(3), 427-448.

Livingstone, P., Hancox, N., Nugent, G., & De Lisle, G. (2015). Toward eradication: the effect of Mycobacterium bovis infection in wildlife on the evolution and future direction of bovine tuberculosis management in New Zealand. *New Zealand veterinary journal, 63*(sup1), 4-18.

Manel, S., Schwartz, M. K., Luikart, G., & Taberlet, P. (2003). Landscape genetics: combining landscape ecology and population genetics. *Trends in ecology & evolution, 18*(4), 189-197.

Mowbray, S. (2002). Eradication of introduced Australian marsupials (brushtail possum and brushtailed rock wallaby) from Rangitoto and Motutapu Islands, New Zealand. *Turning the tide: the eradication of invasive species*, 226-232.

Murphy, E. C., Russell, J. C., Broome, K. G., Ryan, G. J., & Dowding, J. E. (2019). Conserving New Zealand's native fauna: a review of tools being developed for the Predator Free 2050 programme. *Journal of Ornithology, 160*(3), 883-892.

Nugent, G., Fraser, W., & Sweetapple, P. (2001). Top down or bottom up? Comparing the impacts of introduced arboreal possums and 'terrestrial'ruminants on native forests in New Zealand. *Biological Conservation, 99*(1), 65-79.

Ochocki, B. M., & Miller, T. E. (2017). Rapid evolution of dispersal ability makes biological invasions faster and more variable. *Nature Communications, 8*(1), 14315.

Parkes, J. P., Nugent, G., Forsyth, D. M., Byrom, A. E., Pech, R. P., Warburton, B., & Choquenot, D. (2017). Past, present and two potential futures for managing New Zealand's mammalian pests. *New Zealand Journal of Ecology, 41*(1), 151-161.

Parkes, J. P., Nugent, G., & Warburton, B. (1996). Commercial exploitation as a pest control tool for introduced mammals in New Zealand. *Wildlife biology, 2*(3), 171-177.

Pattabiraman, N., Morgan-Richards, M., Powlesland, R., & Trewick, S. A. (2022). Unrestricted gene flow between two subspecies of translocated brushtail possums (*Trichosurus vulpecula*) in Aotearoa New Zealand. *Biological Invasions, 24*(1), 247-260.

Porretta, D., Canestrelli, D., Bellini, R., Celli, G., & Urbanelli, S. (2007). Improving insect pest management through population genetic data: a case study of the mosquito Ochlerotatus caspius (Pallas). *Journal of Applied Ecology, 44*(3), 682-691.

Pracy, L. T. (1962). *Introduction and liberation of the opossum (Trichosurus vulpecula) into New Zealand*: New Zealand Forest Service.

Robertson, B. C., & Gemmell, N. J. (2004). Defining eradication units to control invasive pests. Journal of Applied Ecology, 41(6), 1042-1048.

Rollins, L. A., Woolnough, A. P., Sinclair, R., Mooney, N. J., & Sherwin, W. B. (2011). Mitochondrial DNA offers unique insights into invasion history of the common starling. *Molecular ecology, 20*(11), 2307-2317.

Russell, J. C., & Broome, K. G. (2016). Fifty years of rodent eradications in New Zealand: another decade of advances. *New Zealand Journal of Ecology, 40*(2), 197-204.

Sakai, A. K., Allendorf, F. W., Holt, J. S., Lodge, D. M., Molofsky, J., With, K. A., . . . Ellstrand, N. C. (2001). The population biology of invasive species. *Annual review of ecology and systematics, 32*(1), 305-332.

Sarre, S. D., Aitken, N., Adamack, A. T., MacDonald, A. J., Gruber, B., & Cowan, P. (2014). Creating new evolutionary pathways through bioinvasion: the population genetics of brushtail possums in N ew Z ealand. *Molecular ecology, 23*(14), 3419-3433.

Sato, T., Watari, Y., & Jogahara, T. (2023). Genetic relationships among populations of the small Indian mongoose (Urva auropunctata) introduced in Japan. *Mammal Research*, 1-11.

Searle, J. B. (2008). The genetics of mammalian invasions: a review. *Wildlife research, 35*(3), 185-192.

Taylor, A., Cowan, P., Fricke, B., & Cooper, D. (2000). Genetic analysis of the mating system of the common brushtail possum (*Trichosurus vulpecula*) in New Zealand farmland. *Molecular ecology, 9*(7), 869-879.

Taylor, A., Cowan, P., Fricke, B., Geddes, S., Hansen, B., Lam, M., & Cooper, D. (2004). High microsatellite diversity and differential structuring among populations of the introduced common brushtail possum, *Trichosurus vulpecula*, in New Zealand. *Genetics Research, 83*(2), 101-111.

Tepolt, C. K., & Palumbi, S. R. (2015). Transcriptome sequencing reveals both neutral and adaptive genome dynamics in a marine invader. *Molecular ecology, 24*(16), 4145-4158.

Tompkins, D. M. (2018). *The research strategy for a 'predator free' New Zealand.* Paper presented at the Proceedings of the Vertebrate Pest Conference.

Towns, D., West, C., & Broome, K. (2012). Purposes, outcomes and challenges of eradicating invasive mammals from New Zealand islands: an historical perspective. *Wildlife research, 40*(2), 94-107.

Travis, J. M., & Dytham, C. (2002). Dispersal evolution during invasions. *Evolutionary Ecology Research, 4*(8), 1119-1129.

Triggs, & Green. (1989). Geographic patterns of genetic variation in brushtail possums *Trichosurus vulpecula* and implications for pest control. *New Zealand Journal of Ecology*, 1-10.

Veale, A. J., & Etherington, T. R. (2023). Assessing possum dispersal and the Predator Free Taranaki trapping program me using population genomics. Manaaki Whenua – Landcare Research.

Warburton, B., Cowan, P., & Shepherd, J. (2009). How many possums are now in New Zealand following control and how many would there be without it. *Landcare research contract report LC0910/060*

Wilson, J. R., Dormontt, E. E., Prentis, P. J., Lowe, A. J., & Richardson, D. M. (2009). Something in the way you move: dispersal pathways affect invasion success. *Trends in ecology & evolution, 24*(3), 136-144.

Wright, B., Farquharson, K. A., McLennan, E. A., Belov, K., Hogg, C. J., & Grueber, C. E. (2019). From reference genomes to population genomics: comparing three reference-aligned reduced-representation sequencing pipelines in two wildlife species. *BMC genomics, 20*(1), 1-10.

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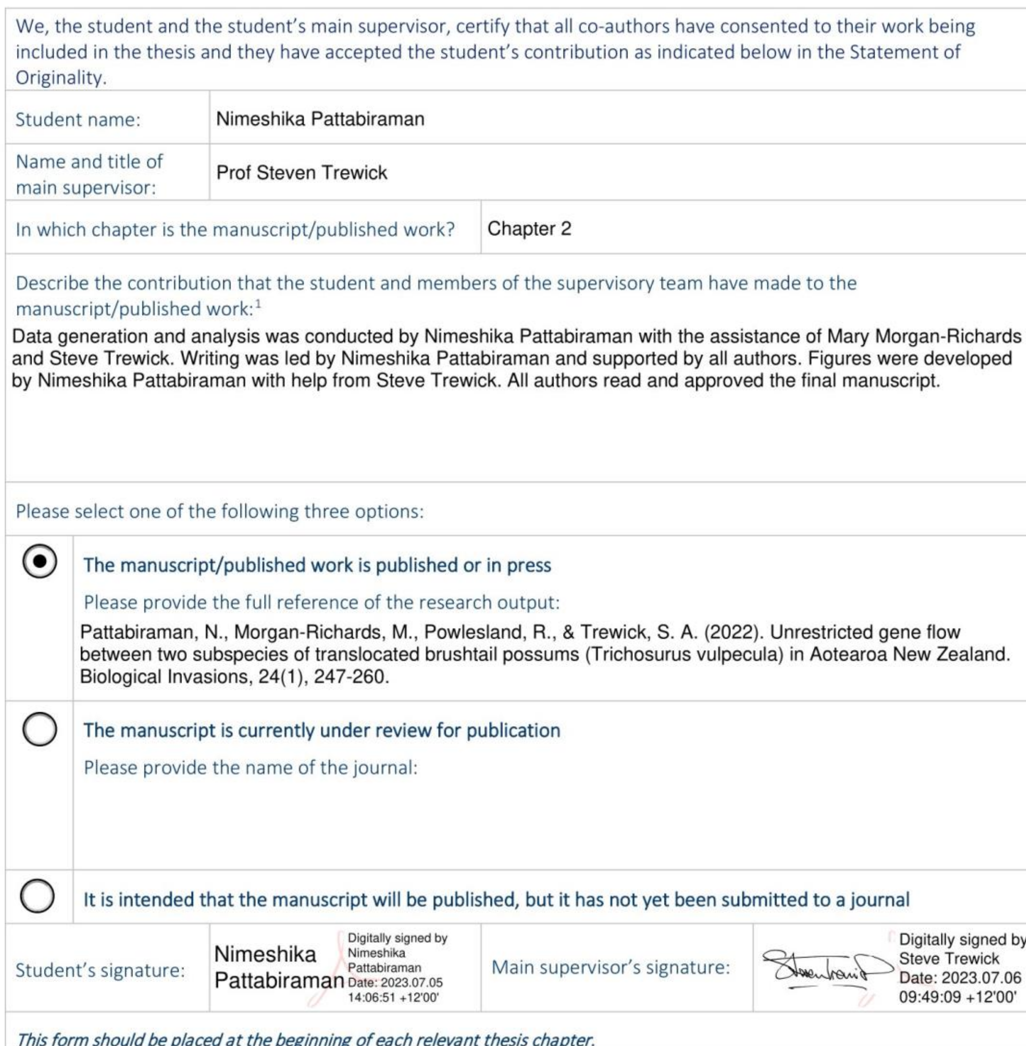

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Data generation and analysis was conducted by Nimeshika Pattabiraman with the assistance of Mary Morgan-Richards and Steve Trewick. Writing was led by Nimeshika Pattabiraman and supported by all authors. Figures were developed by Nimeshika Pattabiraman with help from Steve Trewick. All authors read and approved the final manuscript.

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Chapter Two

Unrestricted gene flow between two subspecies of translocated brushtail possums (*Trichosurus vulpecula*) in Aotearoa New Zealand

Abstract

We looked for evidence of genetic subdivision within invasive brushtail possums in New Zealand indicative of reproductive segregation. In particular we tested the hypothesis that fur colour phenotypes represent distinct lineages (subspecies) as originally introduced from Australia. We also sought evidence of genetic structure from spatial and temporal effects. We examined population samples from within a confined landscape and scored each specimen for phenotype using a number of fur colour traits. This resulted in a bimodal trait distribution expected for segregated grey and black lineages. Genetic structure and rates of gene flow were determined using seven species- specific multilocus nuclear microsatellite markers and mitochondrial DNA control region sequence. Genotype analyses indicated high levels of variation and mtDNA sequences formed two major haplogroups. Pairwise tests for population differentiation of these markers found no evidence of subdivision, indicating that these brushtail possums behave as a single randomly mating unit. Despite maintenance of two main colour types with relatively few colour intermediates, and previous inference of assortative mating and anecdotes of distinct races, our data indicate that New Zealand brushtail possum populations existing under these specific geographic conditions can be considered a single freely interbreeding group for the purposes of efficient pest management towards eradication.

Keywords

Eradication units, Invasions, Microsatellite, mtDNA, Population structure, *Trichosurus vulpecula*

Introduction

In Aotearoa New Zealand deliberate introduction of plants and animals, mostly during European colonisation (Thomson 1922), has resulted in extinctions and population decline across the native biota (Paulay 1994; Spatz et al. 2017; Wodzicki & Wright 1984; Woinarski et al. 2016). The largest of the abundant exotic mammals in New Zealand is the Australian brushtail possum (*Trichosurus vulpecula*) that is both a voracious folivore and an efficient arboreal predator of small vertebrates and invertebrates (Cowan 2005;Tompkins 2018). Initially introduced to establish a fur industry (Pracy 1962), this species is now recognised as a crucial challenge to New Zealand conservation and as a contagious carrier of bovine tuberculosis a major issue for the agriculture economy (Caley et al. 1999).

Brushtail possums introduced to New Zealand came from multiple Australian source populations that are recognised as having distinct phenotypic traits (How & Kerle 1995). Successful introductions were followed by deliberate translocation within New Zealand which is likely to have led to high levels of genetic and phenotypic polymorphism observed across the country (Taylor et al. 2004; Sarre et al. 2014). This diversity is the obverse of the situation where natural range expansion is usually associated with low genetic diversity, leading to the so-called genetic paradox of invasion (Estoup et al. 2016). The success of some biological invasions is attributed to advantages conferred from intraspecific hybridisation (Benvenuto et al. 2012; Rius & Darling 2014), and this might be relevant given the origin of brushtail possums in New Zealand. The scale of genetic diversity and pattern of gene flow that establishes within an invaded territory can significantly affect the adaptive potential of populations just as it does in natural populations (Slatkin 1987). As a result, regional responses of exotic pests to control measures, their reinvasion pathways and subsequent population recovery influence gene flow, and ultimately dictate outcomes for threatened native biota (Adams et al. 2014; Prior et al. 2018).

In New Zealand, brushtail possums have no natural predators and reach high density in both native and modified habitats (Efford 2000; Rouco et al. 2013). Naïve native prey and an abundance of habitats with vegetation palatable to mammalian folivores have enabled the possums to occupy most environments across New Zealand, except at highest elevations (Clout 2000). Possum population management in New Zealand uses aerial broadcast of 1080 toxin (Atkinson et al. 1995; Eason et al. 2011), other synthetic poisons (Goldson et al. 2015; Parkes & Murphy 2003), trapping and hunting. As population control measures create spatial and temporal heterogeneity in density, they may influence gene flow, and understanding this is necessary for efficient and effective pest

eradication efforts (Adams et al. 2024; Owens 2017). However, reproductive behaviour of the pest could also be influential. Where cryptic lineages exist within a pest taxon, differences in ecology and reproductive biology have potential to alter management requirements if responses to population control differ (Bastos et al. 2011; Jarić et al. 2019; Perry et al. 2018).

Reproductive segregation of possum subspecies introduced to different parts of New Zealand could yield differing pest characteristics, and persistence of distinct lineages could mean different management strategies are required. The genetic diversity of possums in New Zealand is spatially structured (Sarre et al. 2014; Taylor et al. 2004; Triggs & Green 1989) (Figure 2.1), and at least some of this structure correlates with possum fur colour variation (Sarre et al. 2014). In their native range six subspecies of *Trichosurus vulpecula* are recognised with distinct geographic ranges and coat colour (How & Kerle 1995). Analysis of size, fur colour, skull morphology, allozymes and karyotypes have revealed few diagnostic features among Australian populations but confirmed the status of regionally distinct subspecies within their native range (Kerle et al. 1991). Possums introduced to New Zealand represent two of these subspecies; *Trichosurus vulpecula vulpecula* from southeastern Australia and *Trichosurus vulpecula fuliginosus* from Tasmania (Pracy 1962). In eastern North Island, New Zealand separate subspecies introductions have retained founder characteristics suggesting pre and/or postzygotic isolation (Sarre et al. 2014). If this were the case more widely in New Zealand, efforts to model outcomes of pest management, population recovery and migration in a metapopulation framework will need to incorporate dynamics of this tension if effective control is to be achieved (Engler et al. 2019; Quilodrán et al. 2018; Ranke et al. 2020).

We sought evidence for reproductive isolation among Australian brushtail possum subspecies in New Zealand within a naturally constrained habitat space. We looked for genetic structure within our sample considering phenotypic effects and also spatial and temporal effects. The prevalence of different fur colours across the study site and colour recorded for each individual sampled allowed us to examine the possibility of non- random mating where coat colour is linked to mate choice (Sarre et al. 2014). If the brushtail possums in this region are randomly mating without strong hybrid fitness disadvantage, then we would expect to detect no genetic structure within the sample. However, should there be a mating bias, we would expect to see significant deviations from Hardy-Weinberg proportions as well as genetic differentiation among phenotype categories.

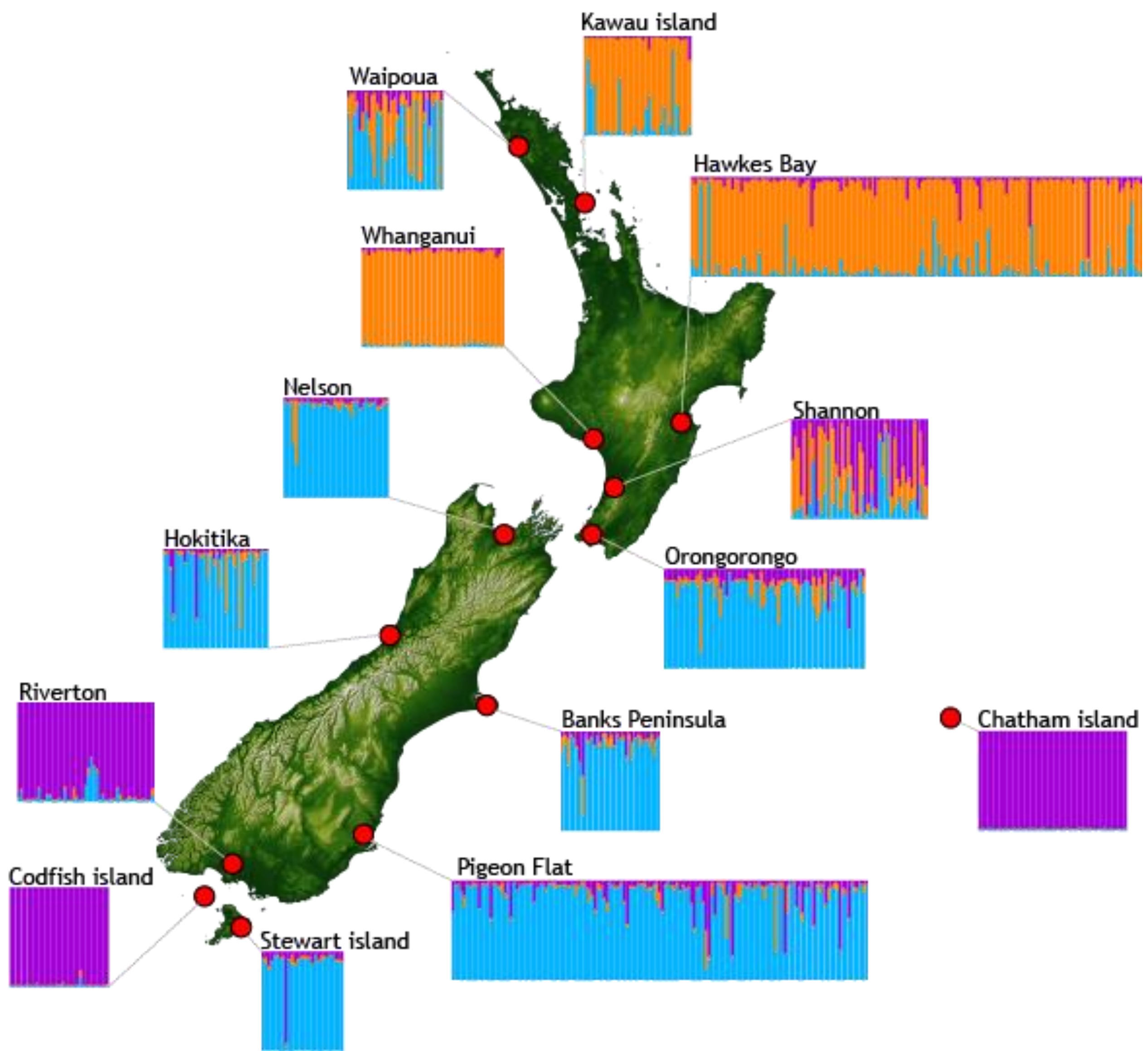


Figure 2.1 Nationwide population genetic structure of invasive brushtail possums (*Trichosurus vulpecula*) in Aotearoa New Zealand inferred using MCMC Bayesian clustering of variation at five microsatellite loci among 799 possums sampled between 1989 and 1997 (Taylor et al. 2004).

Materials and Methods

Location

Kenepuru Peninsula is an area of land (<400km^2) on a narrow isthmus in the Marlborough Sounds region of northern South Island, New Zealand. The region is characterised by drowned valleys resulting from tectonic subsidence (Trewick & Bland 2012). The peninsula is largely separated from the mainland with low potential for dispersal (Adams et al. 2014). Much (~60%) of the original forest on the Kenepuru Peninsula was removed for pastoral farming, but natural regeneration has since formeda mosaic of pasture (~20%), scrub and forest.

We used a sample of 132 individual possums from the Kenepuru Peninsula collected during three different time periods (Table 2.1). The sex and fur colour of each possum was recorded along with the sampling location. Tissue samples, comprising ear clips or muscle biopsies, were taken from fresh possum corpses by landowners and pest controllers. These samples were stored in 95% ethanol. For each sample we sequenced a region of the mitochondrial D-loop and determined allelic variation at seven microsatellite loci.

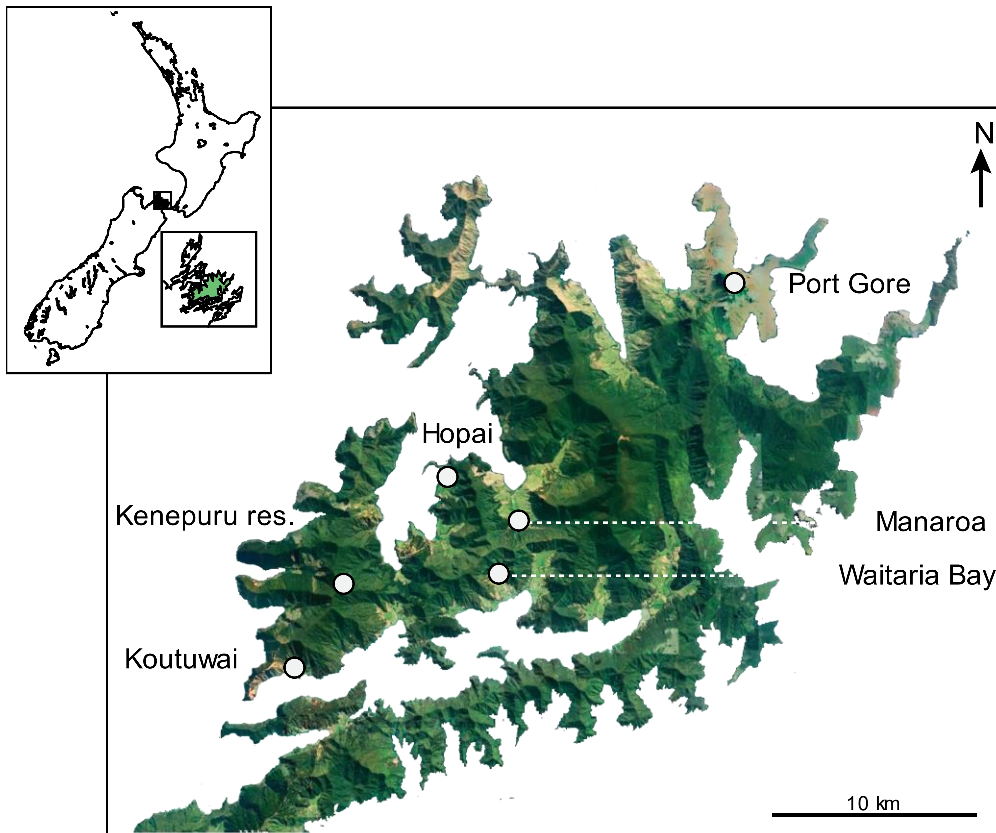


Figure 2.2 The Kenepuru Peninsula, Marlborough Sounds, New Zealand with six locations sampled for invasive brushtail possums (*Trichosurus vulpecula*).

Phenotype variation

Brushtail possums in Australia display spatially structured fur colour polymorphism and size variation that has resulted in their treatment as six subspecies, two of which were introduced to New Zealand (Kerle et al. 1991). In southern and eastern Australia (*Trichosurus vulpecula vulpecula*) the fur of brushtail possums is typically silver-grey above and white-cream on the undersides with a black tail and contrasting pale yellow and black marking on the face and ears (Kerle et al. 1991). In Tasmania (*T. v. fuliginosus*), body size is larger, and the body fur is often almost black, longer and woollier in texture (Kerle et al. 1991; Museums Victoria Sciences Staff 2016). In New Zealand, possum fur traders refer to a range of fur colours from red-grey to dark brown or black (Nixon 1989). Although copper coloured (red) possums are known from Queensland/Northern Territory of Australia (*T. v. johnstonii*) there are no records of possums from this region being brought to New Zealand (Hutching 2008).

We used photographs of freshly killed possums to score their fur colour on seven body sections (ear, tail base, shoulders, rump, flank, belly, and flank to belly transition). For each section we scored predominantly grey characteristics (typical of *Trichosurus v. vulpecula*) as 1 (e.g. grey shoulder = 1; pale belly = 1), and scored the darkest shades (typical of *T. v. fuliginosus*) with higher numbers (e.g. black shoulder = 4, black ear = 3). We scored intermediate/mixed colours of brown and red variants with intermediate values (e.g. brown ear = 2, red-brown flank = 3; Supplementary table 2.1). The flank to belly colour transition was scored from 1 (sharp) to 3 (continuous). Using the combined scores for all sections each possum was categorised as Grey (score of 7–9), Black (scoreof 19–22) or a Ginger-mix (score of 13–18) for population genetic analysis.

Population genetic markers

DNA extraction was conducted using the GeneAid ™ Tissue DNA Isolation Kit (Geneaid Biotech Ltd, Taiwan) following the manufacturer's instructions with a final elution volume of 200µl. The quality and quantity of the DNA extraction was assessed using Invitrogen Qubit 4 Fluorometer (ThermoFisher Scientific).

Mitochondrial locus- Polymerase Chain Reaction (PCR) amplification targeted a fragment of the mtDNA D-loop (~730 bp) using novel PCR primers designed for the short-eared possum (*Trichosurus canninus*) and brushtail possum (*Trichosurus vulpecula*) from whole mtDNA genomes assembled from short read NGS data (Carmelet- Rescan et al. 2022): Tcan_218f (AAGGCAACAACACCTCACCA), Tcan_1049r (AATACGACATCGGCGACCTC), Tvul_228f (CACCTCACCATCAACACCCA) and Tvul_1023r (TCCCGCCCAGTTGATAAACC). Consistent *T. vulpecula* amplifications and DNA sequences were obtained using the primer combination Tcan_218f and Tvul_1023r. Amplification reactions were in 20µl volumes containing 2µl 10x DreamTaq buffer (ThermoFisher Scientific) with a final concentration of 2.5mM Magnesium Chloride (additional 0.4µl of 25mM MgCl2), 200µM each deoxy-nucleotide phosphates (dNTP), 0.05µM of each primer, and 0.05U DreamTaq DNA polymerase. Thermocycling used an initial denaturation step at 95°C for 90 seconds followed by 36 cycles of 94°C for 20 seconds, 51°C for 20 seconds, and 72°C for 1 minute; with a final 8-minute extension step of 72°C. Amplification products were sequenced using BigDye® chemistry (Perkin Elmer) following the manufacturer's protocols on an ABI3730 DNA analyser (Macrogen Inc). Sequences were edited and aligned using the software GENEIOUS 11.5 (Kearse et al.2012). DNA polymorphism tests, overall haplotype (h) and nucleotide (π) diversity, andpopulation size change tests were conducted using the software DNASP v 6.12.01. For visualization of these

results, a haplotype median-joining network (Bandelt et al. 1999) was inferred using POPART (Leigh & Bryant 2015).

Nuclear loci- Eight microsatellite loci developed for this species were screened using available PCR primers (Taylor, Cooper 1998; Taylor et al. 2004). We used multiplex PCR with four loci in each (M1: Tv_16, Tv_58, Tv_53 and Tv_19 and M2: Tv_64, Tv_PnMs, Tv_M1 and Tv_14) using fluorophores FAM™, VIC™ and TAMRA™ (Supplementary table 2.2). PCR reactions comprising 2 µl Thermopol® buffer, 0.4 µl dNTP, 0.1 µl Taq DNA polymerase, 0.5 µl of each forward primer, 0.5 µl of each reverse primer and finally water to make a volume of 10 µl were made with the following steps: denaturation step at 95°C for 4 min, followed by 40 cycles of 94°C for 30s, 60°C for 45s and 72°C for 45s, and a final extension step of 72°C for 10 min. The amplified microsatellite PCR products were genotyped using a fragment analyser at Macrogen Inc. Allele length was determined using the microsat plugin in GENEIOUS 11.5 (Kearse et al. 2012).

The presence of null alleles and long-allele dropout was examined with the use of the software MICROCHECKER v2.2.3 (Van Oosterhout et al. 2004). For Tv_14 some individuals did not amplify, and the presence of null alleles was suggested by observed homozygotes exceeding the expected (MICROCHECKER) and therefore we removed this locus from further analysis. We tested for linkage disequilibrium using pairwise tests with the software GENEPOP on the Web (Rousset 2008). Tests for the mean number of alleles (Na) at seven loci were conducted in ARLEQUIN 3.5.2.2. Mean gene diversity (G) and mean allelic richness (Ar) were calculated using FSTAT v2.9.4 (Goudet 2003).

Data Analyses

Aotearoa New Zealand-wide population structure

We reanalysed an existing dataset obtained for 799 brushtail possums from 14 population samples across New Zealand (Taylor et al. 2004). We used a naïve modelling approach that infers genotypic clusters and assigns individual genotypes using STRUCTURE v2.3.2 (Pritchard et al. 2000). Assigned clusters (K) are based on the assumptions that the populations are in Hardy-Weinberg Equilibrium, the loci under analysis are in complete linkage equilibrium, and each cluster identifies as a subset of allele frequencies (Pritchard et al. 2000). The optimal number of population clusters (optimal K) was estimated using the Bayesian Information Criterion.

We used the Bayesian Markov-Chain Monte Carlo (MCMC) method to cluster the individual

genotypes with the Burnin period was set to 5000 and the MCMC repeats thereafter were set to 50,000 to increase the accuracy of assigning individuals to inferred clusters (Porras-Hurtado et al. 2013). An admixture model was used specifyingthat the pre-defined populations based on location sites were not used as priors when assigning individuals to inferred populations. K-values were tested between 1 to 10 for 799 samples with five loci, with 10 iterations for each K. The K-value best fitting the dataset was calculated using the Evanno method in the software STRUCTURE HARVESTER (Earl 2012). To identify cluster modes and visualise population structure inferences across K values, the software CLUMPAK (Kopelman et al. 2015) was used where the output can be visualised as a stacked bar plot (Figure 2.1).

Kenepuru Peninsula Spatial and temporal population structure

We sequentially divided our Kenepuru possum samples into groups based on geographical location (five groups for mtDNA locus and six groups for microsatellite loci), year of sampling and fur colour (three groups each). For each division we examined the distribution of haplotypes and genotypes to determine whether there was evidence of genetic structure. Geographical structure would suggest we had sampled from more than one possum population within the Kenepuru Peninsula. Temporal genetic structuring would suggest demographic variation over 16 years, and genetic differences among fur colour types would suggest non-random mating with respect tophenotype.

Table 2.1. Location, year of sampling, number of samples (n) of possums (*Trichosurus vulpecula*) used in this study with by nuclear microsatellite diversity values at 7 loci: Mean gene diversity (G), Mean number of alleles (N_a), Mean Allelic Richness per locus based on six diploid individuals (A_r), Observed Heterozygosity (Ho) and Expected heterozygosity (He) of each sample. Fur colour frequency in each sample: Grey (Gr), Ginger-mix (GM), Black (Bl).

Location	Year	Code	n	G	N_a	A_r	H_o	H_e	Fur colour frequency (%)		
									Gr	GM	Bl
Manaroa	2016	Mn16	36	0.68	5.57	3.9	0.66	0.67	38	13.8	47
Manaroa	2019	Mn19	22	0.71	4.43	3.74	0.73	0.7	50	13.63	36.36
Manaroa	Total	Mn	58						43.1	13.79	43.1
Kenepuru	2003	Kn	40	0.67	4.86	3.74	0.67	0.66	57.5	0	42.5
Waitaria Bay	2019	WB	12	0.68	4.43	3.86	0.57	0.64	58.33	16.66	25
Koutuwai	2019	Kt	8	0.56	3.57	3.3	0.58	0.53	87.5	0	12.5
Port Gore	2019	PG	8	0.66	3.71	3.44	0.67	0.64	12.5	37.5	50
Hopai	2019	Hp	6	0.69	4	4	0.71	0.64	33.33	16.66	50
Total			**132**	`							

To analyse mtDNA haplotype differentiation, 110 individuals were organized into geographical location sets (Table 2.2) and then grouped according to time (2003, 2016 and 2019). Spatial and temporal population samples were compared using pairwise ϕ_{ST} with significance of deviations from zero (Supplementary figure 2.1) assessed using 100 permutations, with a 0.05 level of significance in ARLEQUIN and a Bonferroni correction for multiple tests (0.005 and 0.01667 respectively). Using microsatellite genotypes, tests of Hardy-Weinberg expectations were implemented using GeneAlEx 6.503 (Peakall & Smouse 2006). Deviations from Hardy-Weinberg expected proportions were examined for the full sample of possums from Kenepuru Peninsula (n=132) and for subsets (including temporal sampling). We used pairwise F_{ST} semi- matrix with Slatkin's method computed in ARLEQUIN v3.5.2.2 (Excoffier & Lischer 2010) to assess population subdivision. Statistically significant departures from zero of pairwise F_{ST} values would indicate restricted gene flow (Supplementary table 2.3). The analysis used 100 permutations with a significance level of 0.05 and a Bonferroni correction for multiple tests (0.0033 and 0.01667 respectively).

Fur colour variation

110 Kenepuru possums were classified according to fur colour based on their total score from seven body sections: Grey (n=56), Black (n=42), or Ginger-mix (n=12). The mitochondrial diversity of these three phenotype groups was compared using pairwise ϕ_{ST} with significance of deviations from zero assessed using 100 permutations, with a 0.05 level of significance in ARLEQUIN and a Bonferroni correction for multiple tests (0.05/3 = 0.01667). Pairwise F_{ST} was estimated for microsatellite genotypes of 132 individuals grouped according to fur colour (as above plus 9 individuals characterised in the field: 66 Grey, 52 Black, 14 Ginger-mix). Statistically significant departures of F_{ST} values from zero would indicate non-random mating. We used 100 permutations with a significance level of 0.05 a Bonferroni correction for multiple tests (0.05/3 = 0.01667). We also looked for evidence of genotypic partitioning that would beexpected if fur phenotypes were reproductively segregated using the naïve modelling analysis STRUCTURE (Pritchard et al. 2000) with K values of 1 to 3.

Results

Phenotype variation

We found fur colour of four of the seven body sections to be linked, so we reduce our data to five scored sections (belly, ear, tail base-rump, flank-shoulder and flank to belly transition). We found that all possums in our sample with grey body fur had yellow earsand yellow bellies (Figure 2.3). Grey possums had grey fur on their tail bases, rumps, flanks and shoulders with the exception of four individuals who had a mix of red and grey hairs on their flanks and shoulders (Figure 2.3). In the case of dark possums, 14/22 black- eared possums had brown bellies (63%) while the other eight black-eared possums hadred bellies. Similarly, 11/17 brown-eared possums had brown bellies (64%), while the other six brown-eared possums had red bellies. The total scores ranged from 6 to 16 with typical grey and typical black possums at either end of the spectrum. The frequency distribution of fur colour in our sample was bimodal dominated by grey and black individuals with fewer intermediates than expected without segregation (Figure 2.4a).

Figure 2.3. Examples of naturally occurring fur colour variants among invasive brushtail possums in New Zealand.

Aotearoa New Zealand population structure

Modelling of population genetic structure among 799 brushtail possums from 14 populations across New Zealand with 5 microsatellite loci identified a model with K=3 to be optimal. Spatial structure within this sample is readily apparent with most population samples dominated by a single inferred genotype assignment (Figure 2.1). However, two population samples included individual possums assigned to all three genotypic clusters present and many uncertain assignments (Shannon and Waipoua; Figure 2.1).

Lack of spatial and temporal genetic structure on Kenepuru Peninsula

D-loop mtDNA sequences of 684 bp were aligned for 110 possum samples and seven haplotypes were identified. The two common haplotypes differed from one another by 2.49%, and five haplotypes observed in one or two possums varied from the common haplotypes by between one and five mutations (Table 2.2). Overall haplotype diversity (h) was 0.56 and nucleotide diversity (π) was 0.013. Our Koutuwai sample had the highest haplotype diversity (h=0.71) and nucleotide diversity (π =0.014). The Manaroa sample (n=53) had five of the seven haplotypes observed, three of these restricted Manaroa (Table 2.2). When possum samples were grouped by geographic location into five population samples, none of the 10 pairwise ϕ_{ST} estimates from the D-loop locus differed significantly from zero. Similarly, pairwise ϕ_{ST} estimates resulting from haplotype grouping by collection date (2003; 2016; 2019) showed no significant deviation from zero (Supplementary figure 2.1).

Table 2.2 mtDNA (D-loop) polymorphism among 110 *Trichosurs vulpecula* possums from five location in Kenepuru, Marlborough Sounds, New Zealand. Haplotype (h) and nucleotide (π) diversities of ten haplotypes (KPa–Kpg). * Sequence from Hopai.

Location	n	h	π	KPa	KPb	KPc	KPd	KPe	KPf	KPg
Manaroa	53	0.56	0.013	25		1*	1	25		1
Kenepuru	32	0.51	0.012	24				11	1	
Waitaria Bay	12	0.41	0.011	3				9		
Koutuwai	10	0.71	0.014	3	1			5	1	
Port Gore	3	0	0	3						
Total	**110**	**0.56**	**0.013**	**54**	**1**	**1**	**1**	**50**	**2**	**1**

We genotyped 132 possums from Kenepuru using seven microsatellite loci. Total number of alleles per locus ranged from 2 to 13 (Supplementary table 2.2). The Manaroa sample had the highest diversity (H_O= 0.73, He= 0.7) and mean gene diversity (0.71; Table 2.1). Mean allelic richness per locus ranged between 3.3 (Koutuwai) and 4 (Hopai; Table 2.1). Significant deviation from Hardy-Weinberg proportions suggested that there was heterozygous excess in the Manaroa sample for just one locus (n=56; Locus-Tv-58) and heterozygous deficit in the Waitaria Bay population sample for a different locus (n=12; locus-Tv-16; Supplementary table 2.4). Pairwise F_{ST} differentiation tests of genotype data showed no significant deviations from zero for any of the location samples. Naive cluster assignment of genotypes showed no support for more than one cluster among brushtail possums on Kenepuru Peninsula (Figure 2.4e). When considering sampling time events, only the 2019 sample deviated significantly from Hardy-Weinberg equilibrium at one locus with excess of heterozygous individuals at one locus (n=56; locus- Tv_58; Supplementary table 2.4). Pairwise F_{ST} differentiation tests showed no significant deviations from zero when comparing temporal samples (p-value >0.99+-0.003).

Genetic structure in relation to fur colour

Based on scoring fur colour of five body sections we classified 137 possums from Kenepuru Peninsula into three colour groups: Grey, Black and Ginger-mix (Table 2.1). Mitochondrial haplotypes for 110 of these possums found no evidence of genetic differentiation among fur colour groups; pairwise ϕ_{ST} did not deviate significantly from zero (Supplementary figure 2.1) (Figure 2.4b). Genotypic data revealed that the sample of grey possums had fewer heterozygote individuals than expected at one locus (n=66; locus Tv-58) and the sample of black possums had an excess of heterozygote individuals at one locus (n=52; locus- Tv_M1; Supplementary table 2.4), while the Ginger-mix possum sample met all expectations of Hardy-Weinberg genotype frequencies. However, no evidence of genetic differentiation was observed in the nuclear markers with pairwise differentiation; pairwise F_{ST} did not deviate significantly from zero. The optimum number of population clusters (K) present in the sample of possums was inferred usingBayesian clustering and the Evanno ΔK comparison approach (Evanno et al. 2005) suggested K=2. However, K=2 is effectively the default response from ΔK when there islow signal of structure (Janes et al. 2017) and the distribution of assignment probabilities was consistent with a single genetic population (Supplementary figure 2.2).

Therefore, our Bayesian clustering analysis did not provide strong evidence of genotypic clustering among brushtail possums from Kenepuru Peninsula (Figure 2.4c).

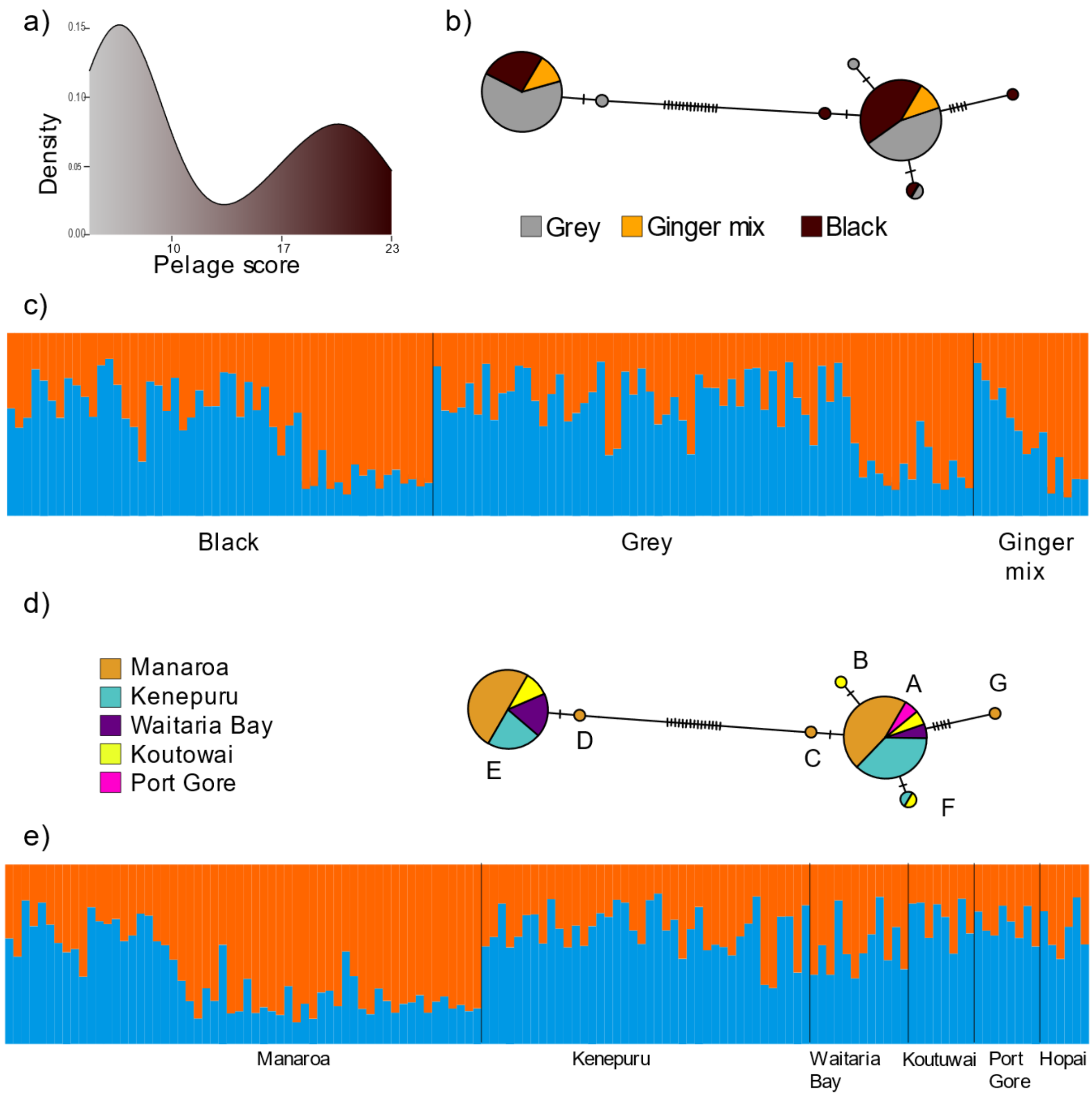


Figure 2.4. a) Density plot showing the fur colour distribution of possums sampled. b) D- Loop haplotype network for brushtail possums with fur colour assignment of sampledindividuals. Pie size proportional to sample size.c) Probability assignment of microsatellite genotypes for the optimal K=2 model, arranged by fur colour type. d) Haplotype network (as above) showing distribution among sample locations, and with codes as given in in Table 2.2. e) Genotype assignment arranged by sampling location.

Discussion

Brushtail possums from Australia were released in New Zealand between 1840 and 1900 (Cowan 2005) to establish a fur industry (Pracy 1962). As a result of dispersal from many release sites, the species went on to spread and establishing throughout New Zealand, reaching a total population estimated to be approximately 70 million at the end of 1900s (Clout 2000). Brushtail possum invasion led to the drastic decline of native flora and fauna (Sadlier 2000), and possums emerged as prominent reservoirs ofbovine tuberculosis in New Zealand (Livingstone et al. 2015). Control measures targeting New Zealand possum populations are spatially and temporally uneven, potentially adding to a complex and poorly understood landscape genetic structure. As genetic composition of invasive populations has pronounced implications for their invasion characteristics and responses to control measures (Lee 2002), developing amodel of gene flow and identifying eradication units within New Zealand possums is essential for their future control and eradication (Adams et al. 2014; Tompkins 2018). Brushtail possums in New Zealand are genetically diverse at neutral loci (Triggs & Green 1989; Taylor et al. 2004) and also exhibit fur colour variation that distinguishesthe two subspecies of *Trichosurus vulpecula* introduced to the country (Figuer 2.1). This raises the possibility that regionally partitioned lineages might have distinct traits and that invasive populations of mixed ancestry may have novel genotypes (e.g- Lu et al. 2011; Smith et al. 2020). However, the fine-scale processes of gene flow and natural selection operating in this invaded landscape remain largely concealed. One study that combined evidence from neutral genetic loci with phenotypic information found evidence that distinct brushtail possums lineages established in the North Island of NewZealand from separate introductions from Australia have remained partitioned (Sarre et al. 2014). Their analysis inferred assortative mating of genotypes characterised by different fur colour (and origin), highlighting the potential for cryptic lineages (Bastoset al. 2011; Jarić et al. 2019; Perry et al. 2018).

Using a fine-scale analysis of possums collected from the Kenepuru Peninsula in the South Island of New Zealand (Figure 2.2) we explored the level at which we could detect genetic structure in this invasive species. We sought evidence of genetic partitioning using seven nuclear microsatellite markers and one mitochondrial marker to resolve reproductive exchange on a small-scale involving possum of mixed ancestry. Phenotypic variation based on morphological data seemed to indicate a preponderance of grey and black types with a deficiency of intermediates. However, our genetic analyses using mitochondrial markers showed no association of haplotype with fur colour, and pairwise differentiation tests using nuclear loci showed no correlation with fur colour (Figure 2.3). Our results contrast with previous evidence for assortative mating (Sarre

et al. 2014) and the presence of a contact zone linked to the genotypic differences between two populations of possums. The stepped cline detected in the eastern North Island ofNew Zealand could be due to spatial effects such as the presence of a large river rather than a mating bias. Within our study, there is no evidence to support different fur colours belonging to different genotypic clusters. We documented for the first time mtDNA haplotype variation among the brushtail possum in New Zealand (Table 2.2). We identified a total of seven haplotypes (Figure 2.4d),with a maximum of 3.2% sequence divergence. Introductions from different source populations in Australia is the most likely explanation for the two distinct mtDNA lineages within the Kenepuru Peninsula population (Carmelet-Rescan et al. 2022). Nuclear markers showed similar levels of allelic variation over the spatial sampling (Table 2.1). Our geographic divisions represent samples from the same population as revealed by the estimates of pairwise differentiation for both sets of markers (ϕ_{ST} and F_{ST} =0). This clearly indicates that possums on the Kenepuru Peninsula are not spatially structured and there seems to be no spatial restrictions to gene flow over this area. This agrees with previous estimates of eradication units of this species in New Zealand that span 20 kms (Adams et al. 2014) and estimates that 20–30% of juvenile possums will disperse up to five kms (Cowan & Clout 2000). Dispersal routes are important in terms of local management, as local eradication is likely to increase opportunities for possum reinvasion, which is possible in the absence of external obstacles. Consequently,this can lead to a new combination of genotypes and potential for high levels of gene flow during reinvasion. Spread of resistance to the poison 1080 in possums (suspected in Tasmanian possums; McIlroy 1983) or development of other genetic advantages will be influenced by an interaction between population connections and population controlmeasures (Allendorf & Leary 1986; Byers et al. 2015).

In conclusion, our study suggests that possums in the Kenepuru Peninsula act as one population. Despite a deficit of intermediate colour morphs, the Kenepuru Peninsula possums do not represent two sympatric but reproductively isolated subspecies. Our genetic data provides enough evidence to refute our initial hypothesis of assortative mating segregating possum subspecies, which substantially simplifies their management. Predator Free 2050 aspires to rid New Zealand of possums and mustelidsby the year 2050 (Tompkins 2018), therefore we need to adopt effective control measures consisting of strategies that consider and eliminate any factors that might render them inefficient, including identification of reinvasion pathways, eradication units and the potential of hybrid advantage.

References

Adams, A., Recio, M., Robertson, B., Dickinson, K., & Van Heezik, Y. (2014). Understanding home range behaviour and resource selection of invasive common brushtail possums (*Trichosurus vulpecula*) in urban environments. Biological Invasions, 16, 1791-1804.

Adams, AL (2013) Spatial ecology and genetic population structure of the common brushtail possum (*Trichosurus vulpecula*) within a New Zealand urban environment. Doctoral dissertation, University of Otago Adams AL, Van Heezik Y, Dickinson KJM, Robertson BC (2014) Identifying eradication units in an invasive mammalian pest species. Biol Invasions, 16:1481-1496

Allendorf FW, Leary RF (1986) Heterozygosity and fitness in natural populations of animals. Conservation biology: the science of scarcity and diversity 57:58-72 Atkinson I, Campbell D, Fitzgerald B et al. (1995) Possums and possum control; effects on lowland forest ecosystems. A literature review with specific reference to the use of 1080, p 32

Ausseil A-GE, Dymond JR, Weeks ES (2011) Provision of natural habitat for biodiversity: quantifying recent trends in New Zealand. Biodiversity loss in a changing planet, pp 201-220

Bandelt H-J, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 16:37-48

Bastos AD, Nair D, Taylor PJ, et al. (2011) Genetic monitoring detects an overlooked cryptic species and reveals the diversity and distribution of three invasive Rattus congeners in South Africa. BMC Genet 12:26

Benvenuto C, Cheyppe-Buchmann S et al. (2012). Intraspecific hybridization, life history strategies and potential invasion success in a parasitoid wasp. Evol Ecol 26:1311-1329 Blackburn TM, Cassey P, Duncan RP et al. (2004) Avian extinction and mammalian introductions on oceanic islands. Science 305:1955-1958

Brzeziński M, Żmihorski M, Zarzycka A et al. (2019) Expansion and population dynamics of a non-native invasive species: the 40-year history of American mink colonisation of Poland. Biol Invasions 21:531-545

Byers JE, Smith RS, Pringle JM et al. (2015) Invasion expansion: time since introduction best predicts global ranges of marine invaders. Sci Rep 5:12436

Caley P, Hickling G, Cowan P et al. (1999) Effects of sustained control of brushtail possums on levels of Mycobacterium bovis infection in cattle and brushtail possum populations from Hohotaka, New Zealand. N Z Vet J 47:133-142

Campbell D, Atkinson IA (2002) Depression of tree recruitment by the Pacific rat (Rattus exulans Peale) on New Zealand's northern offshore islands. Biol Conserv 107:19- 35

Carmelet-Rescan, D., Morgan-Richards, M., Pattabiraman, N., & Trewick, S. A. (2022). Time-calibrated phylogeny and ecological niche models indicate Pliocene aridification drove intraspecific diversification of brushtail possums in Australia. Ecology and Evolution, 12(12), e9633.

Clout M (2000) Anatomy of a disastrous success: the brushtail possum as an invasive species. In: Montague TL (ed) The brushtail possum-biology, impact and management of an introduced marsupial. Manaki Whenua Press: Lincoln, pp 1-9

Courchamp F, Chapuis J-L, Pascal M (2003) Mammal invaders on islands: impact, control and control impact. Biol Rev 78:347-383

Cowan P (2005) Brushtail possum. In: King CM (ed) The Handbook of New Zealand Mammals. Oxford University Press: Melbourne, pp 56-80

Cowan P, Clout M (2000) Possums on the move: activity patterns, home ranges, and dispersal. In: Montague TL (ed) The brushtail possum: biology, impact and management of an introduced marsupial. Manaaki Whenua Press: Lincoln, pp 24–34

Department of Conservation (2020) Towards a Predator Free New Zealand: Predator Free 2050 Strategy.

Dowding JE, Murphy EC (2001) The impact of predation by introduced mammals on endemic shorebirds in New Zealand: a conservation perspective. Biol Conserv 99:47-64

Earl DA & VonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4:359-361

Eason C, Miller A, Ogilvie S et al. (2011) An updated review of the toxicology and ecotoxicology of sodium fluoroacetate (1080) in relation to its use as a pest control tool in New Zealand. N Z J Ecol, pp 1-20

Efford M (2000) Possum density, population structure, and dynamics. In Montague TL (ed) The brushtail possum-biology, impact and management of an introduced marsupial. Manaki Whenua Press: Lincoln, pp 47–61

Engler JO, Sacher T, Coppack T et al. (2019) Assortative mating frames establishment in a young island bird population.R Soc Open Sci 6:190050

Estoup A, Ravigné V, Hufbauer R et al. (2016) Is there a genetic paradox of biological invasion? Annu Rev Ecol Evol Syst 47:51-72

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611-2620 Excoffier L, Lischer HE (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10:564-567

Goldson S, Bourdôt G, Brockerhoff E et al. (2015) New Zealand pest management: current and future challenges. J R Soc N Z 45:31-58

Goodwin BJ (2003) Is landscape connectivity a dependent or independent variable? Landsc Ecol 18:687-699

Goudet J (2003) FSTAT (Version 2.9. 4), a program (for windows 95 and above) to estimate and test population genetics parameters. Department of Ecology & Evolution, Lausanne University, Switzerland, p 53

How R, Kerle J (1995) Common brushtail possum. In: Strahan R (ed) The Mammals of Australia, pp. 273–275

How RK, JA (1995) Common brushtail possum. The mammals of Australia, pp 273-275 Hutching G (2008) Possums. In: Te Ara - the Encyclopedia of New Zealand. http://www.TeAra.govt.nz/en/possums/print

Janes JK, Miller JM, Dupuis JR et al. (2017) The K= 2 conundrum. Mol Ecol 26:3594-3602 Jarić I, Heger T, Monzon F, et al. (2019) Crypticity in biological invasions. Trends Ecol Evol 34:291-302

Kearse M, Moir R, Wilson A et al. (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647-1649

Kerle J, McKay G, Sharman G (1991) A systematic analysis of the brushtail possum, *Trichosurus vulpecula* (Kerr, 1792)(Marsupialia, Phalangeridae). Aust J Zool 39:313-331 King CM (1984) Immigrant killers: introduced predators and the conservation of birds in New Zealand. Oxford University Press

Kopelman NM, Mayzel J, Jakobsson M et al. (2015) Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. Mol Ecol Resour 15:1179-1191

Lassnig N, Colomar V, Picó G et al. (2020) Assessment of the invasion process of the common raccoon Procyon lotor (Carnivora: Procyonidae) on a Mediterranean island a decade after its introduction. Sci Total Environ 699:134191

Lee CE (2002) Evolutionary genetics of invasive species. Trends Ecol Evol 17:368–391. Leigh JW, Bryant D (2015) popart: full-feature software for haplotype network construction. Methods Ecol Evol 6:1110-1116

Levine JM (2008) Biological invasions. Curr Biol 18:R57-R60

Livingstone P, Hancox N, Nugent G et al. (2015) Toward eradication: the effect of Mycobacterium bovis infection in wildlife on the evolution and future direction of bovine tuberculosis management in New Zealand. N Z Vet J 63:4-18

Lu M, Wingfield MJ, Gillette N et al. (2011) Do novel genotypes drive the success of an invasive bark beetle–fungus complex? Implications for potential reinvasion. Ecology 92:2013-2019

Manel S, Schwartz MK, Luikart G et al. (2003) Landscape genetics: combining landscape ecology and population genetics. Trends Ecol Evol 18:189-197

Martin AR, Lea VJ (2020) A mink-free GB: perspectives on eradicating American mink Neovison vison from Great Britain and its islands. Mamm Rev 50:170-179

McIlroy J (1983) The sensitivity of the brushtail possum (*Trichosurus vulpecula*) to 1080 poison. N Z J Ecol, pp 125-131

Museums Victoria Sciences Staff (2016) *Trichosurus vulpecula* Common Brushtail Possum in Museums Victoria Collections. https://collections.museumsvictoria.com.au/species/8449. Accessed 16 July 2020 Nixon A (1989) Fur growth and replacement in the brushtail possum, *Trichosurus vulpecula* Kerr. Unpublished PhD thesis, Massey University, Palmerston North O'Dowd DJ, Green PT, Lake PS (2003) Invasional ‘meltdown’on an oceanic island. Ecol Lett 6:812-817

Owens B (2017) Behind New Zealand’s wild plan to purge all pests. Nature News 541:148

Parkes J, Murphy E (2003) Management of introduced mammals in New Zealand. NZ J Zool 30:335-359

Paulay G (1994) Biodiversity on oceanic islands: its origin and extinction1. Am Zool 34:134-144

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288-295

Perry KD, Baker GJ, Powis KJ et al. (2018) Cryptic Plutella species show deep divergence despite the capacity to hybridize. BMC Evol Biol 18:77

Porras-Hurtado L, Ruiz Y, Santos C et al. (2013) An overview of STRUCTURE: applications, parameter settings, and supporting software. Front Genet 4:98

Pracy LT (1974) Introduction and liberation of the opossum (*Trichosurus vulpecula*) into New Zealand. New Zealand Forest Service, Wellington

Prior KM, Adams DC, Klepzig KD, et al. (2018) When does invasive species removal lead t o ecological recovery? Implications for management success. Biol Invasions 20:267- 283

Pritchard JK, Stephens M, Rosenberg NA et al. (2000) Association mapping in structured populations. Am J Hum Genet 67:170-181

Quilodrán CS, Austerlitz F, Currat M et al. (2018) Cryptic biological invasions: a general model of hybridization. Sci Rep 8:1-12

Ranke PS, Skjelseth S, Hagen IJ et al. (2020) Multi-generational genetic consequences of reinforcement in a bird metapopulation. Conserv Genet, pp 1-10

Rius M, Darling JA (2014) How important is intraspecific genetic admixture to the success of colonising populations? Trends Ecol Evol, 29:233-242

Rouco C, Norbury GL, Smith J et al. (2013) Population density estimates of brushtail possums (*Trichosurus vulpecula*) in dry grassland in New Zealand. N Z J Ecol, pp 12-17 Rousset F (2008) genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol Ecol Resour 8:103-106

Sadleir RMFS (2000) Evidence of possums as predators of native animals. In: Montague TL (ed) The brushtail possum: biology, impact and management of an introduced marsupial. Manaaki Whenua Press: Lincoln, pp 126-131

Sarre SD, Aitken N, Adamack AT et al. (2014) Creating new evolutionary pathways through bioinvasion: the population genetics of brushtail possums in New Zealand. Mol Ecol 23:3419-3433

Simberloff D, Martin J-L, Genovesi P et al. (2013) Impacts of biological invasions: what's what and the way forward. Trends Ecol Evol 28:58-66

Slatkin M (1987) Gene flow and the geographic structure of natural populations. Science 236:787-792

Smith AL, Hodkinson TR, Villellas J et al. (2020) Global gene flow releases invasive plants from environmental constraints on genetic diversity. Proc Natl Acad Sci 117:4218-4227

Spatz DR, Zilliacus KM, Holmes ND et al. (2017) Globally threatened vertebrates on islands with invasive species. Sci Adv 3:e1603080

Taylor-Smith B, Morgan-Richards M, Trewick SA (2020) Patterns of regional endemism among New Zealand invertebrates. NZ J Zool 47:1-19

Taylor A, Cooper D (1998) Microsatellite markers for the Phalangerid marsupial, the common brushtail possum (*Trichosurus vulpecula*). Mol Ecol 7:1780-1782

Taylor A, Cowan P, Fricke B et al. (2004) High microsatellite diversity and differential structuring among populations of the introduced common brushtail possum, *Trichosurus vulpecula*, in New Zealand. Genet Res 83:101-111

Thomson G (1922) The naturalisation of plants and animals in New Zealand. Cambridge University

Tompkins DM (2018) The Research Strategy for a 'Predator Free'New Zealand. In Proc Vertebr Pest Conf 28:11-18

Trewick S, Bland K (2012) Fire and slice: palaeogeography for biogeography at New Zealand's North Island/South Island juncture. J R Soc N Z 42:153-183

Triggs S, Green W (1989) Geographic patterns of genetic variation in brushtail possums *Trichosurus vulpecula* and implications for pest control.N Z J Ecol, pp 1-10

Van Oosterhout C, Hutchinson WF, Wills DP et al. (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535-538

Vitousek PM, D'antonio CM, Loope LL et al. (1997) Introduced species: a significant component of human-caused global change. N Z J Ecol, pp 1-16

Wodzicki K, Wright S (1984) Introduced birds and mammals in New Zealand and their effect on the environment. TUATARA(WELLINGTON) 27:77-104

Woinarski JC, Burbidge AA, Harrison PL (2015) Ongoing unraveling of a continental fauna: decline and extinction of Australian mammals since European settlement. Proc Natl Acad Sci 112:4531-4540

Yamada F, Sugimura K (2004) Negative impact of an invasive small Indian mongoose Herpestes javanicus on native wildlife species and evaluation of a control project in Amami-Ohshima and Okinawa Islands, Japan. Glob Environ Res 8:117-124

Peakall, R., Ruibal, M., & Lindenmayer, D. B. (2003). Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat, Rattus fuscipes. *Evolution, 57*(5), 1182-1195.

SUPPLEMENTARY MATERIAL FOR CHAPTER TWO:

Unrestricted gene flow between two subspecies of translocated brushtail possums (*Trichosurus vulpecula*) in Aotearoa New Zealand

Supplementary table 2.1 Trait scoring schedule for fur colours of seven body sections in brushtail possum (*Trichosurus vulpecula*).

a) Character states

Body segment	Colour variation	Score
Belly	Pale	1
	Red	2
	Brown	3
Ear	Yellow	1
	Brown	2
	Black	3
Tail Base	Grey	1
	Brown	2
	Black Brown	2
	Black	3
Rump	Grey	1
	Black Brown	2
	Black	3
Flank	Grey	1
	Red-Grey	2
	Yellow-Grey	2
	Brown	3
	Red Brown	3
	Red	3
	Black Brown	4
Shoulder	Grey	1
	Red-Grey	2

	Red	3
	Brown	3
	Black Brown	3
	Black	4
	Sharp	1
Flank to Belly Transition	Fuzzy	2
	Continuous	3

b) Fur colour scores for 101 Kenepuru brushtail possums.

Sl.no	belly	ear	tail base	rump	flank	shoulder	flank belly transition	total
4	1	1	1	1	1	1	1	7
15	1	1	1	1	1	1	1	7
22	1	1	1	1	1	1	1	7
53	1	1	1	1	1	1	1	7
61	1	1	1	1	1	1	1	7
75	1	1	1	1	1	1	1	7
78	1	1	1	1	1	1	1	7
81	1	1	1	1	1	1	1	7
82	1	1	1	1	1	1	1	7
92	1	1	1	1	1	1	1	7
1	1	1	1	1	1	1	2	8
2	1	1	1	1	1	1	2	8
16	1	1	1	1	1	1	2	8
23	1	1	1	1	1	1	2	8
24	1	1	1	1	1	1	2	8
25	1	1	1	1	1	1	2	8
26	1	1	1	1	1	1	2	8
27	1	1	1	1	1	1	2	8
28	1	1	1	1	1	1	2	8
29	1	1	1	1	1	1	2	8
30	1	1	1	1	1	1	2	8
45	1	1	1	1	1	1	2	8
54	1	1	1	1	1	1	2	8

55	1	1	1	1	1	1	2	8
60	1	1	1	1	1	1	2	8
63	1	1	1	1	1	1	2	8
64	1	1	1	1	1	1	2	8
67	1	1	1	1	1	1	2	8
69	1	1	1	1	1	1	2	8
70	1	1	1	1	1	1	2	8
73	1	1	1	1	1	1	2	8
74	1	1	1	1	1	1	2	8
76	1	1	1	1	1	1	2	8
83	1	1	1	1	1	1	2	8
84	1	1	1	1	1	1	2	8
86	1	1	1	1	1	1	2	8
87	1	1	1	1	1	1	2	8
89	1	1	1	1	1	1	2	8
98	1	1	1	1	1	1	2	8
99	1	1	1	1	1	1	2	8
14	1	1	1	1	2	2	1	9
51	1	1	1	1	2	2	1	9
62	1	1	1	1	2	2	1	9
68	1	1	1	1	2	2	1	9
91	1	1	1	1	2	2	1	9
3	1	1	1	1	2	2	2	10
17	1	1	1	1	2	2	2	10
18	1	1	1	1	2	2	2	10
19	1	1	1	1	2	2	2	10

31	1	1	1	1	2	2	2	10
43	1	1	1	1	2	2	2	10
56	1	1	1	1	2	2	2	10
71	1	1	1	1	2	2	2	10
77	1	1	1	1	2	2	2	10
85	1	1	1	1	2	2	2	10
90	1	1	1	1	2	2	2	10
93	1	1	1	1	2	2	2	10
97	1	1	1	1	2	2	2	10
52	1	1	1	1	2	3	2	11
41	3	2	1	1	1	1	3	12
44	3	2	1	1	2	2	3	14
94	1	1	3	2	3	3	2	15
7	2	2	2	2	3	3	3	17
21	2	2	2	2	3	3	3	17
48	2	2	2	2	3	3	3	17
57	3	3	1	1	3	3	3	17
72	2	2	2	2	3	3	3	17
88	2	2	2	2	3	3	3	17
95	2	3	2	2	3	3	2	17
5	2	2	2	3	3	3	3	18
8	3	2	2	3	3	3	2	18
37	3	2	2	2	3	3	3	18
46	2	3	2	2	3	3	3	18
50	2	3	2	2	3	3	3	18
79	2	3	2	2	3	3	3	18

13	3	3	2	2	3	3	3	19
66	2	3	3	2	3	3	3	19
10	2	3	3	3	3	3	3	20
11	2	3	3	3	3	3	3	20
32	3	2	3	3	3	3	3	20
33	3	2	3	3	3	3	3	20
34	3	2	3	3	3	3	3	20
35	3	2	3	3	3	3	3	20
36	3	2	3	3	3	3	3	20
40	3	2	3	3	3	3	3	20
59	3	2	3	3	3	3	3	20
65	2	3	3	3	3	3	3	20
6	3	3	3	3	3	3	3	21
9	3	3	3	3	3	3	3	21
12	3	3	3	3	3	3	3	21
38	3	3	3	3	3	3	3	21
42	3	3	3	3	3	3	3	21
47	3	3	3	3	3	3	3	21
49	3	3	3	3	3	3	3	21
58	3	3	3	3	3	3	3	21
80	3	3	3	3	3	3	3	21
100	3	3	3	3	3	3	3	21
101	3	3	3	3	3	3	3	21
39	3	3	3	3	4	3	3	22
20	3	3	3	3	4	4	3	23
96	3	3	3	3	4	4	3	23

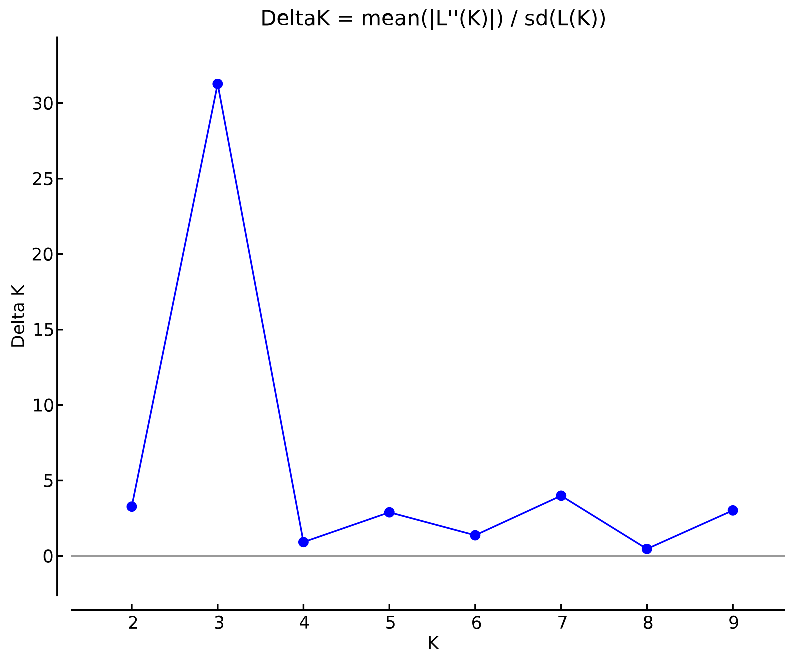
Supplementary table 2.2

Microsatellite primers developed for brushtail possums and used in this study. * (Adams 2013) ** (Taylor, Cooper 1998). Na: Number of alleles per locus.

Multiplex	Locus Name	Primer sequence	Na	Fluorophores
1	Tv_16**	F: GAGGCTACCATTAGACGCAA R: ACCCAAATGAACAGAAAGGC	5	FAM
1	Tv_19**	F: CCTCCTCCCCATCCTTCCTG R: GTTCAATTGCAGGGCTATGG	6	VIC
1	Tv_58**	F: GCACCCAAGGACCCCCAAGA R: CCATATCACAGTGCTTGGCG	11	VIC
1	Tv_53**	F: GGGAGTAGTTGTCTGAGTTCCC R: CCCTGGAGTTTGACAACCTG	13	FAM
2	Tv_64**	F: AGGGAGACTGAGTGCGTTTG R: AGACAGGAAAATTTGTGCCC	7	FAM
2	Tv_PnMS*	F: CCACCCCAATTAGATTAGCTC R: GGATGGTTTGTGACAATTTGC	2	FAM
2	Tv_M1*	F: GACCACAACCTGGGTCTAACCAAC R: CATGACACCTGGGCACTCAGGACT	6	TAMRA
2	Tv_14*	F: CACCTAACCATCATCCTCTC R: CTCACCCATCAGAACCTAGC	-	TAMRA

Supplementary figure 2.1

Delta K plot for 10 Structure runs using the Taylor (2004) dataset.



Supplementary table 2.3

Pairwise ϕ_{ST} of Kenepuru brushtail possums among a) spatial groups, b) temporal groups, c) fur colours. No significant deviation from 0 was observed. ϕ_{ST} values below diagonal and p-values (incl. Bonferroni corrections) above diagonal.

a)

	Mn	Kn	WB	Kt	PG
Mn	-----	0.135 (0.031)	0.065 (0.013)	0.865 (0.024)	0.234 (0.036)
Kn	0.243	-----	0.009 (0.009)	0.36 (0.054)	0.523 (0.035)
WB	0.135	0.018	-----	0.378 (0.053)	0.054 (0.02)
Kt	0.811	0.242	0.288	-----	0.189 (0.037)
PG	0.216	0.522	0.063	0.162	-----

b)

	2003	2016	2019
2003	-----	0.612 (0.041)	0.027 (0.012)
2016	0.034	-----	0.117 (0.023)
2019	0.084	-0.022	-----

c)

	Black	Grey	GM
Black	-----	0.018 (0.012)	0.396 (0.049)
Grey	0.066	-----	0.721 (0.033)
GM	-0.003	-0.046	-----

Supplementary table 2.4

Pairwise F_{ST} of Kenepuru brushtail possums among a) spatial groups, b) temporal groups, c) fur colour. No significant deviation from 0 was observed (all estimates of $F_{ST} < 0$).

a)

	Mn19	Mn16	Kn	WB	Kt	PG
Mn16	-0.018					
Kn	-0.017	-0.013				
WB	-0.033	-0.28	-0.028			
Kt	-0.044	-0.039	-0.038	-0.054		
PG	-0.044	-0.039	-0.038	-0.054	-0.066	
Hp	-0.056	-0.051	-0.051	-0.066	-0.078	-0.077

b

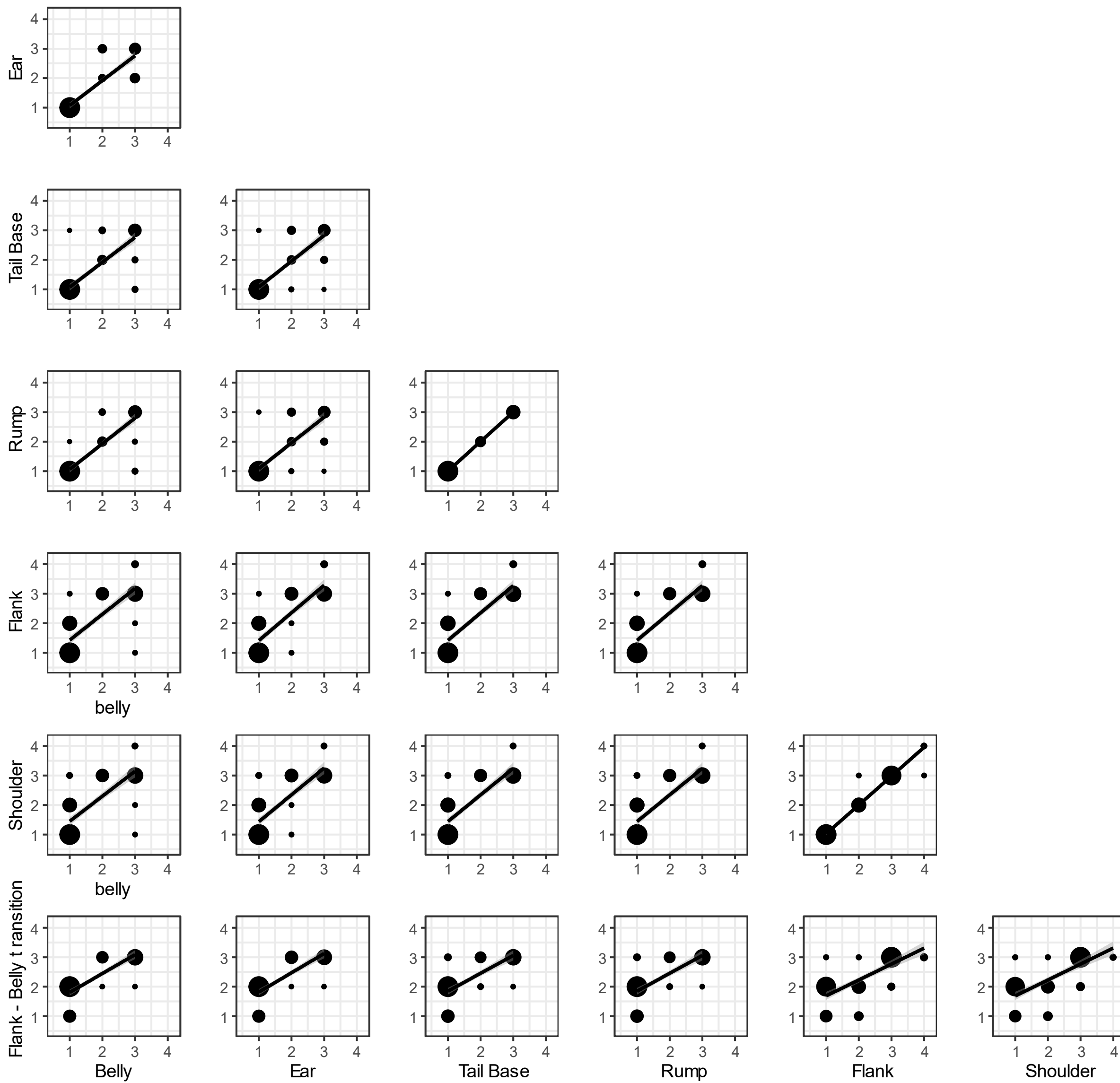
	2003	2016
2016	-0.013	
2019	-0.011	-0.012

c

	Bl/Br	Gr
Gr	-0.008	
GM	-0.023	-0.022

Supplementary figure 2.2

Correlation between fur colour of seven body sections of brushtail possums in New Zealand. Circle size proportional to the number of individual possums with that fur colour score. For example, in the graph comparing the belly fur and ear fur, 60 possums had yellow ears with pale bellies (score 1, 1) and 16 possums had black ears with brown bellies (score 3, 3). Smoothed conditional means are shown with a confidence interval of 0.95.



Supplementary table 2.5

Hardy-Weinberg analysis of Kenepuru brushtail possums genotype data. Significant deviations are indicated in bold.

a. Expected and observed heterozygosity (H_e and H_o) and expected and observed homozygosity (H_e and H_o) at 7 loci for the entire Kenepuru Peninsula population sample (n= 132).

	Tv_16	Tv_58	Tv_53	Tv_19	Tv_64	Tv_PnMS	Tv_M1
Kenepuru Peninsula							
He_e	90	93	96	90	103	56	101
He_o	85	86	86	90	99	57	98
Ho_e	39	37	31	40	25	72	30
Ho_o	44	44	41	40	29	71	33

b. Expected and observed heterozygosity (H_e and H_o) at 7 loci for each of 6 geographically separated populations.

	Tv_16	Tv_58	Tv_53	Tv_19	Tv_64	Tv_PnMS	Tv_M1
Manaroa (56)							
H_o	0.75	0.66	0.68	0.75	0.77	0.48	0.72
H_e	0.72	0.76	0.79	0.71	0.79	0.48	0.78
p	0.19	**0.0001**	0.18	0.046	0.77	0.98	0.038
Kenepuru (40)							
H_o	0.7	0.7	0.65	0.66	0.83	0.38	0.77
H_e	0.68	0.74	0.66	0.67	0.78	0.34	0.75
p	0.09	0.06	0.88	0.56	0.018	0.45	0.72
Waitaria Bay (12)							
H_o	0.3	0.75	0.58	0.58	0.7	0.4	0.75
H_e	0.63	0.76	0.71	0.61	0.67	0.42	0.74
p	**0.0001**	0.03	0.42	0.03	0.83	0.88	0.86
Koutuwai (8)							
H_o	0.5	0.5	0.88	0.62	0.88	0	0.75
H_e	0.63	0.48	0.61	0.65	0.72	0	0.63

p	0.067	0.55	0.15	0.91	0.87	Monomorphic	0.48
Port Gore (8)							
H_o	0.71	0.38	0.75	0.5	0.5	1	0.88
H_e	0.61	0.72	0.65	0.63	0.6	0.5	0.75
p	0.51	0.007	0.24	0.18	0.85	0.005	0.56
Hopai (6)							
H_o	0.5	1	0.67	0.83	0.83	0.5	0.67
H_e	0.65	0.72	0.52	0.69	0.79	0.38	0.71
p	0.51	0.87	0.96	0.32	0.73	0.41	0.17

c. Expected and observed heterozygosity (H_e and H_o) at 7 loci for 3 populations separated by <u>time</u>.

	Tv_16	Tv_58	Tv_53	Tv_19	Tv_64	Tv_PnMS	Tv_M1
2003 (40)							
H_o	0.7	0.7	0.65	0.68	0.83	0.38	0.77
H_e	0.68	0.74	0.66	0.67	0.78	0.34	0.75
p	0.092	0.057	0.88	0.56	0.018	0.44	0.72
2016 (36)							
H_o	0.67	0.64	0.64	0.78	0.78	0.39	0.78
H_e	0.69	0.65	0.74	0.68	0.75	0.44	0.76
p	0.77	0.91	0.70	0.062	0.87	0.45	0.36
2019 (56)							
H_o	0.62	0.67	0.72	0.65	0.73	0.59	0.71
H_e	0.69	0.81	0.68	0.68	0.73	0.46	0.77
p	0.63	0.0001	0.32	0.87	0.95	0.24	0.024

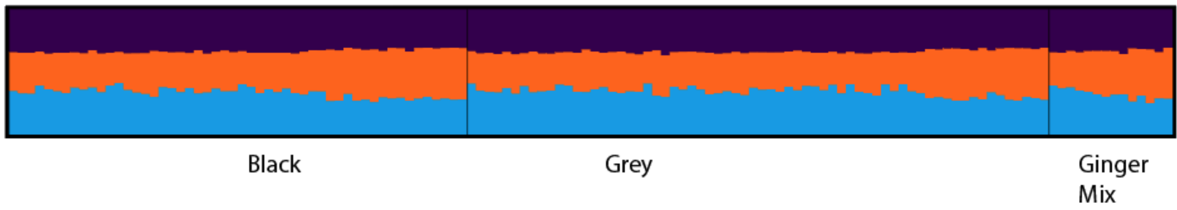
d. Expected and observed heterozygosity (H_e and H_o) at 7 loci for 3 populations separated by fur colour.

	Tv_16	Tv_58	Tv_53	Tv_19	Tv_64	Tv_PnMS	Tv_M1
Black (52)							
H_o	0.65	0.67	0.73	0.71	0.72	0.52	0.73
H_e	0.68	0.84	0.76	0.67	0.81	0.47	0.75
p	0.31	0.021	0.022	0.49	0.05	0.46	0.006
Grey (66)							
H_o	0.64	0.67	0.61	0.67	0.85	0.37	0.77
H_e	0.71	0.85	0.73	0.68	0.79	0.36	0.76
p	0.05	0.0001	0.11	0.41	0.12	0.75	0.81
Ginger-Mix (14)							
H_o	0.78	0.64	0.78	0.71	0.61	0.54	0.71
H_e	0.69	0.74	0.77	0.71	0.77	0.48	0.81
p	0.39	0.13	0.48	0.44	0.77	0.71	0.24

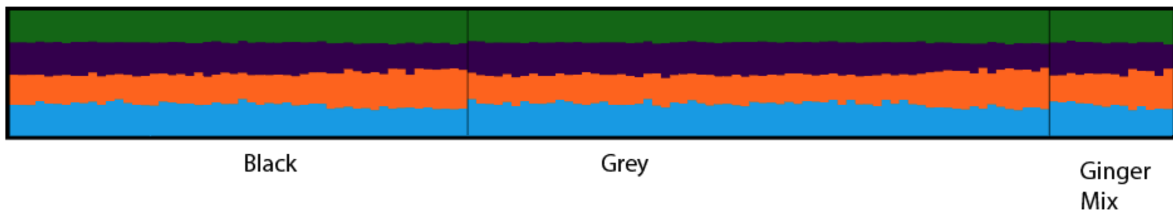
Supplementary figure 2.3

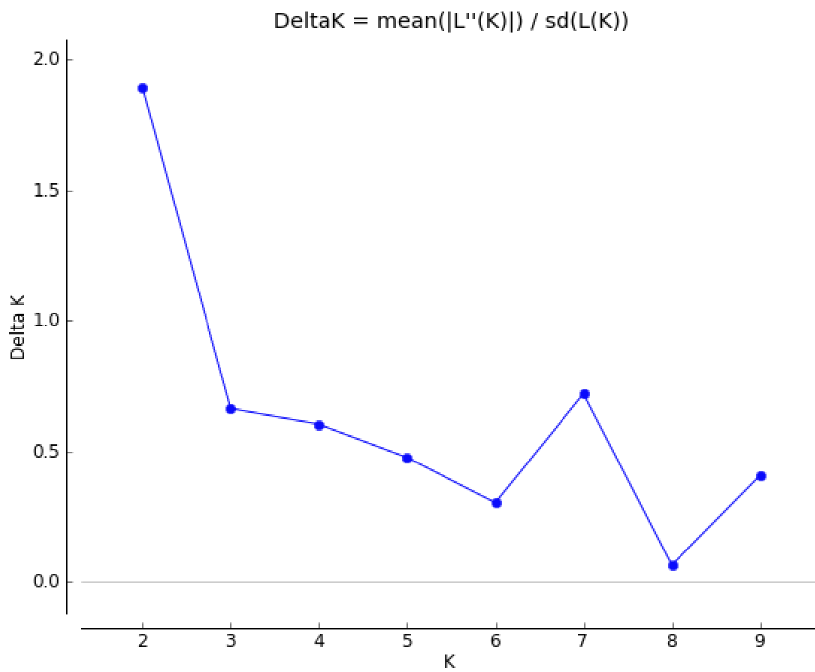
No evidence to support population structure based on fur colour of brushtail possums, under inferred clusters K= 3 and K=4 using genotypes for seven microsatellite loci (including Delta K plot by Evanno method).

K=3



K=4





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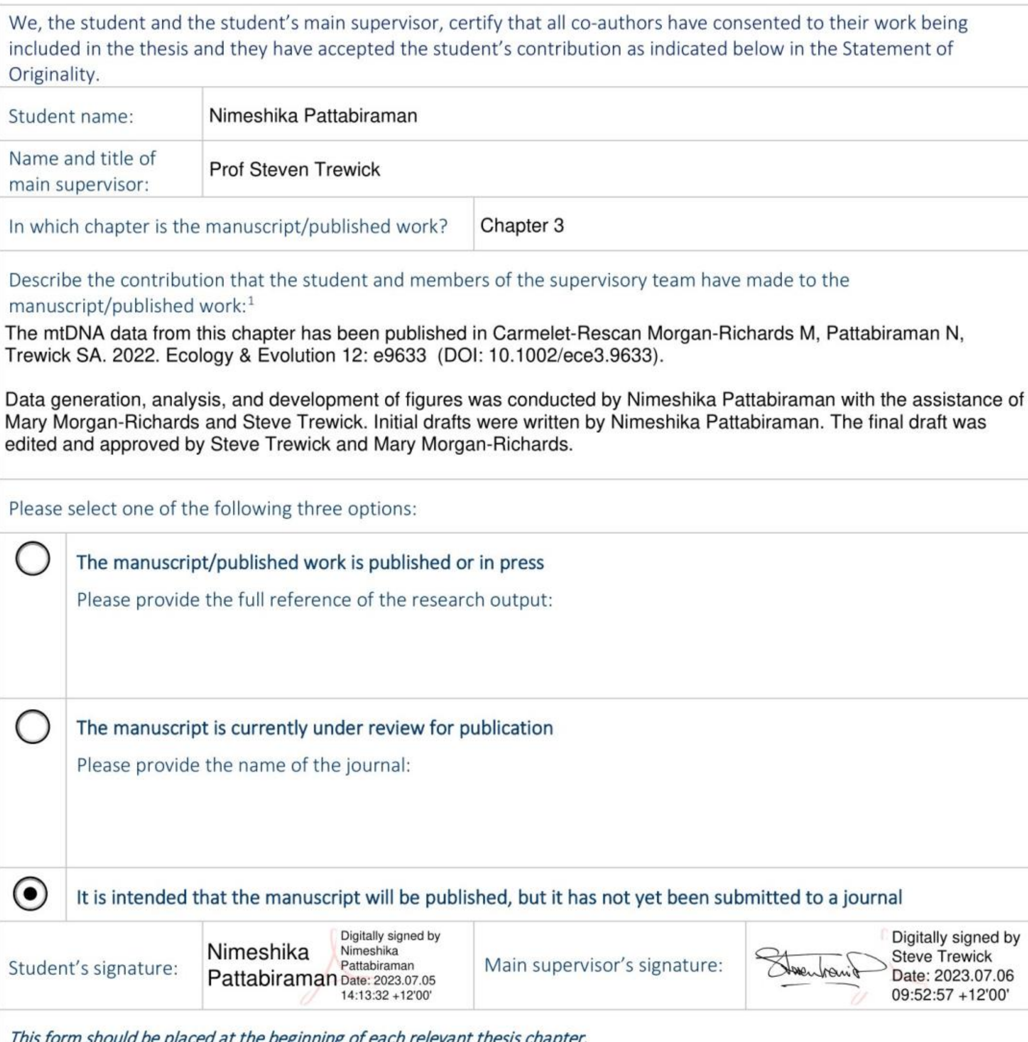

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The mtDNA data from this chapter has been published in Carmelet-Rescan Morgan-Richards M, Pattabiraman N, Trewick SA. 2022. Ecology & Evolution 12: e9633 (DOI: 10.1002/ece3.9633).

Data generation, analysis, and development of figures was conducted by Nimeshika Pattabiraman with the assistance of Mary Morgan-Richards and Steve Trewick. Initial drafts were written by Nimeshika Pattabiraman. The final draft was edited and approved by Steve Trewick and Mary Morgan-Richards.

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Chapter Three

Comparative population genetic structure of native and invasive brushtail possums (*Trichosurus vulpecula*) surveyed with mtDNA haplotype and nuclear microsatellite data

Abstract

The brushtail possum Trichosurus vulpecula is native to Australia where six subspecies are restricted to distinct regions. A large invasive population has established in Aotearoa New Zealand following deliberate introduction. I surveyed mitochondrial and nuclear markers to identify the scale of genetic introductions to New Zealand and the resulting pattern of admixture. mtDNA haplotypic variation shows Australian brushtail possums to be diverse with 17.3% D-loop sequence variation and prominent spatial structure. Some mitochondrial lineages are restricted in distribution, for example, haplotypes from western Australia were seen nowhere else. Haplotypes identified within a sample of 431 New Zealand possums comprise two main lineages that are closely related to haplotypes sampled from Victoria and Tasmania in Australia but only one New Zealand haplotype is identical to one sampled from Australia. Although haplotype diversity in New Zealand is a subset of that in the ancestral population, I found high haplotype diversity (0.71) consistent with multiple successful introductions and rapid population expansion. I found that mitochondrial and genotype diversity was structured within New Zealand. Nuclear microsatellite diversity could be summarised by four genotypic clusters (K=4), but these groups did not correlate with geography. Overall, there is signal of genetic structure within the invasive population but New Zealand possum diversity nests within the larger genetic diversity of Australian populations.

Keywords: *Trichosurus vulpecula*, species invasions, Population structure, mtDNA, Microsatellite

Introduction

Invasive species have negative impacts on people, wildlife, and ecosystems (Zavaleta et al. 2001; McCreless et al. 2016; Gurevitch et al. 2004), impact on the economy and agriculture (Meyerson et al. 2007; Levine & D'Antonio 2003). Management of environmental mammal pest is well developed in Aotearoa New Zealand (Brown & Sherley 2002; Veitch 2002; Clout & Russell 2006) and the resulting benefits for native fauna and flora are well demonstrated (O'Donnell & Hoare 2012; Byrom et al. 2016; Elliot & Kemp 2016), but also the limitations. Pest eradication has been achieved in relatively small, isolated landscapes, primarily offshore islands. Mainland management strategies aim to temporarily reduce local impacts of pests and management strategies have been honed to direct resources optimally (Speedy et al. 2007; Bell et al. 2019). Mainland fenced sanctuaries equipped with predator-proof fences that reduce pest invasion are a valuable tool but one that is applied to a small proportion of the landscape (Day & MacGibbon 2007). The recent proposal by the New Zealand government (Tompkins 2018) to eradicate four invasive mammal species from New Zealand by 2050 significantly raised the stakes for conservationists. Eradicating pests in the country involves the government spending ~ NZD 70 million/year for targeting while undergoing a loss of NZD 3.3 billion in productivity (Owens 2017). The challenges of exterminating all brushtail possums (Trichosurus vulpecula), stoats (Mustela erminea) and rats (Rattus norvegicus, Rattus rattus) cannot be underestimated and requires the full engagement of biologists and landowners (Owens 2017; Linklater & Steer 2018). The pest eradication objective requires novel approaches involving integrated monitoring and control and the most effective way to do this is with population genetics (Combs et al. 2019; Rollins et al. 2006; Burgess et al. 2021; Abdelkrim 2005; Piertney et al. 2015; Desvars-Larrive et al. 2018).

Brushtail possums (Trichosurus vulpecula) were first introduced to Aotearoa New Zealand in 1837, in a concerted effort by the New Zealand government, acclimatization societies and private individuals to establish a fur trade (Clout 2006). More than 100 separate introductions of possums to New Zealand were recorded between 1838 and 1926 from source populations in Victoria and Tasmania (Pracy 1962). Although primarily herbivores able to resist the effects of range of plant defence toxins in Australia (Triggs & Green 1989; McIlroy 1983), brushtail possums found the climate, food resources and absence of predators and competitors in Aotearoa amenable. With rapid population growth, possum numbers were estimated to be 48 million (without control) occupying native forest and exotic pastureland across the country (Warburton et al. 2009). The

negative impacts of the large brushtail possum population in New Zealand include browsing of native plants (Cowan 1989; Cowan 1990; Sweetapple et al. 2002), predation on native birds and their eggs (Brown et al. 1993; McLennan et al. 1996; Clout 2006), and on native invertebrates (Cowan & Moeed 1987; Payton 2000; Sadleir 2000). A significant economic impact arises from the potential for brushtail possums to carry and transmit the notifiable agricultural disease Bovine Tuberculosis (TB) among cattle (de Lisle 1993; Livingstone et al. 2015).

Brushtail possums are distributed across the Australian continent, where distinct subspecies (Kerle et al. 1991) occupy different habitat types from the central arid woodlands to the wet sclerophyll forests (Kerle 1984; Carmelet et al. 2022). Of the six subspecies of Trichosurus vulpecula recognised, it is likely at least two of these were introduced to Aotearoa New Zealand (Pracy 1962). The large number of Brushtail possum introduction events, followed by rapid population growth is likely to have resulted in retention of high allelic diversity, and the mixing of distinct populations could have generated numerous novel genotypes in New Zealand. Although two subspecies have distinctive fur colour traits that can still be seen in New Zealand today, it is now clear that where they occur, they freely interbreed, resulting in high genotypic diversity (Pattabiraman et al. 2021; Sarre et al. 2014).

Identifying the spatial distribution of genetic variants is a first step towards an integrated monitoring and eradication strategy (Taylor et al. 2004; Adams et al. 2014; Pattabiraman et al. 2021; Yarita et al. 2023). To do this, I used mitochondrial haplotype data to test how many distinct matrilines are established in New Zealand and infer their geographic and taxonomic origins in Australia. Inclusion of population samples from a broad range of Australian locations increased the chances of encountering populations genetically similar to the New Zealand possum population. I also examine how the resulting genetic diversity is distributed within New Zealand. After nearly 165 years of high genetic diversity but little geographic structuring due to population growth, bouts of possum culling could promote gene flow that enhances homogenization. Documenting current genetic structure is an important step to infer how past and current management efforts have altered their genetic makeup, which, in turn affects the efficiency of control measures.

Materials and Methods

Brushtail possum samples were collected from their native range in Australia (n= 84) and from non-native range in Aotearoa New Zealand (n=515; Figure 3.1; Table 3.1). Muscle/liver tissue samples of Australian possums were provided by the South Australian Museum, Adelaide. These included samples from Darwin (Northern Territory), Armidale (New South Wales), Kangaroo Island and Adelaide (South Australia), Launceston (Tasmania), Townsville and Brisbane (Queensland), and Sutton Grange and Bendigo (Victoria), and represent five subspecies (Kerle et al. 1991) (Table 3.1). Ear clip samples of New Zealand brushtail possums were collected by Government and private pest management practitioners in 2003, 2016 and 2019, at 16 locations across the country (Table 3.1, Figure 3.1).

DNA extraction using the GeneAid ™ Tissue DNA Isolation Kit (Genaid Biotech Ltd, Taiwan) following the manufacturer's instructions with a final elution volume of 200µl. The quality and quantity of the DNA extracts was assessed using Invitrogen Qubit 4 Fluorometer (ThermoFisher Scientific)

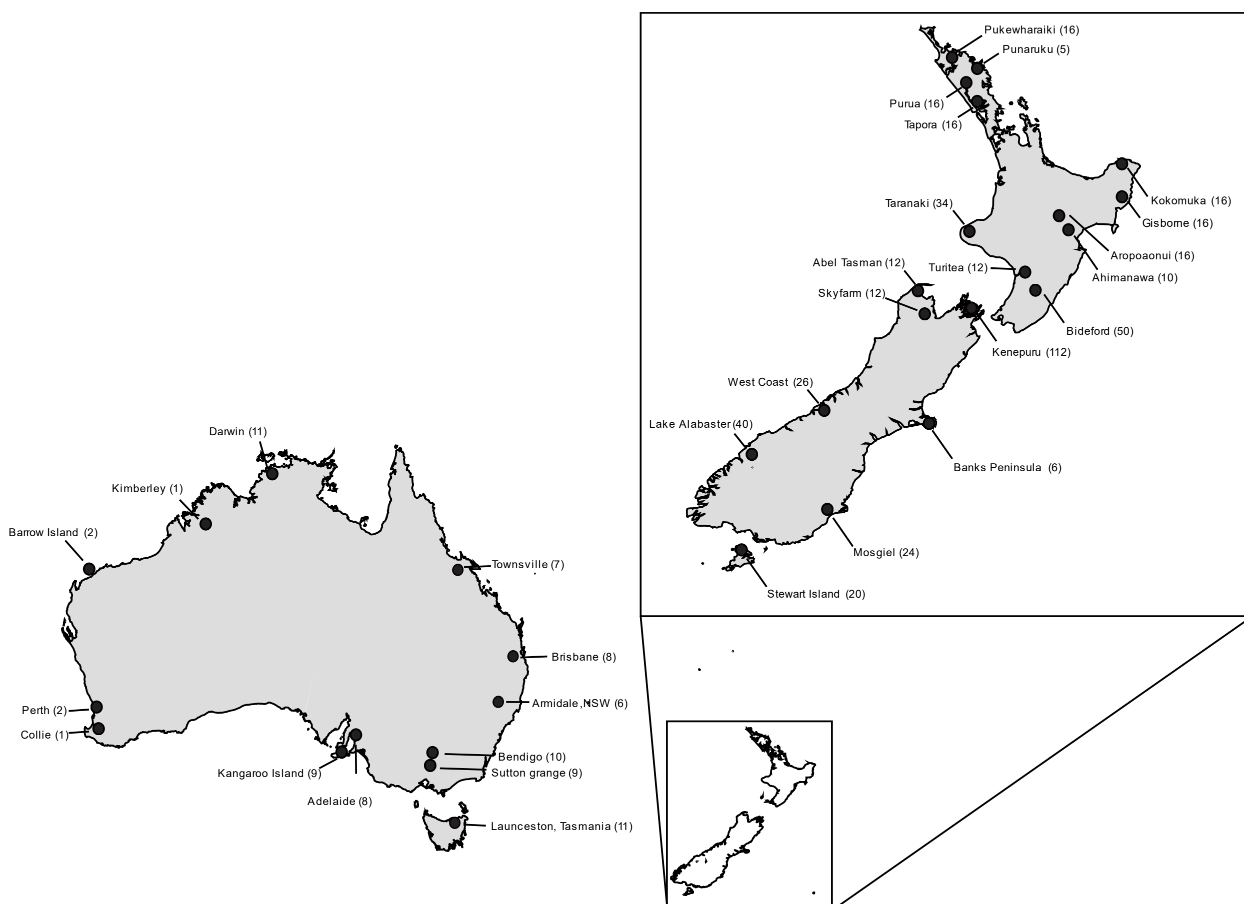


Figure 3.1 Locations and sample sizes (in brackets) of brushtail possums (*Trichosurus vulpecula*) from Aotearoa New Zealand and Australia.

Table 3.1 Locations in Aotearoa New Zealand and Australia from which population samples of brushtail possum (*Trichosurus vulpecula*) were collected.

Location name /Code		subspecies	Coordinates		Number of Individuals	
			Latitude	Longitude	mtDNA	Microsatellites
Punaruku	PUN		-35.37199	174.31295	5	-
Purua	PUR		-35.63253	174.10609	6	16
Pukewharaiki	PUK		-35.0604	173.4157	12	16
Tapora	TAP		-36.2116	174.1829	11	16
Taranaki	TAR		-39.16597	173.95983	17	34
Kokomuka	KOK		-37.68637	178.17109	13	16
Gisborne	GIS		-38.56918	178.22965	10	16
Ahimanawa	AHI		-39.0124	176.4725	10	-
Aropoaonui	ARO		-39.1547	176.5903	-	16
Turitea	TUR		-40.40853	175.65881	-	12
Bideford	BID		-40.907857	175.925866	32	50
Abel Tasman	ABE		-40.4725	172.5828	12	12
Kenepuru	KEN		-41.09844	173.87163	112	40
Skyfarm	SKY		-40.92777	172.8569	-	12
West Coast	WC		-43.2752	170.0108	23	26
Banks Peninsula	BP		-43.86492	172.77970	6	6
Mosgiel	MOS		-45.784045	170.433655	17	24
Lake Alabaster	L.Al		-44.5	168.19	37	40
Stewart Island	STE		-46.543	168.0532	19	20
Townsville	QLD	*johnsonii*	-19.2213	146.7620	8	-
Brisbane	QLD	*vulpecula*	-27.4691	152.8203	7	
Darwin	NT	*arnhemensis*	-12.3805	130.9868	12	-
Armidale	NSW	*vulpecula*	-30.4421	151.6081	6	-
Victoria	VIC	*vulpecula*	-36.9781	144.3563	19	-
Launceston	TAS	*fuliginosus*	-41.4333	147.1333	10	-
Kangaroo Island	SA	*vulpecula*	-35.6500	137.6333	9	-
Adelaide	SA	*vulpecula*	-34.8929	138.7736	8	
Western Australia	WA	*hypoleucus*	-31.9754	115.8526	5	-
Total					**426**	**372**

Mitochondrial haplotype diversity

Polymerase Chain Reaction (PCR) primers originally designed for the short-eared and brushtail possum (Trichosurus canninus and Trichosurus vulpecula respectively) from whole mtDNA (Carmelet-Rescan et al. 2021) were used to target 730 bp of partial mtDNA D-loop. Amplifications were consistently obtained from T. vulpecula using the primer combination Tcan_218f (AAGGCAACAACACCTCACCA) and Tvul_1023r (TCCCGCCCAGTTGATAAACC) (Supplementary figure 3.1a, b, and c; Supplementary table 3.1) in 20µl volumes with final concentrations of 1x DreamTaq buffer (ThermoFisher Scientific), 200µM each deoxy-nucleotide phosphates (dNTP), 2.5mM Magnesium Chloride (additional 0.4µl of 25mM $MgCl_2$), 0.05U DreamTaq DNA polymerase, and 0.25µM each primer. Thermocycling consisted of an initial denaturation step at 95°C for 90 seconds followed by 36 cycles of 94°C for 20 seconds, 51°C for 20 seconds, and 72°C for 1 minute; with a final 8-minute extension step of 72°C. Amplification products were sequenced using BigDye® chemistry (Perkin Elmer) following the manufacturer's protocols on an ABI3730 DNA analyser (Macrogen Inc). Sequences were edited and aligned using the software GENEIOUS 11.5 (Kearse et al. 2012). Total haplotype (h) and nucleotide (π) diversity estimation, and DNA polymorphism analysis were conducted using the software DNASP v 6.12.01. Pairwise population differentiation (ϕST) was tested with ARLEQUIN v3.5.2.2 (Excoffier & Lisher 2010), and the relationship between haplotypes was visualised using a neighbour-joining phylogeny for the full dataset and a median-joining network (Bandelt et al. 1999) inferred using POPART (Leigh & Bryant 2015) for just the New Zealand samples.

Nuclear microsatellite analyses

Two multiplexes of eight species-specific PCR primers (M1: Tv_16, Tv_58, Tv_53 and Tv_19 and M2: Tv_64, Tv_PnMs, and Tv_M1) (Supplementary table 3.2), previously used in brushtail possum studies (Taylor & Cooper 1998; Taylor et al. 2004; Blyton 2012; Duenas et al. 2014) for nuclear DNA diversity analysis (Supplementary table 3.3) (Pattabiraman et al. 2022). PCR reactions in 10 µl volumes comprised 0.05U Dream-Taq DNA polymerase, 0.1 µM each primer, 1x DreamTaq buffer (ThermoFisher Scientific) and 200 µm dNTPs. PCR conditions consisted of an initial denaturation step at 95°C for 4 min, followed by 40 cycles of 94°C for 30s, 60°C for 45s and 72°C for 45s, and a final extension of 72°C for 10 min.

Amplified PCR products were genotyped using a fragment analyser (Macrogen Inc). Allele lengths were determined using the microsat plugin in GENEIOUS 11.5 (Kearse et al. 2012). The presence of null alleles and long-allele dropout was examined using the software MICROCHECKER v2.2.3 (Van Oosterhout et al. 2004) (Supplementary figure 3.2a,b). I tested for linkage disequilibrium using pairwise tests with the software GENEPOP on the Web (Rousset 2008) (Supplementary table 3.4). Mean number of alleles (Na) at seven loci and pairwise population differences were estimated in ARLEQUIN v3.5.2.2 (Excoffier & Lisher 2010). Mean gene diversity (G) and mean allelic richness (Ar) were calculated using FSTAT v2.9.4 (Goudet 2003)

The Garza-Williamson index was used to seek evidence for population bottlenecks (Excoffier & Lischer 2010). This approach uses the ratio of number of alleles to allelic size range to calculate the likelihood that the population has been subject to a bottleneck, under the assumption that during a bottleneck a population is more likely to lose alleles than reduce in allelic range. An index value closer to zero indicates a greater likelihood that the population has gone through a bottleneck and one if the populations have not lost allelic diversity levels (Excoffier & Lischer 2010).

To assign individuals to genotypic clusters based on allele frequencies, I used a naïve Bayesian modelling approach with the software STRUCTURE v2.3.2 (Pritchard et al. 2000). STRUCTURE provides an optimal number of clusters (k) under a given model based on the assumption that populations are in Hardy-Weinberg equilibrium and the loci are in linkage equilibrium (Pritchard et al. 2000).

Bayesian Markov-Chain Monte Carlo (MCMC) clustering of the individual genotypes was run with a the Burnin period set to 15,000 and the MCMC repeats thereafter set to 150,000. The admixture model did not consider pre-defined populations as priors when assigning individuals to the genotypic clusters. Values were tested between 1 to 10 for 799 samples with seven loci, with 15 iterations for each K. The K-value best fitting the dataset was determined using the Evanno method implemented in STRUCTURE HARVESTER (Earl 2012). To identify cluster modes and visualise population structure inferences across K values, the software CLUMPAK (Kopelman et al. 2015) was used where the output can be visualised as a stacked bar plot.

Results

Intraspecific phylogenetic structure using mitochondrial DNA

mtDNA D-loop sequences were obtained from 431 individuals, resulting in a 572bp alignment. Among these I identified a total of 68 D-loop haplotypes of which 38 were unique to brushtail possums sampled in Australia and 29 to possums in New Zealand (Table 3.2). Only one haplotype (Hap 34) was encountered in samples from both countries.

Table 3.2 Haplotype diversity at the mtDNA D-loop locus in Aotearoa New Zealand and Australian brushtail possums. n: Number of individuals, Hap: Number of haplotypes in a population sample, h: Haplotype diversity, π: Nucleotide diversity, S: Number of segregating sites

Population	N	Hap	h	π	S
PUN	5	4	0.9	0.01818	18
PUR	6	3	0.8	0.00186	2
PUK	12	5	0.667	0.00623	12
TAP	11	6	0.855	0.00401	6
TAR	17	2	0.221	0.00039	1
KOK	13	3	0.615	0.00698	11
GIS	10	4	0.711	0.00611	8
AHI	10	2	0.2	0.00035	1
BID	32	3	0.325	0.00824	17
ABE	12	3	0.439	0.00111	3
KEN	112	5	0.528	0.01322	16
WC	23	4	0.767	0.01315	17
BP	6	2	0.533	0.00746	8
MOS	17	3	0.618	0.01411	21
L.AI	37	3	0.156	0.00028	2
STE	19	2	0.491	0.00466	5
QLD	15	9	0.905	0.02716	42
NT	12	5	0.848	0.01526	20
NSW	6	2	0.333	0.01282	22
VIC	19	6	0.836	0.01979	32
TAS	10	6	0.867	0.00937	14
SA	17	7	0.794	0.01661	28
WA	5	4	0.9	0.02922	32
TOTAL NZ	**346**	**31**	**0.717**	**0.012**	**41**
TOTAL AUS	**84**	**39**	**0.973**	**0.035**	**99**

Australia

The total haplotype diversity (0.973) and nucleotide site diversity (0.035) sampled from Australia was higher than observed across the much larger collection of New Zealand samples. The population samples from Northern Territory (T. v. arnhemensis) and Western Australia (T. v. hypoleucus) had distinct mtDNA haplotypes that formed separate clades in the phylogenetic analysis (Figure 3.2). The other brushtail possum population samples from southern and eastern Australia did not form haplotype clades that corresponded to the three subspecies. Both T. v. johnstonii and T. v. fuliginosus nested within the genetic diversity of my T. v. vulpecula samples. In a similar way, the haplotypes from New Zealand samples were nested within the diversity of T. v. vulpecula from New South Wales, South Australia, Brisbane, Victoria, and Tasmania.

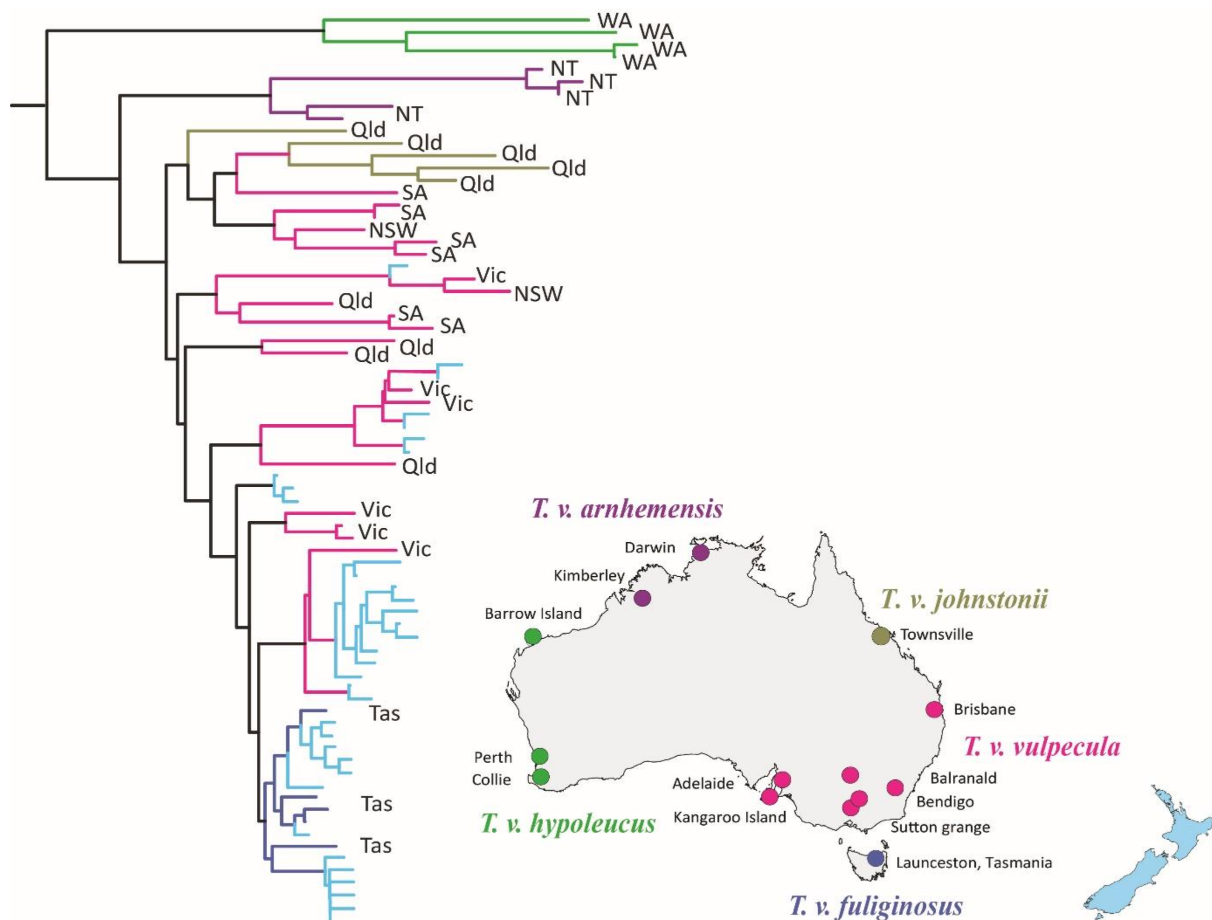


Figure 3.2 Genetic diversity of *Trichosurus vulpecula* possums collected from Aotearoa New Zealand nested within the diversity from its natural range in Australia. Neighbour-joining phylogeny of mtDNA D-loop (572bp) haplotypes from Australia (n=84) and Aotearoa New Zealand (n=346). Colours correspond to regional and subspecies sampling.

New Zealand

A total of 342 samples were screened from 16 locations across New Zealand. Among these were 29 unique D-Loop haplotypes and one shared between West Coast and Tasmania (Figure 3.3). Overall haplotype diversity (h) was 0.717 with the presence of two major common haplotypes, Hap2 (n=166) and Hap44 (n=77), and total nucleotide diversity (π) was 0.012 (Table 3.2). Highest haplotype diversity is seen in Northland population samples of Punaruku (h = 0.9, p=0.018, n = 5) Purua (h=0.8, p =0.001, n = 6) and Tapora (h=0.85, p =0.004, n = 11) and lowest diversity is seen in Fiordland (h=0.15, p = 0.0002, n=37) and Ahimanawa (h=0.2, p =0.0003, n = 10).

Most population samples were genetically distinct from one another, as the majority of pairwise FST were significantly greater than zero implying limited gene flow among the New Zealand populations sampled (Table 3.3). However, 11 pairwise FST did not deviate significantly from zero, suggesting population were not genetically differentiated. For example, the large South Island sample from Lake Alabaster, Fiordland (n = 37) did not differ significantly in terms of mtDNA haplotype composition from the North Island Taranaki (n = 17) and Ahimanawa (n = 10) in samples (380 km apart: Tables 3.2 & 3.3). Likewise, one population sample from Northland, Purua (n = 6) was similar to the Abel Tasman sample (n = 12) in the South Island. In contrast, some geographically adjacent population samples were differentiated, such as pairwise FST between Ahimanawa (n = 10) and Kokomuka (n = 13) of 0.37 despite being separated by just ~280 km.

Table 3.3 Pairwise genetic differences among Aotearoa New Zealand brushtail possum samples (n=346). Above diagonal are F_{ST} estimates from seven microsatellite loci, and below diagonal ϕ_{ST} from D-loop sequences. Values that do not differ significantly from zero are shaded in grey.

	PUN	PUR	PUK	TAP	TAR	KOK	GIS	AHI	ARO	TUR	BID	ABE	KEN	SKY	WC	BP	MOS	L.Al	STE
PUN	-																		
PUR	0.602	-	0.175	0.251	0.171	0.17	0.149		0.066	0.154	0.177	0.192	0.213	0.206	0.146	0.192	0.157	-0.139	0.238
PUK	0.48	0.516	-	0.251	0.147	0.166	0.148		0.108	0.06	0.075	0.154	0.159	0.138	0.108	0.199	0.049	-0.076	0.212
TAP	0.496	0.755	0.314	-	0.245	0.233	0.225		0.169	0.118	0.137	0.216	0.232	0.186	0.177	0.262	0.117	-0.012	0.294
TAR	0.783	0.939	0.769	0.891	-	0.169	0.1		0.138	0.121	0.1	0.141	0.111	0.188	0.114	0.166	0.076	-0.275	0.197
KOK	0.459	0.414	0.351	0.578	0.465	-	0.022		0.142	0.087	0.125	0.119	0.169	0.112	0.075	0.195	0.132	-0.113	0.143
GIS	0.475	0.455	0.386	0.627	0.477	-0.08	-		0.121	0.065	0.108	0.087	0.117	0.117	0.034	0.152	0.076	-0.019	0.109
AHI	0.702	0.927	0.716	0.862	0.031	0.37	0.378	-											
ARO									-	0.069	0.105	0.126	0.163	0.108	0.093	0.171	0.065	-0.165	0.196
TUR										-	0.056	0.055	0.097	0.132	-0.014	0.113	-0.023	-0.084	0.077
BID	0.516	0.678	0.619	0.657	0.757	0.581	0.601	0.724			-	0.081	0.097	0.125	0.083	0.157	0.029	-0.061	0.168
ABE	0.726	0.127	0.619	0.819	0.946	0.536	0.586	0.939			0.725	-	0.176	0.085	0.076	0.204	0.102	-0.094	0.156
KEN	0.323	0.395	0.343	0.435	0.305	0.21	0.213	0.276			0.199	0.442	-	0.148	0.131	0.174	0.075	-0.148	0.187
SKY														-	0.049	0.193	0.041	0.082	0.156
WC	0.309	0.159	0.297	0.445	0.384	0.117	0.109	0.322			0.423	0.251	0.151		-	0.157	0.086	-0.217	0.066
BP	0.366	0.68	0.452	0.647	0.394	0.196	0.195	0.27			0.645	0.776	0.226		0.233	-	0.031	-0.097	0.214
MOS	0.095	0.47	0.31	0.35	0.496	0.277	0.295	0.419			0.543	0.563	0.282		0.269	0.149	-	-0.438	0.141
l..Al	0.875	0.96	0.848	0.931	0.052	0.598	0.62	0.017			0.815	0.962	0.343		0.494	0.556	0.618	-	-0.04
STE	0.566	0.545	0.36	0.638	0.308	0.158	0.129	0.248			0.655	0.62	0.241		0.219	0.113	0.328	0.419	-

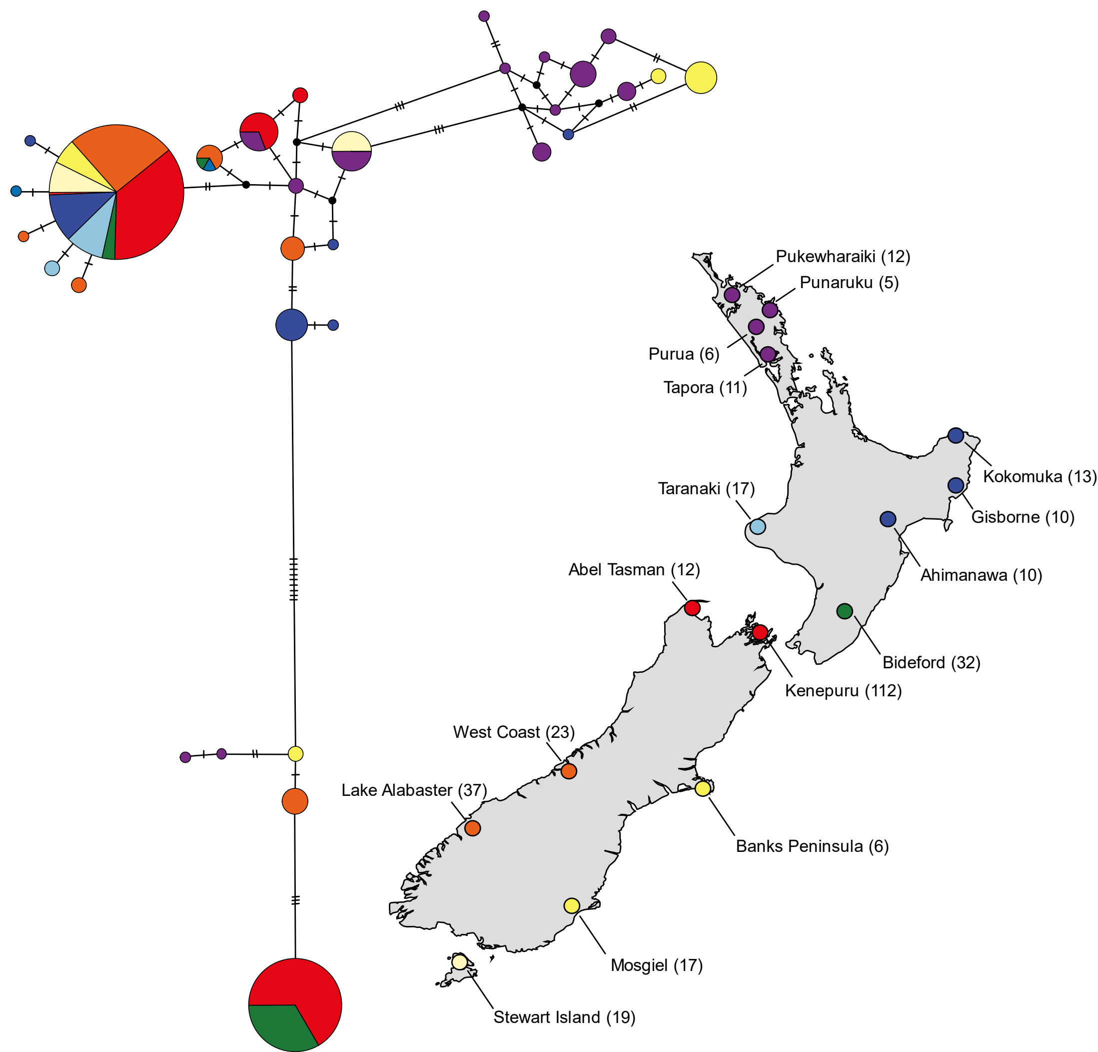


Figure 3.3 Mitochondrial D-loop haplotype (572bp) diversity among 431 brushtail possums sampled at 16 New Zealand locations. Nodes on the median-joining network are proportional to sample sizes. Colours indicate regional population sampling.

Population structure using nuclear microsatellite markers

Initially 515 samples were genotyped, but instances of missing data and homozygote-excess led to exclusion of one population sample from Lake Alabaster (n=40) and two from the Kenepuru region (n= 96) to meet Hardy Weinberg expectations. The remaining 379 individuals screened with seven microsatellite markers in downstream analyses. In this sample, each locus contained between 2 and 10 alleles, with the highest average number of alleles per locus observed in the Bideford population sample (9.57 ± 2.76; n = 50). High allelic diversity was also indicated by the heterozygosity values (Table 3.4), which ranged from 0.55 (n = 19) in the Stewart Island population sample to 0.83 (n= 16) in the Pukewharaiki population sample (Table 3.4). Observed heterozygosities were as expected or lower than expected in 13 of 17 population samples. Garza-

Williamson range values were close to 0 as the number of alleles reduced more than the allelic range, suggesting a recent bottleneck event (Table 3.4) (Excoffier & Lischer 2010).

Table 3.4 Genetic diversity at seven microsatellite loci among 18 New Zealand population samples of brushtail possums. n: Number of individuals, A: Average number of alleles in the population sample, H_o: Mean observed heterozygosity, H_e: Mean expected heterozygosity, G-W Stat: Garza Williamson index.

Location	n	A	H_o	H_e	G-W Stat
PUR	16	4.85 ± 1.77	0.56 ± 0.17	0.58 ± 0.17	0.38 ± 0.15
PUK	16	7.57 ± 1.13	0.83 ± 0.11	0.82 ± 0.03	0.29 ± 0.09
TAP	17	6.28 ± 1.38	0.75 ± 0.78	0.75 ± 0.05	0.33 ± 0.12
TAR	34	5.57 ± 1.72	0.67 ± 0.18	0.65 ± 0.17	0.28 ± 0.18
KOK	16	5.42 ± 1.9	0.66 ± 0.15	0.65 ± 0.17	0.27 ± 0.12
GIS	16	4.74 ± 1.79	0.69 ± 0.18	0.66 ± 0.15	0.28 ± 0.11
ARO	16	6.14 ± 2.6	0.75 ± 0.06	0.73 ± 0.12	0.28 ± 0.06
TUR	12	5.85 ± 2.67	0.68 ± 0.19	0.73 ± 0.11	0.20 ± 0.05
BID	50	9.57 ± 2.76	0.72 ± 0.07	0.84 ± 0.04	0.26 ± 0.05
ABE	17	5.28 ± 0.95	0.59 ± 0.21	0.69 ± 0.12	0.34 ± 0.16
KP	40	6.57 ± 2.22	0.695 ± 0.14	0.696 ± 0.11	0.24 ± 0.06
SKY	10	3.85 ± 2.61	0.57 ± 0.31	0.58 ± 0.21	0.27 ± 0.11
WC	26	7.28 ± 3.04	0.63 ± 0.15	0.71 ± 0.17	0.29 ± 0.08
BP	6	3.85 ± 1.25	0.55 ± 0.23	0.59 ± 0.25	0.19 ±0.07
MOS	24	6.85 ± 3.48	0.68 ± 0.13	0.73 ± 0.11	0.32 ±0.09
L.Al	40	4.43 ± 2.64	0.693 ± 0.18	0.698 ± 0.16	0.40 ± 0.18
STE	21	4.42 ± 1.27	0.55 ± 0.28	0.57 ± 0.25	0.21 ± 0.09
TOTAL	**379**	**5.6 ± 1.66**		**0.66 ± 0.08**	**0.28 ± 0.05**

Naïve Bayesian genotype assignment

From 15 iterations of genotype assignment for 379 individuals across New Zealand using seven microsatellite markers, the optimal number of clusters, K was 4 according to the Evanno method (Supplementary figure 3.3). In this Bayesian method of clustering, the K value is estimating the most likely number population clusters within the data (Verity & Nichols 2016), rather than precisely representing biological clusters among these possum populations samples, and a similar population structure was apparent for K = 3, 4 and 5 (Supplementary figure 3.4). In the optimal model (K = 4) the genotypic clusters did not, in general, reflect geographical proximity (Figure 3.4). Two of the three Northland samples had individuals with high assignment probability to their own distinct cluster. Likewise, the population sample from Lake Alabaster had all individuals with

high assignment probability to a unique cluster (admixture coefficient of 0.0001, 0.03 and 0.08 for three minor clusters). Three non-adjacent populations (Turitea (TUR), Bideford (BID) in North Island, and Skyfarm (SKY) in South Island) were dominated by individuals with high assignment probability to one genetic cluster. However, many possum samples had high assignment probability to more than one cluster so that most of the four genotypic clusters was represented to some degree in most population samples (Figure 3.4). The population samples from Taranaki and Mosgiel show the most admixture.

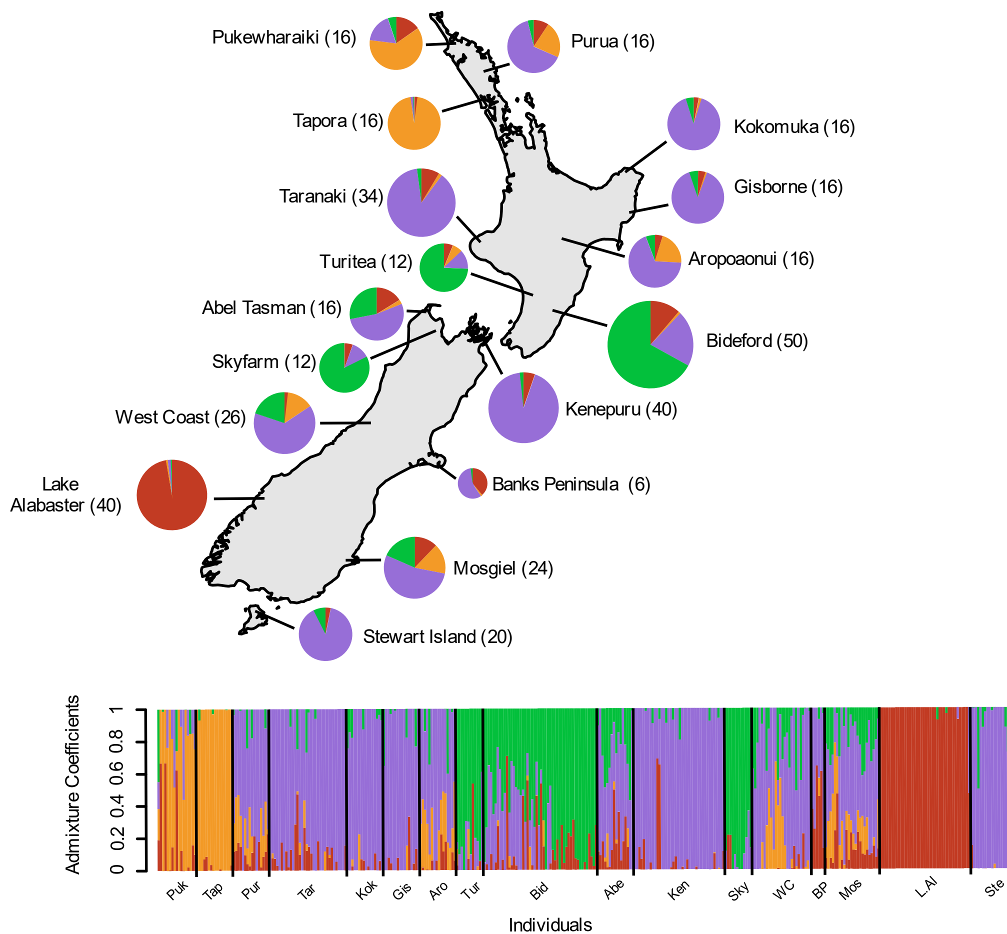


Figure 3.4 Genetic structure in brushtail possum (*Trichosurus vulpecula*) population samples across Aotearoa New Zealand (n=379). Assignment probability of each individual for K=4 genotypic clusters inferred from seven microsatellite loci as shown by the barplot. Summed assignment probabilities for each of four clusters are represented as pie plots where their area is proportional to sample size.

Discussion

I expected the founding populations of possums in Aotearoa New Zealand would have only a subset of genetic diversity that existed in Australia and this is what the mitochondrial sequence data has confirmed. The haplotypes detected in New Zealand are consistent with the known history of multiple translocations from southern Australia and Tasmania. The phylogenetic analysis shows that New Zealand samples are most closely related to possums from Victoria and Tasmania, concurring with the historical records at the time of introduction. I have no evidence that source populations were from anywhere other than southeast Australia and Tasmania. Within New Zealand the largest haplotype differences are between two distinct Victorian mtDNA lineages.

The pattern of release and translocations of possums within New Zealand would be expected to aid intermixing with the potential for producing a homogenized single invasive population. Compared to Australia, the mtDNA distribution shows little structure and no correlation to geography (Supplementary figure 3.5a), e.g. some New Zealand populations a long way apart have identical haplotype composition. However, pairwise genetic distances as estimated by F_{ST} revealed many populations in New Zealand retain mtDNA differences. Relative haplotype and nucleotide diversity for New Zealand population samples is generally lower than that of Australian population samples, as expected of a bottleneck-like effect from either or both translocation history and control measures since the mid-1900s.

The nuclear microsatellite diversity results also show that there is genotypic population structure within the New Zealand possums. Reanalysis of genotypes from five microsatellite loci (possums collected 1989-1997) revealed three genetic clusters across New Zealand concordant with geography (Chapter Two). In contrast, with an increase in number of loci, this study resolved four genotypic clusters but with little geographic concordance (Supplementary figure 3.5b). The difference between the two studies could be the result of 20 more generations of interbreeding and mixing or a difference in sampling locations. Comparing between nuclear microsatellite diversity and mitochondrial diversity resulted in a slight (but not statistically significant) positive correlation (Supplementary figure 3.6). The fact that both datasets identify genetic structure with a small number of loci supports the idea that the New Zealand possum population is not homogenous despite intermixing for the last 165 years.

Comparison between the distribution of haplotypes versus the genotypic clusters can be explained by the male-biased nature of dispersal in possums (Clout et al. 1984; Stow et al. 2006).

The current population structure of brushtail possums in Aotearoa New Zealand results from a combination of their introductions in the 19th century and the subsequent spread and mixing. The extent of admixture among regional populations is not constrained by limitations in gene flow among ancestral lineages based on the findings in my study (Pattabiraman et al. 2021), which can vary within regional population interactions (Sarre et al. 2014). Therefore it must be modulated by population densities that must fluctuate in time and space given the intensity of control measures applied in Aotearoa (Goldson et al. 2015). While properties of the landscape, isolation by distance, historical introduction and contemporary population fluctuations are expected to influence patterns of gene flow (Campbell et al. 2021; With 2019), pest management may also be influential. Indeed, rapid changes in local population density may stimulate migration rates (Smith et al. 2016). The population structure reported here is a result of allele frequencies with seven microsatellite markers. For a more detailed look at population clustering, using a larger dataset (eg. SNPs) would allow us to discern distinct genotypes/ population clusters. Creating a foundational study on a large scale for the genetic structure of brushtail possums in New Zealand can inform us of the current state of possum distribution which could help us develop strategies to control their populations effectively.

Conclusion- Genetically distinct populations translocated to Aotearoa New Zealand have not fully mixed. Despite fine scale mixing (Pattabiraman et al. 2021), the original source and translocation history has left a strong signal on the current genetic structure (Sarre et al. 2014). Mitochondrial diversity shows presence of multiple matrilines from different source backgrounds. Multiple distinct genotypes that are spatially contrasted indicate that regional releases could have resulted in geographic structure among genotypes from local intermixing. However, regional structure could be the consequence of initial introductions. Sampling from different time points with high-resolution markers will help identify the mechanisms that maintain strong genetic structure of possums in Aotearoa New Zealand.

References

Abdelkrim, J., Pascal, M., Calmet, C., & Samadi, S. (2005). Importance of assessing population genetic structure before eradication of invasive species: examples from insular Norway rat populations. *Conservation Biology, 19*(5), 1509-1518.

Adams, A., Recio, M., Robertson, B., Dickinson, K., & Van Heezik, Y. (2014). Understanding home range behaviour and resource selection of invasive common brushtail possums (*Trichosurus vulpecula*) in urban environments. *Biological Invasions, 16*, 1791-1804.

Bandelt, H.-J., Forster, P., & Röhl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular biology and evolution, 16*(1), 37-48.

Bell, P., Nathan, H., & Mulgan, N. (2019). Island eradication within large landscapes: the remove and protect model. *Island invasives: scaling up to meet the challenge, 62*, 604.

Blyton, M. D., Banks, S. C., Peakall, R., & Lindenmayer, D. B. (2012). Using probability modelling and genetic parentage assignment to test the role of local mate availability in mating system variation. *Molecular ecology, 21*(3), 572-586.

Brown, K., Moller, H., & Innes, J. (1996). Sign left by brushtail possums after feeding on bird eggs and chicks. *New Zealand Journal of Ecology*, 277-284.

Brown, K., & Sherley, G. (2002). The eradication of possums from Kapiti Island, New Zealand. *Turning the tide: the eradication of invasive species*, 46-52.

Burgess, B. T., Irvine, R. L., Howald, G. R., & Russello, M. A. (2021). The promise of genetics and genomics for improving invasive mammal management on islands. *Frontiers in Ecology and Evolution, 9*, 704809.

Byrom, A. E., Innes, J., & Binny, R. N. (2016). A review of biodiversity outcomes from possum-focused pest control in New Zealand. *Wildlife research, 43*(3), 228-253.

Carmelet-Rescan, D., Morgan-Richards, M., Pattabiraman, N., & Trewick, S. A. (2022). Time-calibrated phylogeny and ecological niche models indicate Pliocene aridification drove intraspecific diversification of brushtail possums in Australia. *Ecology and Evolution, 12*(12), e9633.

Clout, M. (2006). 17 Keystone Aliens? The Multiple Impacts of Brushtail Possums. *Biological Invasions in New Zealand, 186*, 265.

Clout, M., & Efford, M. (1984). Sex differences in the dispersal and settlement of brushtail possums (*Trichosurus vulpecula*). *The Journal of Animal Ecology*, 737-749.

Clout, M., & Russell, J. (2006). The eradication of mammals from New Zealand islands. *Assessment and control of biological invasion risks*, 127-141.

Combs, M., Byers, K., Himsworth, C., & Munshi-South, J. (2019). Harnessing population genetics for pest management: *Theory and application for urban rats. Human–Wildlife Interactions, 13*(2), 11.

Cowan, P. (1990). Fruits, seeds, and flowers in the diet of brushtail possums, *Trichosurus vulpecula*, in lowland podocarp/mixed hardwood forest, Orongorongo Valley, New Zealand. *New Zealand journal of zoology, 17*(4), 549-566.

Cowan, P., & Moeed, A. (1987). Invertebrates in the diet of brushtail possums, *Trichosurus vulpecula*, in lowland podocarp/broadleaf forest, Orongorongo Valley, Wellington, New Zealand. *New Zealand journal of zoology, 14*(2), 163-177.

Day, T., & MacGibbon, R. (2007). Multiple-species exclusion fencing and technology for mainland sites. *Managing vertebrate invasive species*, 8.

de Lisle, G. (1993). Bovine tuberculosis-the New Zealand problem. Paper presented at the Proceedings of the New Zealand Grassland Association.

Desvars-Larrive, A., Baldi, M., Walter, T., Zink, R., & Walzer, C. (2018). Brown rats (Rattus norvegicus) in urban ecosystems: are the constraints related to fieldwork a limit to their study? *Urban Ecosystems, 21*, 951-964.

Dueñas, J. F., Cruickshank, R., & Ross, J. (2015). Optimisation of a microsatellite panel for the individual identification of brushtail possums using low template DNA. *New Zealand Journal of Ecology, 39*(1), 93-102.

Earl, D. A., & VonHoldt, B. M. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation genetics resources, 4*, 359-361.

Elliott, G., & Kemp, J. (2016). Large-scale pest control in New Zealand beech forests. *Ecological Management & Restoration, 17*(3), 200-209.

Excoffier, L., & Lischer, H. E. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular ecology resources, 10*(3), 564-567.

Goudet, J. (2003). Fstat (ver. 2.9. 4), a program to estimate and test population genetics parameters. Updated from Goudet

Gurevitch, J., & Padilla, D. K. (2004). Are invasive species a major cause of extinctions? *Trends in ecology & evolution, 19*(9), 470-474.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., . . . Duran, C. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics, 28*(12), 1647-1649.

Kerle, J. (1984). Variation in the ecology of *Trichosurus*: its adaptive significance. *Possums and gliders*, 115-128.

Kerle, J., McKay, G., & Sharman, G. (1991). A systematic analysis of the brushtail possum, *Trichosurus-vulpecula* (Kerr, 1792)(Marsupialia, Phalangeridae). *Australian Journal of Zoology, 39*(3), 313-331.

Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular ecology resources, 15*(5), 1179-1191.

Leigh, J. W., & Bryant, D. (2015). POPART: full-feature software for haplotype network construction. *Methods in Ecology and Evolution, 6*(9), 1110-1116.

Levine, J. M., & D'Antonio, C. M. (2003). Forecasting biological invasions with increasing international trade. *Conservation Biology, 17*(1), 322-326.

Linklater, W., & Steer, J. (2018). Predator Free 2050: A flawed conservation policy displaces higher priorities and better, evidence-based alternatives. *Conservation Letters, 11*(6), e12593.

Livingstone, P., Hancox, N., Nugent, G., & De Lisle, G. (2015). Toward eradication: the effect of Mycobacterium bovis infection in wildlife on the evolution and future direction of bovine tuberculosis management in New Zealand. *New Zealand Veterinary Journal, 63*(sup1), 4-18.

McCreless, E. E., Huff, D. D., Croll, D. A., Tershy, B. R., Spatz, D. R., Holmes, N. D., . . . Wilcox, C. (2016). Past and estimated future impact of invasive alien mammals on insular threatened vertebrate populations. *Nature Communications, 7*(1), 12488.

McIlroy, J. (1982). The sensitivity of Australian animals to 1080 poison. III. Marsupial and eutherian herbivores. *Wildlife Research, 9*(3), 487-503.

McLennan, J., Potter, M., Robertson, H., Wake, G., Colbourne, R., Dew, L., . . . Miller, P. (1996). Role of predation in the decline of kiwi, Apteryx spp., in New Zealand. *New Zealand Journal Of Ecology*, 27-35.

O'Donnell, C. F., & Hoare, J. M. (2012). Quantifying the benefits of long-term integrated pest control for forest bird populations in a New Zealand temperate rainforest. *New Zealand Journal Of Ecology*, 131-140.

Owens, B. (2017). Behind New Zealand's wild plan to purge all pests. *Nature,* 541(7636)

Pattabiraman, N., Morgan-Richards, M., Powlesland, R., & Trewick, S. A. (2022). Unrestricted gene flow between two subspecies of translocated brushtail possums (*Trichosurus vulpecula*) in Aotearoa New Zealand. *Biological Invasions, 24*(1), 247-260.

Payton, I. (2000). Damage to native forests. The brushtail possum: biology, impact and management of an introduced marsupial. *Manaaki Whenua Press, Lincoln, New Zealand*, 111-125.

Piertney, S. B., Black, A., Watt, L., Christie, D., Poncet, S., & Collins, M. A. (2016). Resolving patterns of population genetic and phylogeographic structure to inform control and eradication initiatives for brown rats Rattus norvegicus on South Georgia. *Journal Of Applied Ecology, 53*(2), 332-339.

Pracy, L. T. (1962). Introduction and liberation of the opossum (*Trichosurus vulpecula*) into New Zealand: New Zealand Forest Service.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics, 155*(2), 945-959.

Reaser, J. K., Meyerson, L. A., Cronk, Q., De Poorter, M., Eldrege, L., Green, E., . . . Mauremootoo, J. (2007). Ecological and socioeconomic impacts of invasive alien species in island ecosystems. *Environmental Conservation, 34*(2), 98-111.

Rollins, L. A., Woolnough, A. P., & Sherwin, W. B. (2006). Population genetic tools for pest management: a review. *Wildlife Research, 33*(4), 251-261.

Rousset, F. (2008). genepop’007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources, 8*(1), 103-106.

Sadlier, R. (2000). Evidence of possums as predators of native animals (chapter 11). In: The Brushtail possum: Biology, Impact and Management on and Introduced ….

Sarre, S. D., Aitken, N., Adamack, A. T., MacDonald, A. J., Gruber, B., & Cowan, P. (2014). Creating new evolutionary pathways through bioinvasion: the population genetics of brushtail possums in N ew Z ealand. *Molecular Ecology, 23*(14), 3419-3433.

Smith, A. L., Landguth, E. L., Bull, C. M., Banks, S. C., Gardner, M. G., & Driscoll, D. A. (2016). Dispersal responses override density effects on genetic diversity during post-disturbance succession. Proceedings of the Royal Society B: *Biological Sciences, 283*(1827), 20152934.

Speedy, C., Day, T., & Innes, J. (2007). Pest eradication technology-the critical partner to pest exclusion technology: the Maungatautari experience. *Managing Vertebrate Invasive Species*, 49.

Stow, A., Minarovic, N., Eymann, J., Cooper, D., & Webley, L. (2006). Genetic structure infers generally high philopatry and male-biased dispersal of brushtail possums (*Trichosurus vulpecula*) in urban Australia. *Wildlife Research, 33*(5), 409-415.

Sweetapple, P., Nugent, G., Whitford, J., & Knightbridge, P. (2002). Mistletoe (Tupeia antarctica) recovery and decline following possum control in a New Zealand forest. *New Zealand Journal Of Ecology*, 61-71.

Taylor, A., & Cooper, D. (1998). Microsatellite markers for the Phalangerid marsupial, the common brushtail possum (*Trichosurus vulpecula*). *Molecular Ecology, 7*(12), 1780-1782.

Taylor, A., Cowan, P., Fricke, B., Geddes, S., Hansen, B., Lam, M., & Cooper, D. (2004). High microsatellite diversity and differential structuring among populations of the introduced common brushtail possum, *Trichosurus vulpecula*, in New Zealand. *Genetics Research, 83*(2), 101-111.

Taylor, P. D., Fahrig, L., & With, K. A. (2006). Landscape connectivity: a return to the basics. *Conservation Biology Series-Cambridge-*, 14, 29.

Tompkins, D. M. (2018). The research strategy for a ‘predator free’New Zealand. Paper presented at the Proceedings of the Vertebrate Pest Conference.

Triggs, & Green. (1989). Geographic patterns of genetic variation in brushtail possums *Trichosurus vulpecula* and implications for pest control. *New Zealand Journal Of Ecology*, 1-10.

van Oosterhout, C., Hutchinson, W., Wills, D., & Shipley, P. (2004). et al (2004) MICRO--CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, 4

Veitch, C. R., & Clout, M. N. (2002). Turning the tide: The eradication of invasive species: Proceedings of the International Conference on Eradication of Island Invasives: IUCN.

Verity, R., & Nichols, R. A. (2016). Estimating the number of subpopulations (K) in structured populations. *Genetics, 203*(4), 1827-1839.

Warburton, B., Cowan, P., & Shepherd, J. (2009). How many possums are now in New Zealand following control and how many would there be without it. *Landcare Research Contract Report* LC0910/060

Yarita, S., Morgan-Richards, M., & Trewick, S. A. (2023). Genotypic detection of barriers to rat dispersal: Rattus rattus behind a peninsula predator-proof fence. *Biological Invasions*, 1-16.

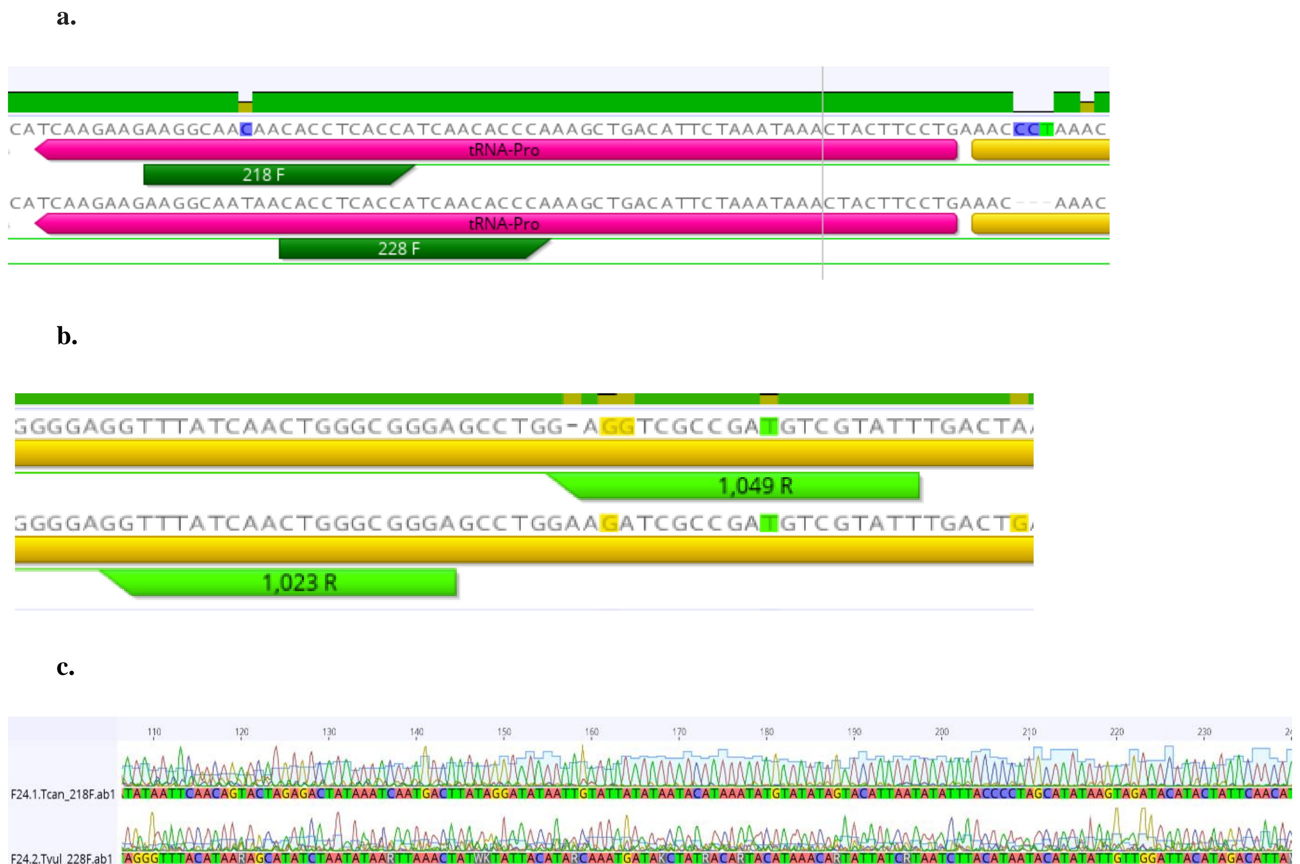
Zavaleta, E. S., Hobbs, R. J., & Mooney, H. A. (2001). Viewing invasive species removal in a whole-ecosystem context. *Trends In Ecology & Evolution, 16*(8), 454-459.

SUPPLEMENTARY MATERIAL FOR CHAPTER THREE:

Comparative population genetic structure of native and invasive brushtail possums (*Trichosurus vulpecula*) surveyed with mtDNA haplotype and nuclear microsatellite data

Supplemetary figure 3.1

Position of **a.** the two forward primers and **b.** the two reverse primers designed for amplification of the brushtail possum D-loop region of the mitochondrial genome: Tcan_218 F (*T. canninus*) and Tvul_228 F (*T. vulpecula*). **c.** DNA sequence chromatograms produced using different primers: Tcan_218 F (top chromatogram) and Tvul_228 F (bottom chromatogram) for the same sample, amplified with the reverse primer (Tvul_1023 R).



Supplementary table 3.1

Primer sequences designed to amplify control region (D-loop) mtDNA of two species of possums. Tcan- Short-eared possum (*T. canninus*); Tvul- Brushtail possum (*T. vulpecula*).

Primer name	Primer sequence
Tcan_218 (Forward)	AAGGCAACAACACCTCACCA
Tcan_1049 (Reverse)	AATACGACATCGGCGACCTC
Tvul_228 (Forward)	CACCTCACCATCAACACCCA
Tvul_1023 (Reverse)	TCCCGCCCAGTTGATAAACC

Supplementary table 3.2

Microsatellite primers developed for brushtail possums and used in this study. F: Forward Primer R: Reverse Primer * As given by Adams et al. (2013).

Locus name	Primer template	Allele size range*	Fluorophores
Tv_16	F: GAGGCTACCATTAGACGCAA R: ACCCAAATGAACAGAAAGGC	144-146	FAM
Tv_19	F: CCTCCTCCCCATCCTTCCTG R: GTTCAATTGCAGGGCTATGG	254-294	VIC
Tv_58	F: GCACCCAAGGACCCCCAAGA R: CCATATCACAGTGCTTGGCG	124-168	VIC
Tv_53	F: GGGAGTAGTTGTCTGAGTTCCC R: CCCTGGAGTTTGACAACCTG	222-272	FAM
Tv_64	F: AGGGAGACTGAGTGCGTTTG R: AGACAGGAAAATTTGTGCCC	138-199	FAM
Tv_PnMs	F: CCACCCCAATTAGATTAGCTC R: GGATGGTTTGTGACAATTTGC	220-251	FAM
Tv_M1	F: GACCACAACCTGGGTCTAACCAAC R:CATGACACCTGGGCACTCAGGACT	224-252	TAMRA
Tv_27	F: R: AGTGGAACCACATGTCAGGGC GGACTGAAATGACTGCACAAC	163-193	TAMRA

Supplementary table 3.3

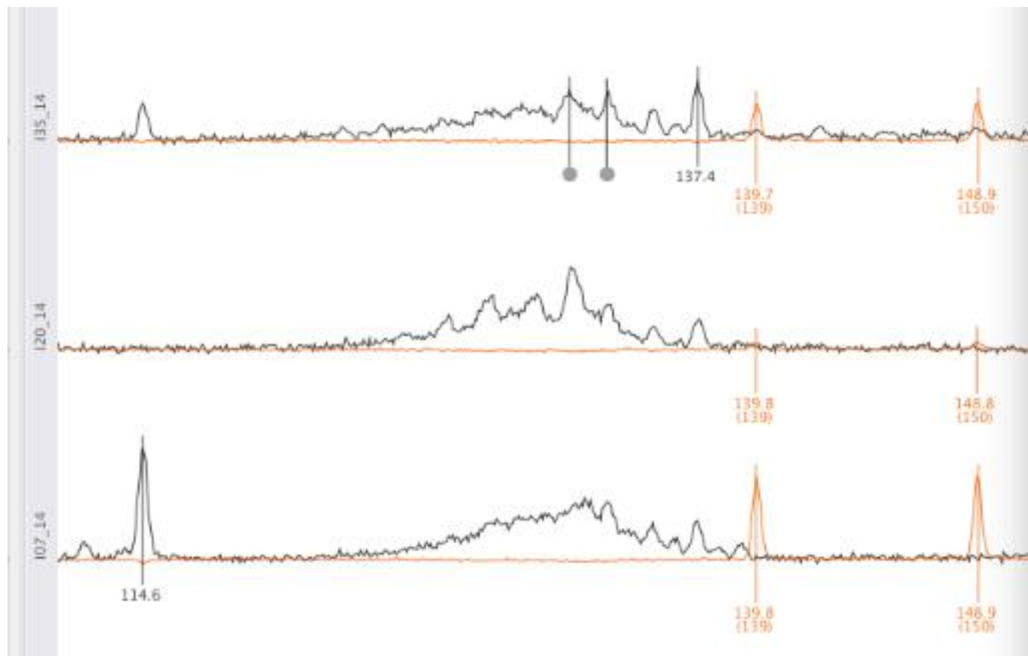
Nuclear microsatellite markers used in previous research to study genetic diversity in Brushtail Possums *(T. vulpecula)* in Aotearoa New Zealand. 1: Markers were used in the study. 0: Markers were not used in the study.

References	Tv 16	Tv 19	Tv 27	Tv 53	Tv 54	Tv 58	Tv 64	Tv M1	Tv 14	Tv 5.64	Tv PnMS	Tv 38.1
Adams 2013	1	1	1	1	1	1	1	1	1	1	1	1
Clinchy et al. 2004	1	1	1	1	0	1	1	0	0	0	0	0
DeGabriel et al. 2014	1	1	1	1	0	1	1	0	0	0	0	0
Duenas et al. 2014	1	1	1	1	1	1	0	1	0	1	0	0
Sarre et al. 2014	1	1	0	1	0	1	1	1	0	1	1	1
Taylor et al. 2004	1	1	1	0	0	1	1	0	0	0	0	0
Taylor et al. 2000	1	1	1	1	0	1	1	0	0	0	0	0
Blyton 2012	1	1	1	0	0	1	1	1	1	0	0	0
Total	8	8	7	6	2	8	7	4	2	3	2	2

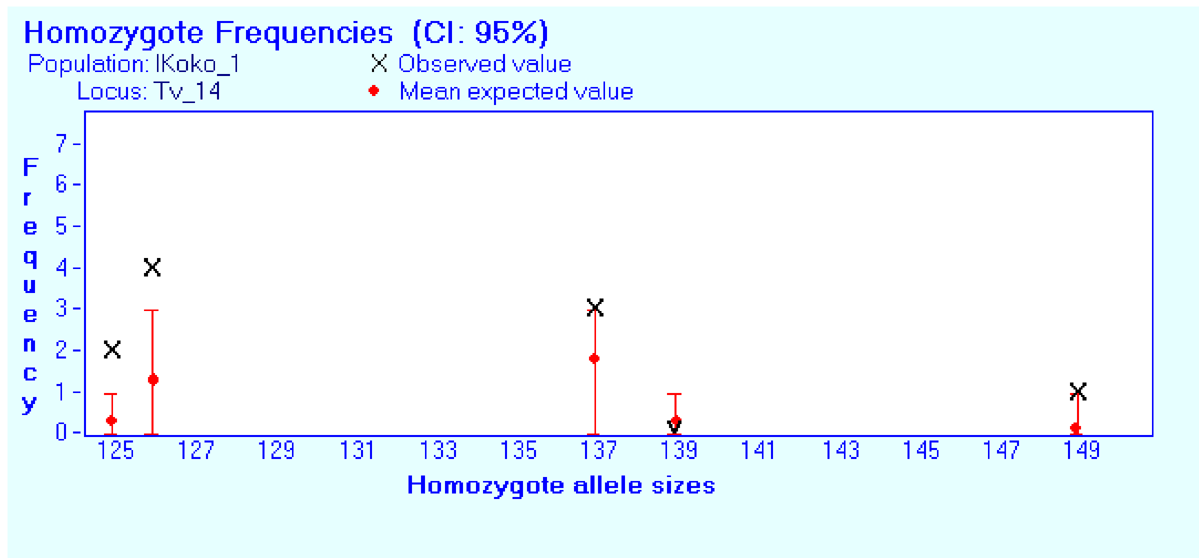
Supplementary figure 3.2

a. Distorted allele peaks for locus Tv_14, using GENEIOUS v 6.05. The allelic range for Tv_14 was 116 to 145, while the allelic range for Tv_27 was 128 to 154. **b.** Homozygote frequencies as shown in MICROCHECKER for locus Tv_14, where the observed values exceed the expected range of homozygotes in the Kokomuka population.

a.



b.



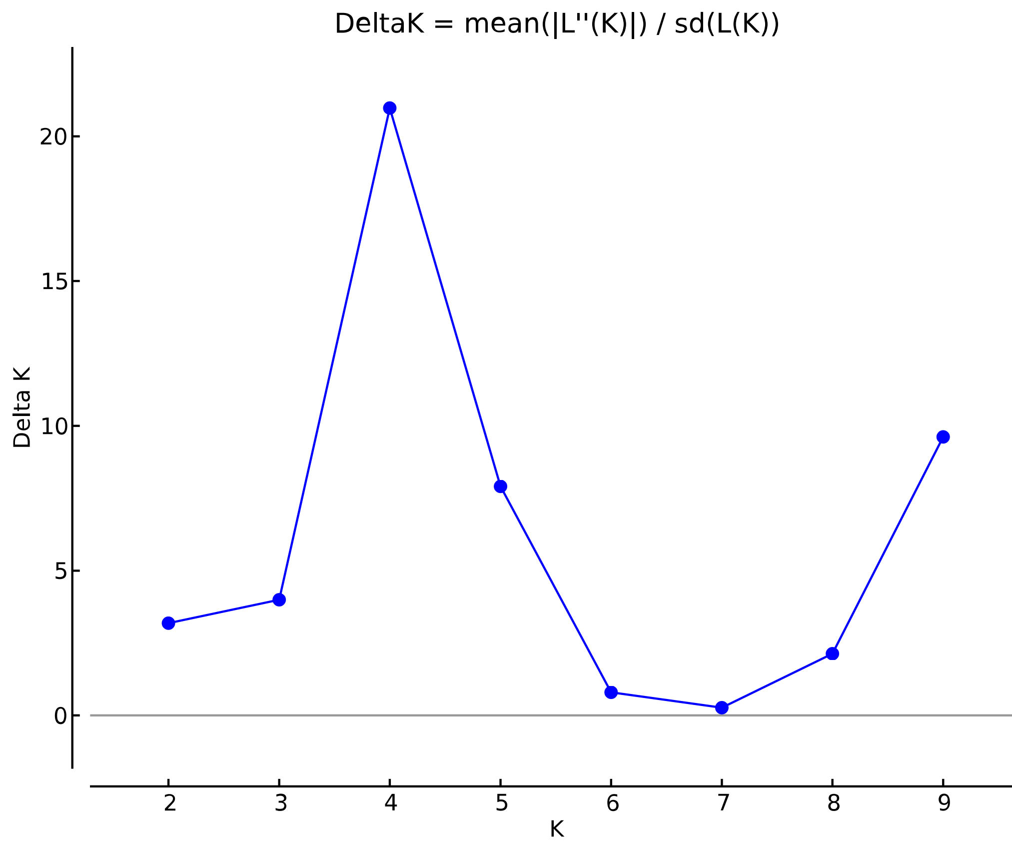
Supplementary table 3.4

Linkage disequilibrium checked for seven microsatellite markers in the Kenepuru population using Fisher's method in GENEPOP v 4.7.2 (Rousset 2008). Tv_16 and Tv_27 (**in bold**) are linked, with a p-value of 0.

Locus Pair	P-value	S.E
Tv_16 &Tv_58	0.828	0.021
Tv_16 & Tv_53	0.188	0.027
Tv_58 & Tv_53	0.074	0.017
Tv_16 & Tv_19	0.034	0.007
Tv_58 & Tv_19	0.833	0.021
Tv_53 & Tv_19	0.275	0.034
Tv_16 & Tv_64	0.705	0.029
Tv_58 & Tv_64	0.321	0.033
Tv_53 & Tv_64	0.769	0.029
Tv_19 & Tv_64	0.192	0.025
Tv_16 & Tv_PnMS	0.265	0.014
Tv_58 & Tv_PnMS	0.531	0.015
Tv_53 & Tv_PnMS	0.752	0.016
Tv_19 & Tv_PnMS	0.801	0.009
Tv_64 & Tv_PnMS	0.967	0.005
Tv_16 & Tv_M1	0.041	0.013
Tv_58 & Tv_M1	0.999	0.001
Tv_53 & Tv_M1	0.882	0.024
Tv_19 & Tv_M1	0.719	0.033
Tv_64 & Tv_M1	0.249	0.037
Tv_PnMs & Tv_M1	0.485	0.017
Tv_16 & Tv_27	**0**	**0**
Tv_58 & Tv_27	0.226	0.031
Tv_53 & Tv_27	0.171	0.032
Tv_19 & Tv_27	0.201	0.025
Tv_64 & Tv_27	0.822	0.031
Tv_PnMS & Tv_27	0.209	0.015
Tv_M1 & Tv_27	0.011	0.009

Supplementary figure 3.3

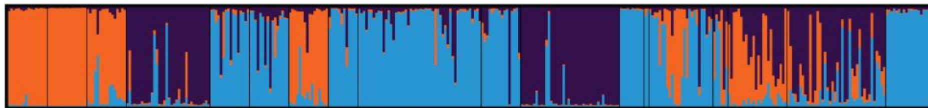
The number of optimal clusters for the, ΔK= 4, for the nuclear microsatellite dataset (n=379) analysed with 7 microsatellite markers, as shown by the Evanno method (Earl & VonHoldt 2012).



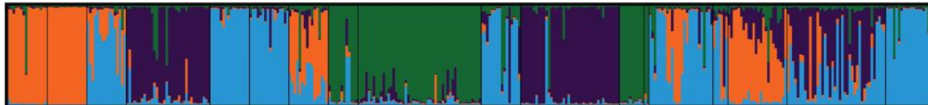
Supplementary figure 3.4

Comparison of assignment plots for models with differing number of population clusters (K= 3, K= 4, and K= 5). Each bar represents and individual, each colour a genotypic cluster. The length of the colours in the bar (admixture coefficient) is the assignment probability that the individual's genotype belongs to those clusters. The x-axis has the names of the population groups to which the samples belong. The plots show that all three plots indicate a similar distribution of genotypic clusters, supporting K= 4 as the optimal number of clusters in this dataset.

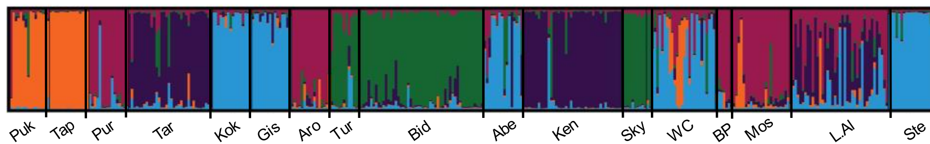
K=3



K=4



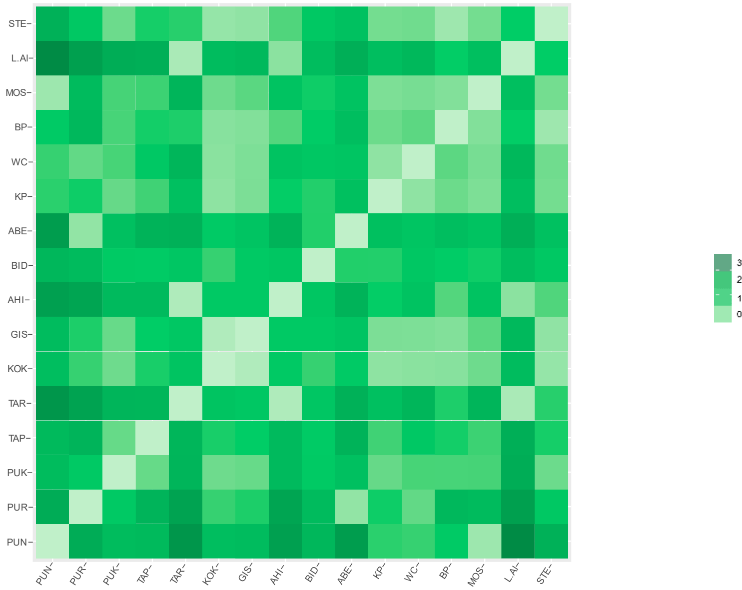
K=5



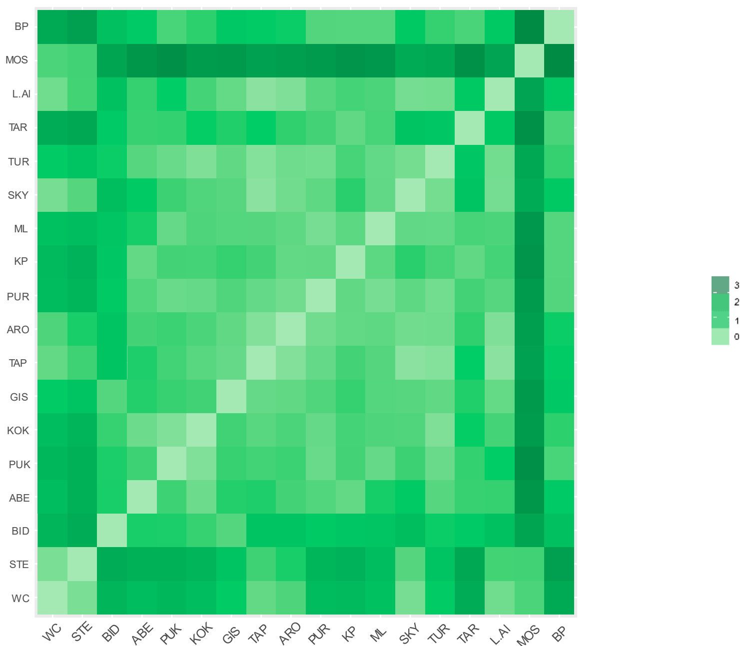
Supplementary figure 3.5

a. A visual representation showing the lack of correlation between pairwise φst estimates against population samples based on their latitude using the Corrplot package on R (Wei et al. 2017) **b.** A visual representation showing the lack of correlation between pairwise Fst estimates against population samples based on their latitude using the Corrplot package on R (Wei et al. 2017).

a.



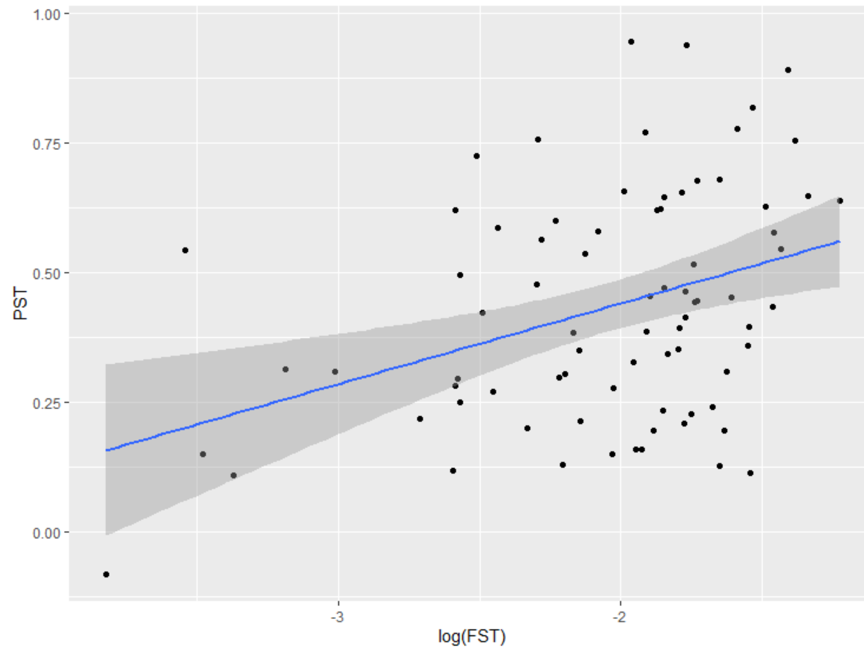
b.

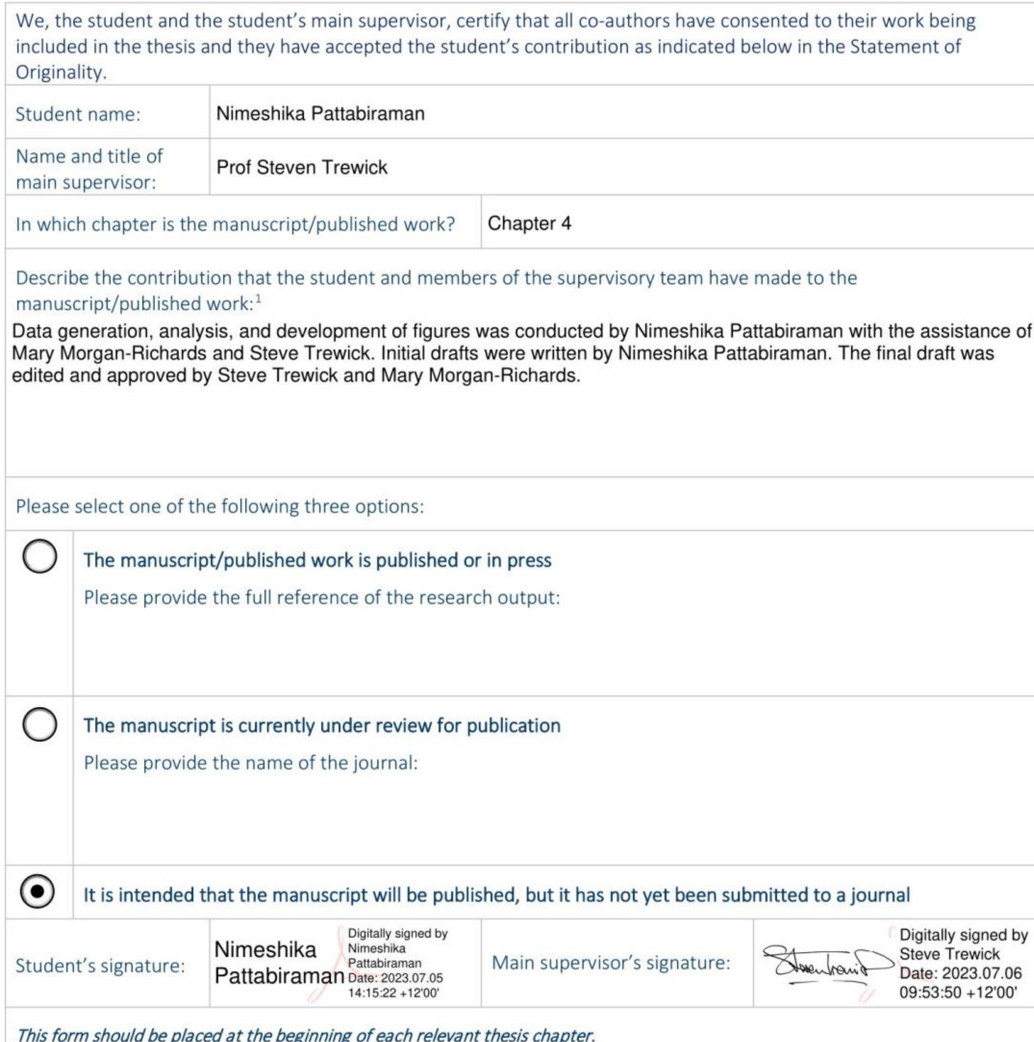


⋆ Wei, T., Simko, V., Levy, M., Xie, Y., Jin, Y., & Zemla, J. (2017). Package ‘corrplot’. *Statistician*, *56*(316), e24.

Supplementary figure 3.6

Pairwise comparison of Phist (mtDNA haplotypes) and F_{ST} (microsatellite loci) for 14 common locations with 999 replicates, p-value:0.583, std observation: -0.2364 variance: 0.04157**.**

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Student name:	Nimeshika Pattabiraman
Name and title of main supervisor:	Prof Steven Trewick
In which chapter is the manuscript/published work?	Chapter 4

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Data generation, analysis, and development of figures was conducted by Nimeshika Pattabiraman with the assistance of Mary Morgan-Richards and Steve Trewick. Initial drafts were written by Nimeshika Pattabiraman. The final draft was edited and approved by Steve Trewick and Mary Morgan-Richards.

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May 2023

Chapter Four

High resolution genetic structure of native and invasive populations of brushtail possums (*Trichosurus vulpecula*) using genotyping-by-sequencing

Abstract

Genetic variation in four subspecies of the most widespread Australian marsupial, the brushtail possum (*Trichosurus vulpecula*) was analysed using genotyping-by-sequencing (GBS). Single nucleotide polymorphism (SNP) data representing four subspecies of brushtail possums in Australia reveal that *Trichosurus vulpecula* from Darwin, Northern Territory are more strongly differentiated from individuals sampled in southeast Australia and Tasmania than those populations are from each other. The population sample from Kangaroo Island (*T. v. vulpecula)* was also genetically distinct, which supports the expectation that island isolation is a barrier to migration and gene flow. After nearly 165 years of mixing following translocation to Aotearoa New Zealand, there remains clear population genetic structure but with little concordance to geography. Most possums sampled from New Zealand were genetically similar to the Tasmanian population sample. However, some New Zealand population samples formed separate genetic clusters differentiated from all native population samples, suggesting that there may be additional, unidentified source Australian populations used for translocations.

Keywords

Genotyping-by-sequencing, *Trichosurus vulpecula*, invasions, population structure, single nucleotide polymorphisms

Introduction

Investigating genetic variation among natural and invasive populations of the same species is highly informative (Facon et al. 2008; Guillimaud 2010; Lee 2002) as it can pinpoint the locations of original native populations, and the spatial and temporal scale of natural barriers to gene flow can be investigated (Smith et al. 2020). Effective biological control of invasive species, such as the introduction of host-specific parasites, requires accurate information about genetic variation among native populations in order to select suitable strains (Browett et al. 2020). Unlike the situation of native species where biogeography is estimated from current distributions (Hortal et al. 2012; Pearson et al. 2007), historical records can be informative when reviewing past changes in species distribution associated with human activity and in particular species introductions (Stott 1981; Van Driesche et al. 1996). Population genetic tools widely used to interpret gene flow in native species' ranges allows inferences to be made about or explicitly test predictions based on introduction history. Depending on the resolving power of the markers used it may be feasible to demonstrate the relative success of different lineages of the organism of interest in the novel habitat (Cao et al. 2019; Combs et al. 2019; Cordeiro et al. 2019; Elameen et al. 2020; Rollins et al. 2006). As such the study of introduced species in this way can have valuable applied outcomes in terms of management planning (Burgess et al. 2021), but also provide empirical data on the way population expansion and gene flow operate in a known environment (Burgess et al. 2021; Díez-del-Molino et al. 2013; Hofmeister et al. 2021; Smith et al. 2020).

An exceptional example is presented by the brushtail possum that is native to Australia and introduced in Aotearoa New Zealand. Understanding where the successful lineages originated has potential to aid in attempts to control the exotic population (O'Callaghan & Moore 1986), especially as possums show varied, ancestral susceptibility to plant defence toxins and their synthetic analogues (McIlroy 1982). In addition, genetic structure within the natural range of a species will improve our understanding of the type and scale of spatial features that influence density and gene flow in a metapopulation framework (Calazans et al. 2017; Gagnaire 2020; Urvois et al. 2022; Zalewski et al. 2009). This is an important factor in the development and execution of invasive pest management strategies.

The brush-tailed possum, Trichosurus vulpecula Kerr, 1792 is one of the most widespread marsupial species in Australia (Flannery & Schouten 1994; Kerle 2001). Within its seven million km2 range, six distinct morphotypes are associated with climatically distinct geographic regions and differentiated by size and colour (Kerle et al. 1991). Taxonomic treatment of Trichosurus vulpecula as a set of subspecies or species, has fluctuated over time, with regional variants originally established as distinct species (e.g. Phalangista johnstonii (Ramsay 1888); Trichosurus arnhemensis (Collett 1897)). Only recently have deep evolutionary lineages been demonstrated within T. vulpecula suggesting divergence during the Pliocene (Carmelet-Rescan et al. 2022), lending credence to earlier treatments as a set of distinct species. Mitochondrial haplotype evidence hints at mixing among populations from the south and east of Australia, putatively T. v. vulpecula, T. v. fuliginosus and T. v. johnstonii (Chapter Three). It is not known whether this mixing results from recent exchange, but niche modelling indicated potential habitat formerly existed in eastern Australia that could have facilitated gene flow (Carmelet-Rescan et al. 2022).

Brushtail possums were introduced to Aotearoa New Zealand between 1838 and 1924, purportedly from south-eastern Australia and Tasmania, although records of exact locations are not recorded (Campbell et al. 2021; Pracy 1962). To establish a feral population for the fur industry, further translocations among introduced sites in Aotearoa were undertaken over the course of 70 years into a landscape lacking predators, resulting in a population increase to 75 million by 2005 (Efford et al. 2000).

Genotyping-by-sequencing (GBS) (Elshire et al. 2011) provides a way to generate rich multilocus data applicable to many levels of population genetic analysis (Vaux et al. 2023). Here, GBS is used to develop a single nucleotide polymorphism (SNP) population genomic dataset suitable for investigating the population structure of brushtail possums. In this approach, genome-wide SNPs are applied to assess intraspecific diversity among native populations of brushtail possums in Australia. This provides a context for comparison and a robust basis for inferring the source(s) of possums brought to Aotearoa New Zealand. The same dataset is then used to explore the resulting diversity and spatial structure that has developed since introduction to New Zealand. The four sampled subspecies provide an expectation of four main lineages, but the existing habitat could allow gene flow between the mainland subspecies Trichosurus v. arnhemensis from Darwin and the south-eastern populations of T. v. vulpecula and T. v. johnstonii. In contrast, possums that are isolated on Australian islands that brushtail possums were brought to Aotearoa from the southeastern part of Australia – mainly the states of Victoria and Tasmania. Possums that were

brought to Aotearoa would possess a subset of that genetic diversity but how small a subset depends not only on what individuals were translocated but how they have contributed to subsequent generations. Theory predicts that rapid population expansion after establishment would retain founding diversity, and novel admixture could yield novel genotypic permutations (Uller & Leimu 2011; Dlugosch et al. 2008; Roman & Darling 2007; Lavergne & Molofsky 2007; Szucs et al. 2017). Thus after many translocations and phases of range expansion, the current population genetic structure might, after approximately ~110 generations, have coalesced into one or more distinct New Zealand types. Alternatively, landscape features and differential reproductive success might have yielded regional genotypes that reflect introduction patterns.

Materials and Methods

Brushtail possum sampling comprised 84 individuals collected from nine locations representing four subspecies in Australia (South Australian Museum, Adelaide), and 104 samples of invasive possums from nine locations in Aotearoa New Zealand (Table 4.1).

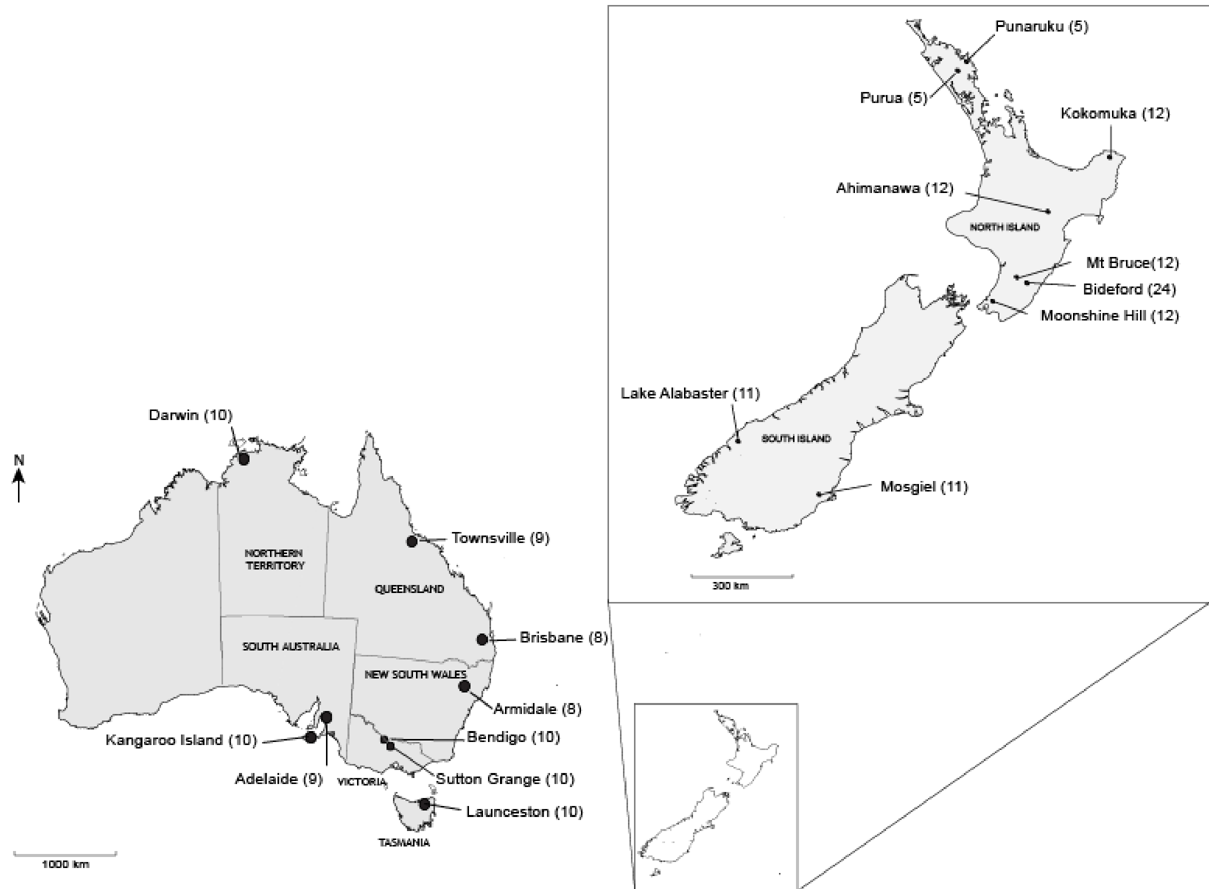


Figure 4.1 Eighteen locations in Australia and Aotearoa New Zealand sampled for genotyping-by-sequencing (GBS) analysis of brushtail possums (*Trichosurus vulpecula*). Number of individuals per population sample in brackets (n).

Whole genomic DNA was extracted using the Geneaid genomic DNA kit following the manufacturer's instructions and were eluted to a final volume of 200 µl of DNA. Preliminary assays were undertaken to identify the most suitable endonucleases for DNA fragmentation. Library validation tests, conducted using an Agilent DNA 1000 Kit (5067-1504) assay on the Agilent 2100 BioAnalyzer instrument, suggested a high proportion of repetitive elements were produced (i.e. a spike in produced DNA fragments around 500bp) using *PstI* and *EcoT22i.* In contrast, the endonuclease *ApeKI*, gave an optimal distribution of fragment sizes (Supplementary figure 4.1). The GBS data were generated following the Elshire et al. (2011) method used 100 ng

of genomic DNA and a total of 3.6 ng adapters. The genomic DNAs were restricted with *ApeKI* endonuclease and adaptors ligated before pooling. The library was amplified with 18 PCR cycles, samples pooled for multiplexed PCRs, and sequenced in one lane by Illumina NovaSeq platform generating 150 bp paired end reads. GBS libraries and high-throughput sequencing were performed by KCCG Sequencing Laboratory, Darlinghurst, NSW, Australia. Initial SNP calling was performed by Tea Break Bioinformatics before further bioinformatic filtering.

Table 4.1 Sample information for brushtail possums (*Trichosurus vulpecula*) collected for this study including sample location, region/state, grid reference, number of samples/individuals collected, year of sampling, and subspecies of possums found in the region. North Island NI, South Island SI.

Location	Dataset	Region/State	Grid Ref	n	year	Subspecies
	Australia (native)					
Darwin	A1,A2	Northern Territory	-12.38, 130.98	10	2015	*T. v. arnhemensis*
Townsville	A1,A2,C1,C2	Queensland	-19.2293, 146.7681	9	2015	*T. v. johnstonii*
Brisbane	A1,A2,C1,C2	Queensland	-27.5709,152.8659	8	2015	*T. v. vulpecula*
Sutton Grange	A1,A2,C1,C2	Victoria	-36.9789,144.3543	10	2015	*T. v. vulpecula*
Bendigo	A1,A2,C1,C2	Victoria	-36.9789,144.2943	10	2015	*T. v. vulpecula*
Armidale	A1,A2,C1	New South Wales	-30.5147, 151.7505	8	2015	*T. v. vulpecula*
Kangaroo Island	A1,A2	South Australia	-35.6482,137.5865	10	2015	*T. v. vulpecula*
Adelaide	A1,A2,C1,C2	South Australia	-35.1352,138.5383	9	2015	*T. v. vulpecula*
Launceston	A1,A2,C1,C2	Tasmania	-41.3847, 147.2158	9	2015	*T. v. fuliginosus*
				84		
	Aotearoa New Zealand (invasive)					
Punaruku	B1,B2,C1,C2	Northland NI	-35.374494, 174.306335	5	2019	
Purua	B1,B2,C1,C2	Northland NI	-35.583897, 174.126434	5	2003	
Kokomuka	B1,B2,C1,C2	East Cape NI	-37.68637, 178.17109	12	2003	
Ahimanawa	B1,B2,C1,C2	Hawke's Bay NI	-39.0124, 176.4725	12	2019	
Mount Bruce	B1,B2,C1,C2	Wellington region NI	-40.745046, 175.640659	12	2003	
Bideford 2016	B1,B2,C1,C2	Wellington region NI	-40.858551, 175.879869	12	2016	
Bideford 2017	B1,B2,C1,C2	Wellington region NI	-40.858551, 175.879869	12	2017	
Moonshine Hill	B1,B2,C1,C2	Wellington region NI	--41.107295, 175.023193	12	2003	
Lake Alabaster	B1,B2,C1,C2	Lake Alabaster SI	-44.54553, 168.14397	11	2020	
Mosgiel	B1,B2,C1,C2	East Otago SI.	-45.784045, 170.433655	11	2019	
				104		

SNP Calling, Annotation and Filtering

The availability of the full sequenced possum (*Trichosurus vulpecula*) genome allowed a reference-based pipeline for SNP calling to be used (GCA_011100635.1; NCBI Genome ID: 7126). The GBS data were first demultiplexed using AXE-DEMUX v0.3.3 (Murray & Borevitz 2018); Murray 2021) into pairs of forward and reverse reads for each individual. Reads were subsequently trimmed for adapter and reverse-barcode sequences, and then aligned to the reference genome using single-end mode with BOWTIE2 (Langmead & Salzberg 2012). The mapping rate was successful, with >90% of all reads mapping to the genome, and about two-thirds of them mapping uniquely. The SNPs had a median call rate of over 50%, which was high for such a low coverage dataset. The raw data will be available via Dryad. The alignments were put through SNP discovery using STACKS v2.5 (Catchen et al. 2013), and to exclude close genetic linkage among SNPs, the dataset was reduced by retaining one SNP per locus using the STACKS 'write single snp' function.

An initial STACKS output with no further filtering yielded a large dataset of > 12 million SNPs with plenty of scope for optimising SNP coverage to suit analyses. In particular, the contrast in allele frequencies across the sequenced individuals, which represented populations spanning most of the spatial and taxonomic diversity of *T. vulpecula* in Australia, made it necessary to optimise the SNP dataset appropriately for particular analyses.

The high-frequency restriction endonuclease *ApeKI* resulted in loci with relatively low coverage depth per sample, and the large reference genome (3359.35 Mb) which can affect coverage and missingness-based filtering of SNPs. The proportion of loci that are not represented in an individual or population sample can influence the downstream analysis, especially where missingness is not randomly distributed. For a widely distributed species such as *T. vulpecula* where it is possible that gene flow has been limited among some spatially separated populations, a relatively high level of genomic divergence might have this outcome. Such divergence could manifest as increased allelic variation at consistent loci among all individuals, but particular populations (and numerous permutations of multiple populations) could exhibit exclusive loci and private alleles. Coverage depth and missing data are therefore crucial parameters to consider as it can be challenging to distinguish low yields from the limited scale of sequencing from genuine signatures of genotypic divergence. To maximise signal quality, two approaches were applied to each of the datasets (A, B and C).

For the first set of analyses (A1, B1, and C1), a series of filtering steps were implemented in the STACKS populations pipeline based primarily on the pattern of population sampling used. The filtering of SNPs was initially optimised using three parameters: -p considers the minimum number of population samples at which a locus must be present for inclusion, -r sets the minimum proportion of individuals within a population sample with data for that locus, and --min-maf sets the minimum minor allele frequency (MAF) required to retain an allele within a locus (Catchen et al. 2013). The SNP data from 188 samples were first considered as a single group for analysis, effectively treating all individuals as belonging to the same sample. In this case, parameters were set so that loci present in at least 90 % of individuals were retained (r = 0.9, p = 1). The MAF value was adjusted to reflect different model expectations: MAF set to 0.01 allowed more loci to be included (for the dataset where all individuals were considered as a single sample group) than a MAF of 0.05, and it resulted in a reduction in missing data (Supplementary figure 4.2a, b). As the MAF value has a strong effect on the results of downstream population genetic analyses (Linck & Battey 2019), population structure was investigated for the data resulting from both options.

SNP filtering was repeated treating each location sample as a separate population sample in datasets A1, B1 and C1. Values of -p ranging from one to four for the invasive possum sample and one to six for native Australian possums, and those for -r ranged as low as 0.3 to 0.9 depending on the number of samples included in the sample groups created (Supplementary table 4.1a). For instance, considering only the native Australian sample, -p = 1 and -r = 0.5 resulted in 650,404 SNPs with 63.8 % missing data whereas -p = 6 and -r = 0.3 resulted in 260,968 SNPs with 38.7 % missing data. Settings -p = 6 and -r = 0.5 resulted in 78,930 SNPs with only 28.7 % missing data. Datasets with fewer total SNPs tended to have a smaller proportion of missing data, leading to less computationally intensive processes for downstream analyses.

While the datasets generated from this analysis resulted in a large number of loci to be analysed, we also tested for more stringent parameters under MAF values of 0.01 and 0.05 using VCFtools (Danecek et al., 2011). Datasets generated by filtering for minimum coverage depth values and missing data for both individuals and loci (without prior population sample grouping) provided us with fewer loci, but robust datasets that mostly concurred with our initial results.

In this second set of analyses (A2, B2 and C2), we filter for proportion of loci included and the number of individuals included by checking coverage depth and missingness for both individuals and loci. Loci associated with sex chromosomes were removed from the datasets to avoid any analytical complexities in downstream analyses. We only used the first filtering step in Stacks for generating VCF files with varying MAF and minor allele count (MAC) values with no division of samples into population groups. The VCF file was then subject to a combination of three parameters: minDP- minimum depth of coverage required for a variant to be considered, max missingness – threshold for maximum allowed missing genotype calls, and missingness per individual – exclusion of individuals with missing data exceeding a certain percentage (Table 4.2b). The datasets corresponding to the those in the initial analysis were chosen considering the trade-off between number of loci and representation for each location.

For the first approach, three multi-locus datasets were used to address different levels of population structure:

For dataset A1, the 84 available Australian possum samples (i.e. the native population) were divided into six groups, and each locus was required to be present in all the six groups (-p = 6) (Table 4.1) and in fifty percent of individuals per population sample (-r = 0.5). Based on the assessment of alternative parameter settings (Table 4.2), this permutation yielded the highest genotypic variation and lowest proportion of missing data.

Dataset B1 focused on population structure present in the invasive population in Aotearoa, without the influence of native population genotypes of Australia in the analysis. The 104 Aotearoa possum samples were each assigned to one of four geographic groups (Table 4.1) and required each locus to be present in all the four groups (-p = 4) and in at least ninety percent of individuals per population (-r = 0.9).

Finally, a dataset was created that comprised a subset of possums sampled from their native range and all the invasive population samples from Aotearoa New Zealand were considered together and run as a single group (dataset C1) to identify any common genotypic clusters spanning the geographic spread of sampling and to help clarify the source-population hypothesis. The data from 134 individual possum samples were assigned to one of six geographic groups (Table 4.1), and the settings required each locus to be present in at least five of those groups (-p = 5) and in at least sixty percent of individuals per population sample (-r = 0.6).

Population genetic analysis

A summary of the genotypic variation among all the samples was generated using Principal Component Analysis (PCA) in R (Jombart & Ahmed 2011). The number of 'meaningful' principal components (PCs) to retain for interpretation was determined by comparing PC eigenvalues. Discriminant Analysis with Principal Components (DAPC) was then conducted to identify maximum variation among population groups (Jombart & Collins 2015). For the DAPC, cross-validation tests with each run determined the number of principal components to be analysed (Jombart & Collins 2015).

To obtain ancestry coefficients from large genotypic matrices generated from our dataset, and evaluate the number of ancestral populations to which our samples belong, we used the 'snmf' (sparse non-negative matrix factorization) function in the R package 'LEA' (Latent Environmental & Genetic Approaches) (Frichot & François 2015). A cross-entropy criterion is computed by the snmf() function using a cross-validation technique to fit a statistical model to the data. We provided an upper limit of ten 'ancestral populations' to which the populations could cluster/ assign, with 10 replicates of each run. The 'cross.entropy' function was used to identify the best-supported model for each K, resulting in plots that show estimated individual ancestry to genotypic clusters based on signal from input data .
Pairwise population genetic distance (F_{ST}) (Nei, 1987), population inbreeding coefficient (F_{IS}), observed (Ho) and expected heterozygosity (He) for all populations was calculated using the HIERFSTAT package in R (Goudet et al. 2015) (Table 4.3; Supplementary table 4.2). The proportion of missingness and mean coverage depth per population group were calculated with the VCFR package in (Knaus & Grünwald 2017) (Table 4.2 a,b).

Results

An initial analysis included all 188 samples pooled as a single group, with no filters, yielded over 12 million SNPs (using STACKS). To meaningfully test population structure within the native (Australia) and invasive populations (Aotearoa New Zealand), and to clarify the relationship between these two regions (Table 4.2).

Table 4.2. a. The loci datasets generated for brushtail possums for the first set of analyses using STACKS. The table lists the number of samples (n), native population to which the dataset belongs, number of groups, *population* settings (-p = the minimum number of population samples at which a locus must be present for inclusion; -r =the minimum proportion of individuals within a population sample with data for that locus), and number of loci excluded and retained for each data set after applying filters. The next column lists the mean missing data per sample for each data set, as estimated by VCFR v1.12.0. The final column identifies K, the 'ideal' number of genotypic clusters for the dataset.

n		Dataset	# Total groups	(-p)	(-r)	# Total loci	#Total variant loci (i.e. SNPs)	Missing data (%)	# Genotypic clusters
84		Australia	1	1	0.9	360,652	586	7.8	4
84	A1	Australia/ native	6	6	0.5	1,235,602	78,930	28.7	4
104		Aotearoa	1	1	0.9	745,575	3,756	7.7	3
104	B1	Aotearoa/ invasive	4	4	0.9	444,921	772	5.2	3
104		Aotearoa	4	4	0.5	1,695,800	187,655	29.1	4
188		combined	1	1	0.9	510,199	776	7.8	6
168		combined	8	8	0.6	1,190,097	40,163	21.6	4
134	C1	combined	6	5	0.6	1,512,593	104,911	29.0	6

b. The loci datasets generated for brushtail possums for the second set of analyses using VCFtools. The table lists the number of samples (n), invasive/native population to which the dataset belongs, minimum minor allele frequency (MAF), minimum read depth at a locus (minDP) , maximum missingness allowed per locus, maximum missingness allowed per individual, and number of loci retained for each data set after applying filters. The next column lists the mean coverage depth for the dataset, followed by mean missing data per sample for each data set, as estimated by VCFR v1.12.0. The final column identifies K, the 'ideal' number of genotypic clusters for the dataset.

n		Dataset	MAF	minDP	Max miss/ locus	Max miss/ind	#Total variant loci (i.e. SNPs)	Mean Coverag e Depth	Missing data (%)	# Genotypic clusters K
70	A2	Native	0.05	6	30%	70%	955	22x	0.06	4
102	B2	Invasive	0.01	5	30%	50%	577	20.5x	0.069	3
140	C2	Combined	0.01	5	60%	70%	5184	10.23x	0.09	3

Native brushtail possums in Australia:

Dataset A1: Data filtering of the native Australian population dataset (n = 84) with parameters -p = 6 and -r = 0.5 yielded 78,930 SNPs with a moderate level of missing data (28.3 %). Missing data results from low sequencing coverage, meaning that for each individual there was insufficient information for a fraction of loci (SNPs). Analysis of the SNP dataset from these settings identified four genetic clusters (K = 4). The possums from Darwin (*Trichosurus v. arnhemensis*) were genetically distinct and the possums from Kangaroo Island (*T. v. vulpecula*) also formed a separate genetic cluster (Figure 4.2a, c). The proportion of missing data for each sample yielded a distinct genetic cluster (grey in Figure 4.2a), but no other genotypic cluster was identified among the remaining Australian samples despite these comprising three subspecies (*T. v. vulpecula, T. v. johnstinii, T. v. fuliginosus;* Figure 4.2a).

Varying the parameter settings in STACKS had little effect on clusters inferred for the native sample, as apparent from the LEA analysis that resulted in the same number of optimal genotypic clusters (K= 4). Although changing the minor allele frequency (MAF) from 0.01 to 0.05 yielded substantially more loci, there were no observed differences in the resulting population structure (Supplementary figure 4.3). This dataset (A1), in comparison with dataset B1 and dataset C1 showed the lowest observed heterozygosity (0.06) and mean allelic diversity (0.11) but population differentiation (F_{ST}) was high (Table 4.3). Population samples from Launceston and Darwin showed the lowest observed and expected heterozygosity, while Kangaroo Island showed the highest (Supplementary table 4.2a).

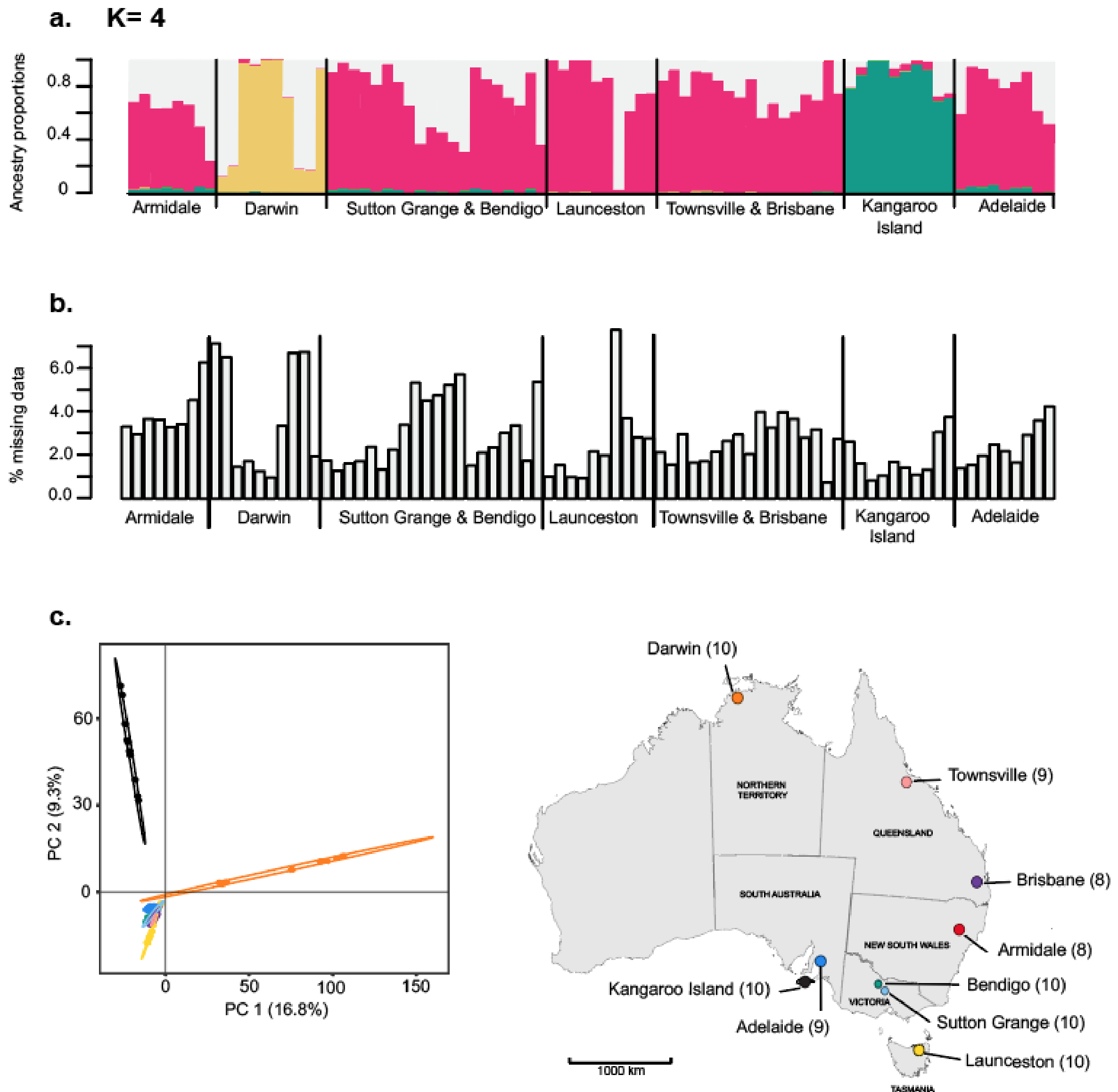


Figure 4.2 Genotypic variation in four subspecies of *Trichosurus vulpecula* across eastern Australia. **a.** Ancestry proportions bar graph depicting the assignment of Australian samples of brushtail possums (n = 84) to three genotypic clusters, with 78,930 variants (28.3% missing data) under the conditions r 0.5 and p 6. **b.** Proportion of missing data for each sample in the same dataset reflecting the pattern indicated by the grey genetic cluster in **a**. **c.** Principal components of variation. PC1 = 16.8%, PC2 = 9.3 %. Sample sites corresponding to clusters on **c** shown in map.

Dataset A2: All the samples into one population sample group with the following filtering conditions: minimum read depth at a locus (minDP) 6 and missingness allowed per locus 30%. This yielded 955 SNPS (n=70) with a mean coverage depth of 22x. Analysis of the SNP dataset from these settings identified four genetic clusters (K = 4). As in dataset A1, samples from Darwin (*Trichosurus v. arnhemensis*) and Kangaroo Island (*T. v. vulpecula*) formed distinct clusters, while possums from Tasmania (Launceston) mostly comprised of a distinct cluster (Figure 4.3). Unlike dataset A1, there was no distinct genetic cluster represented missingness in the data. When we

analysed Australian samples with different filtering conditions that resulted in varying depth, missingness, MAF and individuals included (Supplementary table 4.1b) we see a consistent distribution of genotypic clusters for this group.

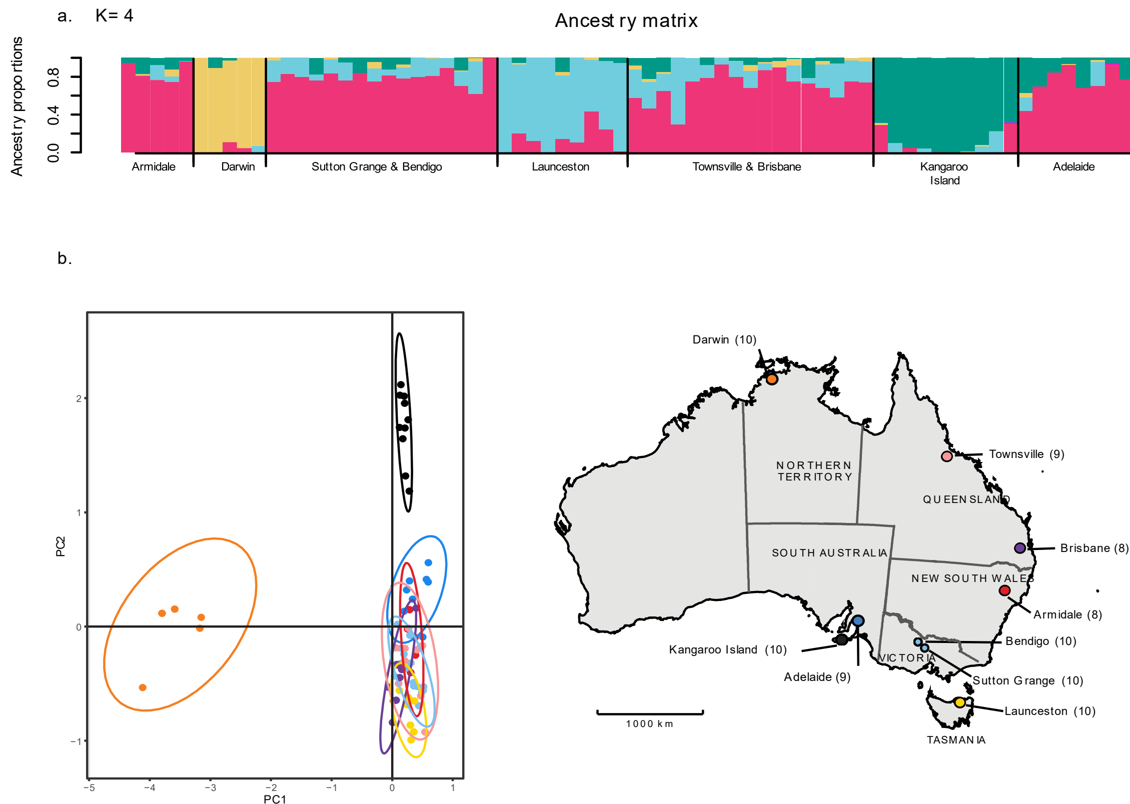


Figure 4.3 Genotypic variation in four subspecies of *Trichosurus vulpecula* across eastern Australia. **a.** Ancestry proportions bar graph depicting the assignment of Australian samples of brushtail possums (n = 70/84) to four genotypic clusters, with 955 variants (~5% missing data) under the conditions minDP 6, and mean coverage depth 22x. K = 4 was the optimal number of clusters obtained. **b.** Principal components of variation. PC1 =10.1 %, PC2 = 6.4 %. Sample sites corresponding to clusters shown in map.

Invasive brushtail possums in Aotearoa New Zealand:

Dataset B1: Analysis of possums introduced to Aotearoa New Zealand (invasive range) was performed by grouping population samples into four regional groups (Table 4.1; Figure 4.4). Filtering SNPs under the conditions -r = 0.9 and -p = 4 (n = 104), yielded 772 SNPs with a low proportion of missing data (5.1%; Table 4.2). The ancestry matrix revealed that three genetic clusters was the optimal model (K = 3) for this dataset. Despite grouping population samples into regional groups (Table 4.1; Figure 4.4) the three genetic clusters are not concordant with geography. About 75% of individuals from New Zealand showed evidence of genetic mixing with assignment to more than one genetic cluster. The two time-separated samples from Bideford (2016 and 2017) formed a unique cluster with some admixture with Moonshine Hill (Figure 4.4a), while

the possums from the adjacent site at Mount Bruce were genetically distinct. Similarly, in northern North Island samples from Purua and Punaruku (~60 km and 16 years apart) had high assignment probabilities to two distinct genetic clusters. However, evidence of genetic mixing was high in the population samples from Ahimanawa and in the southern samples (Lake Alabaster and Mosgiel; mean ancestry coefficient 0.98). The first two principal components of variation (total variance 3.54%) failed to separate the samples into discrete groups (Figure 44.4c), but by classifying individuals based on the population sample to which they belong, the discriminant analysis could distinguish Bideford 2016 & 2017 from other samples and grouped Kokomuka and Punaruku samples together (Figure 4.4b).

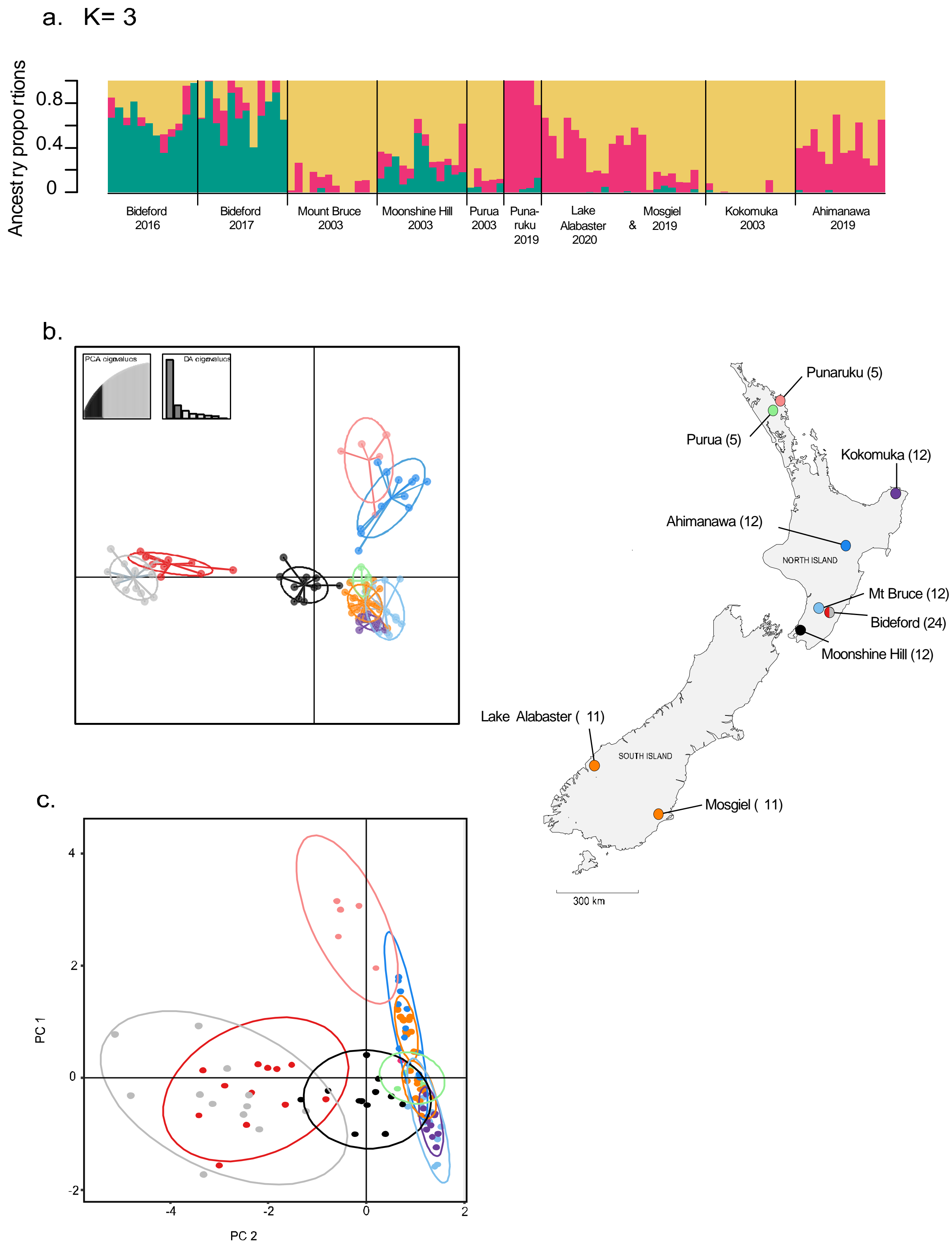


Figure 4.4 Genotypic variation of invasive populations of brushtail possums, *Trichosurus vulpecula* across Aotearoa New Zealand. **a.** Ancestry proportions bar graph depicting the assignment of Aotearoa New Zealand samples of brushtail possums (n= 104) to three genotypic clusters, with 772 SNPs (filtered under r 0.9 and p 4 with 5.15% missing data). The four geographic groups used for filtering data are indicated below the assignment plots (-p = 4). **b.** Discriminant Principal components analysis plot with the same GBS data colour coded by population sample as shown in map **c.** The first two principal components of variation explain little of the variation in the dataset (PC1 = 2.63%, PC2 = 0.91%). Sample site colour corresponding to clusters on **b** and **c** shown in map and sites group for SNP filtering.

Visualisation of K = 4 from this SNP dataset revealed a similar pattern to K = 3 but with more admixture within the Bideford samples (Supplementary figure 4.4). When K = 2 the samples from Bideford (2016 & 2017) formed a separate genetic cluster, with admixture showing in samples from Mount Bruce and Moonshine Hill (Supplementary figure 4.4).

When dataset B1 was re-processed under more lenient conditions (-r 0.5 and -p 4), it resulted in 187,655 SNPs with moderate missing data (29.0%; Table 4.2). Results for the ancestry analysis were similar to that showed by the filtering of dataset B1 (-p = 4, -r = 0.9) retaining 772 SNPs (K = 4) (Supplementary figure 4.5a). The dataset implemented with stricter conditions is shown here because it reduced the proportion of missing data and resolved the population structure most effectively. Discriminant analysis resolved the same genetic clusters observed in the naive LEA analysis and had the advantage that it places the Moonshine Hill samples between the Bideford samples and the southern samples (Mosgiel, Lake Alabaster; Supplementary figure 4.5b), as expected of genetic mixing from gene flow.

This invasive population dataset showed the highest estimates of overall observed heterozygosity (0.16) and the lowest overall F_{ST} (0.07), along with an F_{IS} value of -0.10 (Table 4.3). Sample groups in this dataset showed higher estimates of observed heterozygosity compared to expected heterozygosity (except for Mount Bruce samples), and only two pairwise comparisons of population differentiation (Bideford2016-Bideford2017, and Mount Bruce-Purua) showed non-significant differentiation from zero (Supplementary table 4.2b).

Dataset B2: The filtering conditions for this dataset analysis included: minDP of 5, MAF of 0.01, 30% missingness allowed per locus, and 50% missingness allowed per individual. This resulted in 577 SNP loci for this dataset (n = 102/104), with a high coverage depth of 20.5x and an overall missingness in the dataset of 0.069%. Analysis of the SNP dataset from these settings identified three genetic clusters (K = 3). Cluster distribution among samples were similar to that of dataset B1, with one cluster uniquely present among Bideford and Moonshine Hill samples (Fig 4.5 a). Bideford groups separately to the other population samples as seen in the PCA and DAPC analyses (Fig 4.5 b,c) and Punaruku is seen to group separately in the DAPC plot (Fig 4.5 b).

Dataset B2, with fewer loci than dataset B1, shows to further resolve the sample groups under higher mean coverage depth and lower overall missingness.

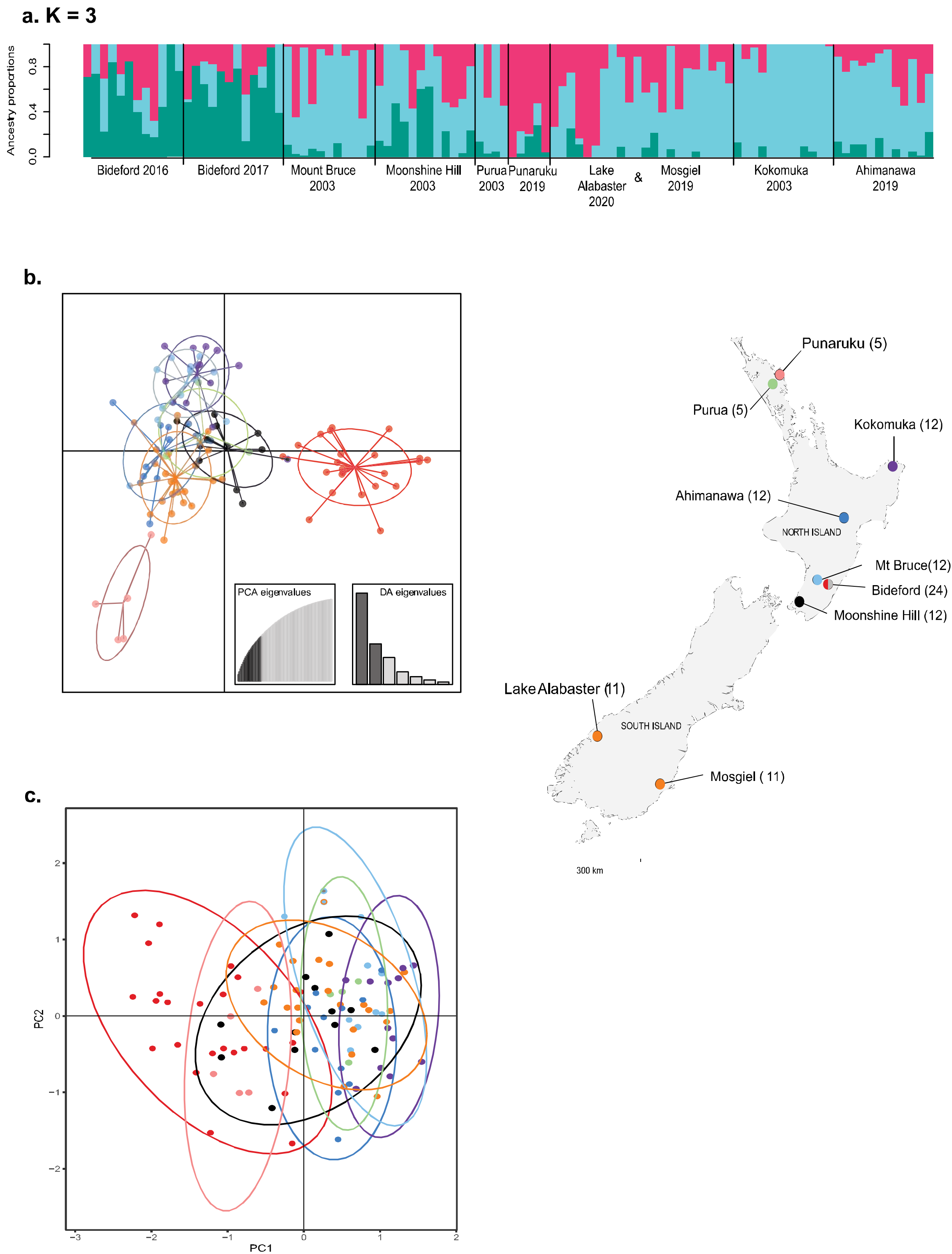


Figure 4.5 Genotypic variation of invasive populations of brushtail possums, *Trichosurus vulpecula* across Aotearoa New Zealand. **a.** Ancestry proportions bar graph depicting the assignment of Aotearoa New Zealand samples of brushtail possums (n= 102/104) to three genotypic clusters, with 577 variants (0.069% missing data) under the conditions minDP 5, and mean coverage depth 20.5x resulting in K = 3 optimal clusters. **b.** Discriminant Principal components analysis plot with the same GBS data**.** The first two principal components of variation explain little of the variation in the dataset (PC1 = 0.87%, PC2 = 0.44%). Sample site colour corresponding to clusters on **b** and **c** shown in map.

Invasive brushtail possums and their origins:

Dataset C1: To clarify the relatedness of Aotearoa New Zealand possums with those from Victoria and Tasmania (as early records suggest), we created dataset C1 which included samples from Australia (Sutton Grange, Bendigo, and Launceston) and all Aotearoa samples (n = 134) (Table 4.1). The population samples were grouped into six geographic regions for SNP selection (Table 4.1). This dataset was analysed under new conditions (-r = 0.6, -p = 5) which yielded 104,911 SNPs (29.0% missing data) used for analysis of population genetic structure. The optimal number of genotypic clusters was six (K = 6) (Figure 4.6a). Given the larger proportion of missing data (29.0%) one of the genotypic clusters identified matched the proportion of missing loci, as seen in dataset A1. Most individuals were assigned to a genotypic cluster shared by only two population samples, e.g. individuals from Sutton Grange and Bendigo were part of one unique cluster, individuals from Mosgiel and Lake Alabaster were part of another unique cluster and individuals from Purua and Punaruku were part of another unique cluster (Figure 4.6a). The population sample from the native range of *T. v. fuliginosus* (Launceston in Tasmania) formed a genotypic cluster which also comprised samples from the invasive range (Ahimanawa, Kokomuka, Mount Bruce, Moonshine Hill in New Zealand). However, signatures of genetic admixture were high in four population samples from the invasive range (Ahimanawa, Kokomuka, Mount Bruce, Moonshine Hill; Figure 4.6a).

By classifying individuals based on population sample, the discriminant analysis could not separate seven population samples but Bideford, Ahimanawa and Kokomuka individuals were identified as distinct from one another (Figure 4.6b). The naïve principal component analysis (showing a total of 7.3% variance), shows a similar signal, however, Ahimanawa samples did not differ from the Tasmanian samples on these axes (Figure 4.6c).

When considering ancestry coefficients with a model with five genotypic clusters (K = 5), Purua showed admixture patterns similar to Bideford (2016, 2017) and Moonshine Hill samples (Supplementary figure 4.6).

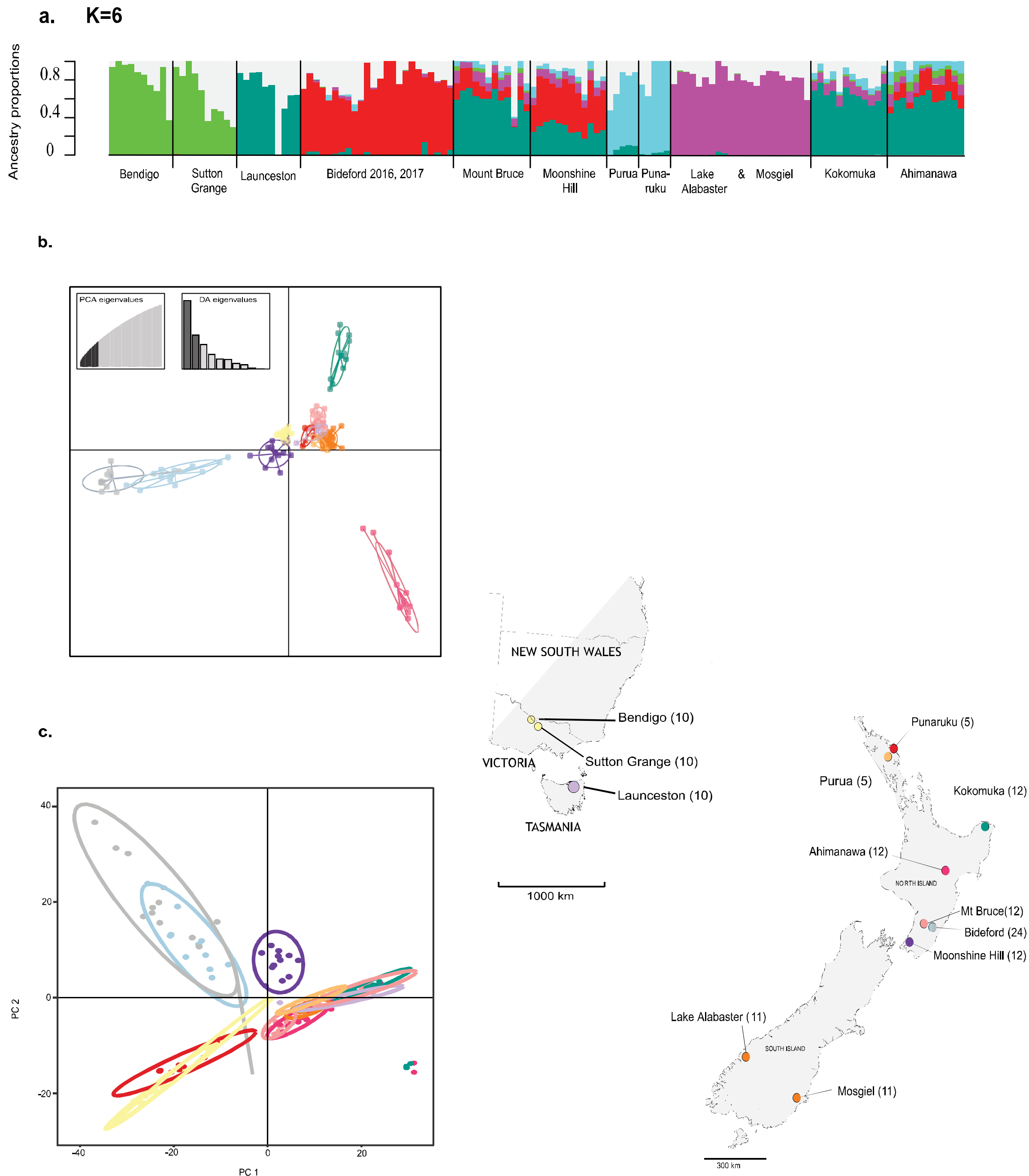


Figure 4.6 Genotypic variation of invasive populations of brushtail possums *Trichosurus vulpecula* in native and invasive range. **a.** Ancestry proportions bar graph depicting the assignment of Australian and Aotearoa New Zealand samples of brushtail possums (n= 134) to six genotypic clusters, where r = 0.6 and p = 5 with 104,911 SNP loci (29.0% md), resulted in K = 6 optimal clusters, with one (grey) cluster representing missing data (Supplementary figure 4.6). **b.** Plot of first two Discriminant Principal components for dataset C1 **c.** Principal components of variation. PC1 = 4.8%, PC2 = 2.5%. Sample sites corresponding to clusters on **b** and **c** shown in map. Lines below LEA plot indicate regional groups used for SNP filtering (-p= 6).

Dataset C1 contained possums from putative source locations in the native range (Victoria and Tasmania) however, assignment probabilities to genotypic clusters showed genotypic clusters in Aotearoa New Zealand apparently not sampled in Australia. Therefore another dataset was created for analysis to explore origins of invasive genotypes. Additional native population samples (Brisbane, Townsville, Kangaroo Island, Adelaide) were analysed with the invasive population samples using the naive summary of genetic variation in the form of a principal components of variation (PCA; Figure 4.7). The largest two principal components explained only 10.67% of all variation but these axes show that despite population sample overlap, the possums collected from Bideford are genetically distinct from all other samples. In contrast, the population sample from Townsville (*T. v. arnhemensis*) is very similar to the population sample from Brisbane (*T. v. vulpecula*) (Figure 4.7).

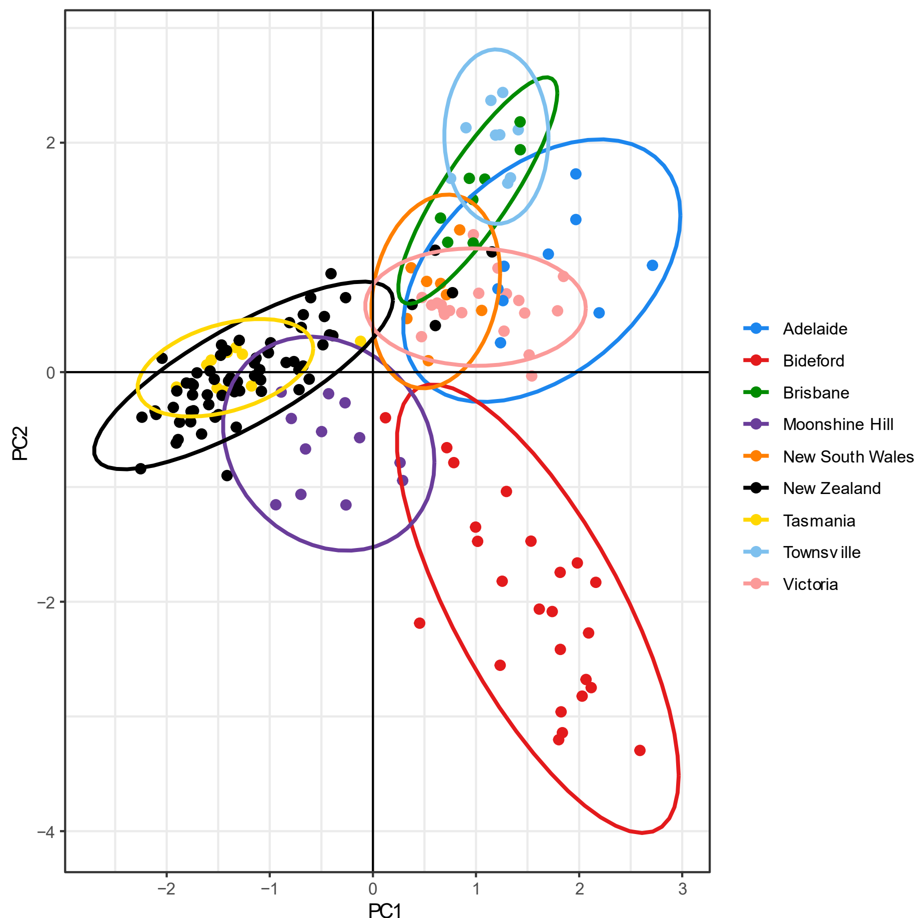


Figure 4.7 Genotypic variation of native populations of Adelaide, Bideford, Brisbane, New South Wales, Tasmania, Townsville, and Victoria, and invasive populations of Bideford, Moonshine Hill, and other New Zealand samples (Purua, Punaruku, Kokomuka, Ahimanawa, Mount Bruce, Lake Alabaster, and Mosgiel) of brushtail possums, *Trichosurus vulpecula* using principal components of variation at 812 SNPs. PC1 = 6.23%, PC2 = 4.44%.

Overall, dataset C1 showed moderate estimates of Observed Heterozygosity (0.11), F_{ST} (0.11) and F_{IS} (0.3) compared to datasets A and B (Table 4.3). Sutton Grange & Bendigo have higher diversity indices compared to those in dataset A1 (Supplementary table 4.2c). In addition to Bideford 2016 & Bideford 2017 and Mount Bruce & Purua pairwise comparisons, Purua & South Island samples also showed a non-significant deviation from zero (Supplementary table 4.2c).

Dataset C2: For this dataset, possum samples from Southeast mainland Australia (Townsville, Brisbane, Sutton Grange, Bendigo, and Adelaide), Launceston, and all of Aotearoa New Zealand were included with no prior assignment of population sample groups. The filtering conditions for this analysis included: minDP of 5, MAF of 0.01, 60% missingness allowed per locus, and 70% missingness allowed per individual. This yielded 5184 SNP loci (n = 140/168), with a mean coverage depth of 10.23x and an overall missingness in the final dataset of 0.09%. The optimal number of genotypic clusters was three (K = 3) (Fig 4.8 a). PCA and DAPC analyses indicate most individuals in New Zealand were assigned to the same genotypic cluster as individuals from Launceston, Tasmania. One cluster that was mainly assigned to Southeast Australian samples was present in lower proportions in New Zealand samples. Individuals from Bideford and Moonshine Hill had higher ancestry proportions of a third cluster. Samples from Southeastern Australia and Bideford clustered distinctly from the other population samples (Fig 4.8 b,c).

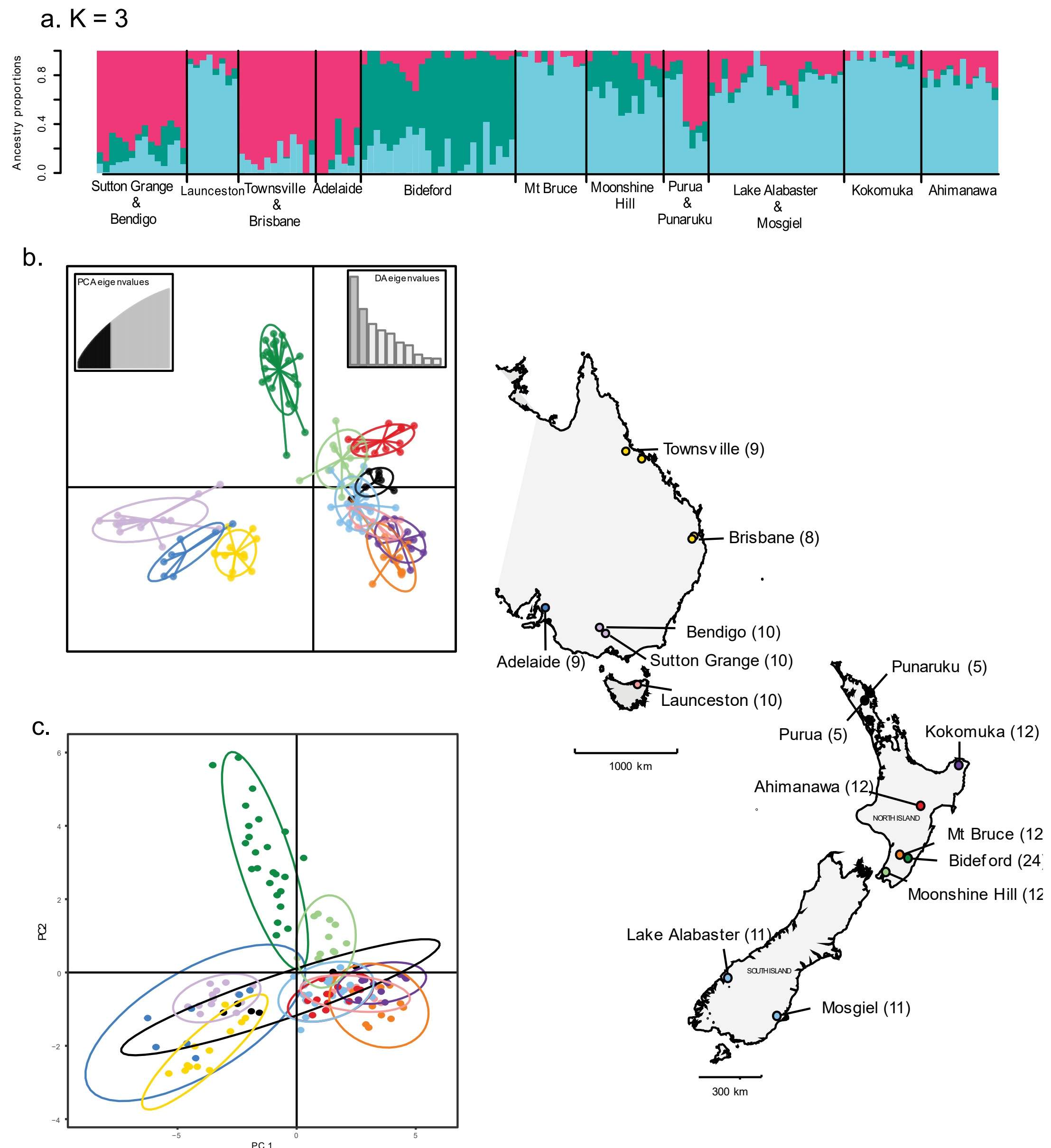


Figure 4.8 Genotypic variation of invasive populations of brushtail possums *Trichosurus vulpecula* in native and invasive range (Australia and Aotearoa New Zealand. **a.** Ancestry proportions bar graph depicting the assignment of Australian and Aotearoa New Zealand samples of brushtail possums (n= 140/168) to three genotypic clusters, with 5184 variants under the conditions minDP 6, and mean coverage depth 10.23x. **b.** Plot of first two Discriminant Principal components for dataset C2 **c.** Principal components of variation. PC1 = 7.23%, PC2 = 2.98 %. Sample sites corresponding to clusters on **b** and **c** shown in map.

Table 4.3 Genetic diversity indices calculated for the three datasets A1, B1, and C1. Ho: Observed Heterozygosity, Hs: Mean allelic diversities within populations, Ht: Overall allele diversities, F-statistics (F_{ST} and F_{IS}) calculated according to Nei (1987).

	# SNPs	Ho	Hs	Ht	F_{ST}	F_{IS}
Dataset A1 (Native)	78930	0.06	0.11	0.18	0.42	0.41
Dataset A2 (Native)	955	0.12	0.08	0.09	0.11	-0.47
Dataset B1 (Invasive)	772	0.16	0.15	0.16	0.07	-0.10
Dataset B2 (Invasive)	577	0.32	0.21	0.22	0.03	-0.54
Dataset C1 (combined)	104911	0.11	0.16	0.18	0.11	0.30
Dataset C2 (combined)	5184	0.16	0.14	0.16	0.10	-0.15

Discussion

Previous studies of local genetic diversity in brushtail possums of Aotearoa New Zealand (Chapter Two; Sarre et al. 2014) have used genetic markers to understand population structure and movement of possums within habitat patches, to understand connectivity and gene flow (Adams et al. 2014; Adams 2013; Campbell et al. 2021). Studies of possums in Aotearoa that sampled on a broader, nation-wide geographic scale have shown the presence of multiple genotypic clusters in different regions of the country, although this clustering is not concordant with phenotype or geography (Taylor et al. 2004; Chapter Three). North Island possum population samples exhibited higher rates of heterozygosity and allelic richness than populations sampled in the South Island using 45 nuclear loci (Triggs & Green 1989) and five microsatellite markers (Taylor et al. 2004). Overall, existing research indicates that brushtail possums in Aotearoa New Zealand cannot be expected to be genetically homogeneous.

Previous studies have also included both Australian and Aotearoa sampling with contrasting results; either samples from South Australia (Adelaide) were found to be not closely related to Aotearoa possums (Triggs & Green 1989), or they were (Chapter Three; Campbell et al. 2022). Genetically distinct populations of possum in Hawke's Bay are most likely descended from *T. v. vulpecula* in mainland Australia and from *T. v. fuliginosus* from Tasmania (Campbell et al. 2021). In the current study, the population genetic structure of possums in southeastern Australia were explored with larger numbers representing four subspecies and samples from two natural island populations. Population structure of possums from across their invasive range Aotearoa New Zealand was examined in relation to genetic diversity in the source population. Genotyping-by-sequencing resulted in >90 % of all reads successfully mapped to the reference genome, with two-thirds mapping uniquely and yielded over 12 million SNPs. This is a substantially higher yield of SNPs than reported from DArTseq analysis of brushtail possums (27,339 SNPs; Campbell et al. 2022), and most likely reflects the choice of restriction enzymes used to fragment genomic DNA and the use of a reference genome for this study. The large number of SNPs did result in moderately high levels of missing data for each sample due to their large genomes and low sequencing coverage, however my analyses were able to identify the downstream effect of missing data to avoid inappropriate inferences. After iteratively adjusting the STACKS filters to get a maximise SNP numbers with a low proportion of missing data, three datasets were formed (A, B and C) to investigate the theories posited by previous records and research. All results presented were obtained after exhaustive analyses to ensure SNP selection is not biasing the structure observed.

Genetic diversity within Australia

Analysis of genetic diversity with the native range of brushtail possums (dataset A1 and A2) showed that samples from Northern Australia (Darwin, *T. v. arnhemensis*) were genetically distinct from all other possum samples, consistent with their current and recent isolation in a fragmented habitat as well as the large distance separating them from other sampled brushtail possum populations (Carmelet-Rescan et al. 2022). Despite only sampling one individual, the same relationship for *T. v. arnhemensis* was observed by Campbell et al. (2022) – indicating concordance in our restriction-enzyme derived genomic data. The lack of genetic differentiation between population samples from the south-eastern populations of *T. v. vulpecula* and *T. v. johnstonii* further north confirm that continuous habitat in the recent past has facilitated gene flow between these regional forms. In contrast, I found individuals from Kangaroo Island (*T. v. vulpecula*) to be genetically distinct from the mainland *T. v. vulpecula* population samples (the nearest individuals were sampled in Adelaide). Results from genetic analysis with a range of alternative STACKS filtering parameters all concur. The Kangaroo Island population is currently geographically isolated, but just as modelled for the Tasmanian population, it would have been in contact with mainland *T. v. vulpecula* during the last glacial maximum – permitting gene flow. Dataset A1 shows my samples from Tasmania (*T. v. fuliginosus*) are genetically similar to mainland possums as expected from recent land connection Given these results, the observed differentiation of the Kangaroo Island population is probably more than just genetic drift in isolation since the LGM. Population bottlenecks in this geographically isolated population is one possible explanation but the sample from Kangaroo Island (n=10) had the highest observed and expected heterozygosity levels of all native populations (Supplementary table 4.2), so another explanation may be required to explain the identified novel genotypic cluster.On the eastern coast of Australia two subspecies occur, *T. v. vulpecula* and *T. v. johnstonii*, but my samples from Brisbane (*T. v. vulpecula*) and Townsville *(T. v. johnstonii)* were genetically very similar, suggesting that gene flow in fact occurs between these populations (Figure 4.7). Similarly, my samples from Adelaide, South Australia clustered with southeastern Australian samples (Sutton Grange and Bendigo, Armidale, Townsville and Brisbane) suggesting gene flow among these populations, which contrasts with previous results from a smaller set of nuclear loci (allozyme data;(Triggs & Green 1989)) (Supplementary figure 4.6).
The difference between datasets A1 and A2 is the cluster distribution among population samples. For dataset A2, there are no samples assigned to a cluster that matches the pattern of missingness as in dataset A1. Additionally, the fourth cluster in blue (Fig 4.3a) was mainly assigned to samples

from Tasmania, which are represented in again in dataset C2 to potentially distinguish the two lineages introduced to New Zealand.

Genetic diversity within Aotearoa New Zealand

Exploring genetic structure of the invasive population (dataset B1 and B2) used samples from 104 Aotearoa New Zealand individuals and reveal that populations sampled in close proximity can be genetically distinct. Despite the very large, apparently continuous population of brushtail possums across Aotearoa New Zealand (Efford et al. 2000), my population samples were genetically distinct from one another, and formed three genotypic clusters. This highlights that possums have not attained panmixia within Aotearoa New Zealand despite the last 150 years of translocations and opportunities for admixture. Recent fine-scale analysis on a relatively small area of land with no large landscape barriers found a mixture of possum phenotypes within single randomly mating population suggesting no behavioural barriers to gene flow(Pattabiraman et al. 2022). However in Aotearoa, population genetic structure did not correlate with geographical proximity. For example, Lake Alabaster and Mosgiel are situated on opposite sides of the lower South Island of Aotearoa New Zealand (~210 km apart), and yet they were found to be more similar to each other than populations located at a similar distance from each other in the East and Northern North Island. This pattern could reflect the complicated introduction history of brushtail possums to Aotearoa New Zealand. In Hawke's Bay, a genetic cline was concordant with fur colour variation, which was thought to result from source population differences and introduction history (Sarre et al. 2014). Alternatively, the rapid range expansion of brushtail possums in Aotearoa may have resulted in neutral genetic processes such as allele surfing (Excoffier & Ray 2008), which could cause populations at the extremities of the geographic distribution (e.g. the far southern South Island) to exhibit similar allele frequencies. These results also concur with the findings of previous studies, which found North Island populations to have higher estimates of diversity than South Island populations (Taylor et al. 2004; Triggs & Green 1989). There was a difference in sample size between the two islands, and inclusion of more South Island individuals would likely provide more insight towards the population genetic variation in that region (Supplementary table 4.2).

In this study, three main genotypic clusters were observed, one major cluster and a smaller proportion of the other clusters that exists in all populations across Aotearoa New Zealand, while a third genotypic cluster contained only possums from the lower North Island (Bideford, Mount

Bruce and Moonshine Hill). My results differed from previous microsatellite studies as the Aotearoa possums actually exhibited higher estimates of observed heterozygosity compared to their counterparts in their native population in Australia (Supplementary table 4.2).

Simultaneously, dataset A1 showed a positive mean F_{IS} value of 0.41 and dataset B1 shows a negative value of -0.10, indicating that Australian possums tend to exhibit higher estimates of inbreeding than Aotearoa populations. In concordance with previous studies, however, the North Island samples show higher mean observed heterozygosity than South Island ones, and greater genetic distances between populations of the former (Supplementary table 4.2) (Taylor et al. 2004; Triggs & Green 1989). A future study with larger sample sizes and a more even spread of geographic sampling for both invasive and native populations could test the reliability of these population genetic estimates.

Origin of invasive possums in Aotearoa New Zealand

Datasets C1 and C2 were used to investigate whether population structure across Australia and Aotearoa New Zealand supported the hypothesis that invasive Aotearoa New Zealand possums were brought from mainland southeast Australia and Tasmania. Analysis of 426 possum samples (Chapter Three) revealed mainly two mitochondrial haplogroups in Aotearoa that nested within Sutton Grange and Bendigo, and Launceston haplotypes. The current nDNA dataset indicated that Sutton Grange-Bendigo and Launceston are part of two different genotypic clusters (Figure 4.6a), but the genotypes typical of Tasmania are spread through both North and South Island populations of Aotearoa New Zealand. In contrast assignment to the Sutton Grange and Bendigo genotypic cluster is a minor part of ancestry coefficients from the LEA analysis. Dataset C2, which included all Southeastern mainland samples (except Armidale in New South Wales) and the more robust of the two datasets, highlights the evidence provided by dataset C1. Furthermore, the discriminate principal components analysis conducted with dataset C2 suggests many of the New Zealand possum samples are genetically distinct to the Southeastern mainland genotypes., Both ancestry matrix and DAPC suggest that individuals from Bideford (2016, 2017) were genetically distinguished from all other samples within Aotearoa and Australia. This differentiation might be the result of bottlenecks (as suggested for Kangaroo Island) or it could represent allelic variation inherited from a native population in Australia that has not yet been sampled. Again, a bottleneck does not seem a likely explanation as the observed and expected heterozygosity level for the Bideford 2017 sample is the highest seen in New Zealand.

While the datasets from this study have some contradictory results from the previous genetic research, they are concordant with the previous DartSeq study suggesting that RAD-seq results are internally consistent in that the mainland Australian possums are genetically distinct from those in Tasmania and Aotearoa. (Campbell et al. 2021). The results presented here may be more reliable as nuclear RAD-seq and GBS markers have been previously shown to provide higher resolution than microsatellite and mtDNA markers (Alvarez-Aleman et al. 2022; Farquharson et al. 2021; Lemopoulos et al. 2019; McGowan et al. 2023; Morin & McCarthy 2007), and the loci used for this study are more robust than the eight markers (combined) used previously for possum genetics. Direct comparison of genetic diversity estimates from the two types of markers may not be ideal especially, as Farquharson et al. (2021) note, for species management suggestions. Many previous studies have compared estimates of population structure and genetic diversity for RAD-seq markers versus microsatellites and mitochondrial markers (Vaux et al. 2023). RAD-seq data tend to produce results that agree with microsatellites on a population-level, and in particular they seem to fare well for smaller populations with moderate diversity, but SNPs derived from RAD-seq data have been found to surpass microsatellites for insights on an individual-level (e.g. to clarify kinship and heterozygosity) (Lemopoulos et al. 2019; Riccioli 2020). A good example of this application for GBS data exists for brushtail possums, where SNP data has been used to determine population dynamics around Mt Taranaki ring-plains, which is currently being used to inform management plans to effectively eradicate possums in the area (Veale & Etherington 2023). The data from that study also suggested that possum morphotypes (which have previously influenced species characterisation, and in turn, control efforts), were not correlated with genetic relatedness, and therefore did not influence population structure (Chapter Two; Veale & Etherington 2023). Despite mixing of subspecies (fur colour variation) in Aotearoa New Zealand, the invasive populations are not all genetically the same. Genotypic clusters in close geographic proximity suggest that despite the appearance of one large continuous pest population in Aotearoa New Zealand the species is subdivided suggesting dispersal is not even across the country. Consequently, when considering genetic knowledge for pest management, as is the case for conserving endangered species, the comparison of data generated from different genetic markers can help researchers to evaluate the effectiveness of management efforts.

References

Adams, A., Recio, M., Robertson, B., Dickinson, K., & Van Heezik, Y. (2014). Understanding home range behaviour and resource selection of invasive common brushtail possums (*Trichosurus vulpecula*) in urban environments. *Biological Invasions, 16*, 1791-1804.

Adams, A. L. (2013). *Spatial ecology and genetic population structure of the common brushtail possum (Trichosurus vulpecula) within a New Zealand urban environment.* University of Otago,

Alvarez-Aleman, A., Hunter, M. E., Frazer, T. K., Powell, J. A., Alfonso, E. G., & Austin, J. D. (2022). The first assessment of the genetic diversity and structure of the endangered West Indian manatee in Cuba. *Genetica*, 1-15.

Browett, S. S., O'Meara, D. B., & McDevitt, A. D. (2020). Genetic tools in the management of invasive mammals: recent trends and future perspectives. *Mammal Review, 50*(2), 200-210.

Burgess, B. T., Irvine, R. L., Howald, G. R., & Russello, M. A. (2021). The promise of genetics and genomics for improving invasive mammal management on islands. *Frontiers in Ecology and Evolution, 9*, 704809.

Calazans C, S. H., Walters, L. J., Fernandes, F. C., Ferreira, C. E., & Hoffman, E. A. (2017). Genetic structure provides insights into the geographic origins and temporal change in the invasive charru mussel (Sururu) in the southeastern United States. *PloS one, 12*(7), e0180619.

Campbell, C. D., Cowan, P., Gruber, B., MacDonald, A. J., Holleley, C. E., & Sarre, S. D. (2021). Has the introduction of two subspecies generated dispersal barriers among invasive possums in New Zealand? *Biological Invasions, 23*(12), 3831-3845.

Cao, L. J., Gao, Y. F., Gong, Y. J., Chen, J. C., Chen, M., Hoffmann, A., & Wei, S. J. (2019). Population analysis reveals genetic structure of an invasive agricultural thrips pest related to invasion of greenhouses and suitable climatic space. *Evolutionary Applications, 12*(10), 1868-1880.

Carmelet-Rescan, D., Morgan-Richards, M., Pattabiraman, N., & Trewick, S. A. (2022). Time-calibrated phylogeny and ecological niche models indicate Pliocene aridification drove intraspecific diversification of brushtail possums in Australia. *Ecology and Evolution, 12*(12), e9633.

Catchen, J., Hohenlohe, P. A., Bassham, S., Amores, A., & Cresko, W. A. (2013). Stacks: an analysis tool set for population genomics. *Molecular ecology, 22*(11), 3124-3140.

Combs, M., Byers, K., Himsworth, C., & Munshi-South, J. (2019). Harnessing population genetics for pest management: Theory and application for urban rats. *Human–Wildlife Interactions, 13*(2), 11.

Cordeiro, E. M., Campbell, J. F., Phillips, T., & Akhunov, E. (2019). Isolation by distance, source-sink population dynamics and dispersal facilitation by trade routes: impact on population genetic structure of a stored grain pest. *G3: Genes, Genomes, Genetics, 9*(5), 1457-1468.

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., . . . Sherry, S. T. (2011). The variant call format and VCFtools. Bioinformatics, 27(15), 2156-2158

Díez-del-Molino, D., Carmona-Catot, G., Araguas, R.-M., Vidal, O., Sanz, N., García-Berthou, E., & García-Marín, J.-L. (2013). Gene flow and maintenance of genetic diversity in invasive mosquitofish (Gambusia holbrooki). *PloS one, 8*(12), e82501.

Efford, M., Warburton, B., & Spencer, N. (2000). Home-range changes by brushtail possums in response to control. *Wildlife research, 27*(2), 117-127.

Elameen, A., Klütsch, C. F., Fløystad, I., Knudsen, G. K., Tasin, M., Hagen, S. B., & Eiken, H. G. (2020). Large-scale genetic admixture suggests high dispersal in an insect pest, the apple fruit moth. *PloS one, 15*(8), e0236509.

Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., & Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PloS one, 6*(5), e19379.

Excoffier, L., & Ray, N. (2008). Surfing during population expansions promotes genetic revolutions and structuration. *Trends in ecology & evolution, 23*(7), 347-351.

Facon, B., Pointier, J.-P., Jarne, P., Sarda, V., & David, P. (2008). High genetic variance in life-history strategies within invasive populations by way of multiple introductions. *Current biology, 18*(5), 363-367.

Farquharson, K. A., McLennan, E. A., Wayne, A., Smith, M., Peel, E., Belov, K., & Hogg, C. J. (2021). Metapopulation management of a critically endangered marsupial in the age of genomics. *Global Ecology and Conservation, 31*, e01869.

Flannery, T. F., & Schouten, P. (1994). *Possums of the World*: GEO Productions in association with the Australian Museum.

Frichot, E., & François, O. (2015). LEA: An R package for landscape and ecological association studies. *Methods in Ecology and Evolution, 6*(8), 925-929.

Gagnaire, P. A. (2020). Comparative genomics approach to evolutionary process connectivity. *Evolutionary Applications, 13*(6), 1320-1334.

Goudet, J., Jombart, T., & Goudet, M. J. (2015). Package 'hierfstat'. *R package version 0.04-22. Retrieved from http://www. r-project. org, http://github. com/jgx65/hierfstat*

Guillimaud, E. (2010). Reconstructing routes of invasion using genetic data: why, how and so what? *Molecular ecology, 19*(19), 4113-4130.

Hofmeister, N. R., Werner, S. J., & Lovette, I. J. (2021). Environmental correlates of genetic variation in the invasive European starling in North America. *Molecular ecology, 30*(5), 1251-1263.

Hortal, J., Lobo, J. M., & Jiménez-Valverde, A. (2012). Basic questions in biogeography and the (lack of) simplicity of species distributions: putting species distribution models in the right place. *Natureza & Conservação, 10*(2), 108-118.

Jombart, T., & Ahmed, I. (2011). adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. *Bioinformatics, 27*(21), 3070-3071.

Jombart, T., & Collins, C. (2015). A tutorial for discriminant analysis of principal components (DAPC) using adegenet 2.0. 0. *London: Imperial College London, MRC Centre for Outbreak Analysis and Modelling*

Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC genetics, 11*(1), 1-15.

Kerle, A. (2001). *Possums: the brushtails, ringtails and greater glider*: UNSW Press.

Kerle, J., McKay, G., & Sharman, G. (1991). A systematic analysis of the brushtail possum, *Trichosurus-vulpecula* (Kerr, 1792)(Marsupialia, Phalangeridae). *Australian Journal of Zoology, 39*(3), 313-331.

Knaus, B. J., & Grünwald, N. J. (2017). vcfr: a package to manipulate and visualize variant call format data in R. *Molecular ecology resources, 17*(1), 44-53.

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature methods, 9*(4), 357-359.

Lee, C. E. (2002). Evolutionary genetics of invasive species. *Trends in ecology & evolution, 17*(8), 386-391.

Lemopoulos, A., Prokkola, J. M., Uusi-Heikkilä, S., Vasemägi, A., Huusko, A., Hyvärinen, P., . . . Vainikka, A. (2019). Comparing RADseq and microsatellites for estimating genetic diversity and relatedness—Implications for brown trout conservation. *Ecology and Evolution, 9*(4), 2106-2120.

Linck, E., & Battey, C. (2019). Minor allele frequency thresholds strongly affect population structure inference with genomic data sets. *Molecular ecology resources, 19*(3), 639-647.

Mastretta-Yanes, A., Arrigo, N., Alvarez, N., Jorgensen, T. H., Piñero, D., & Emerson, B. C. (2015). Restriction site-associated DNA sequencing, genotyping error estimation and de novo assembly optimization for population genetic inference. *Molecular ecology resources, 15*(1), 28-41.

McGowan, A. M., Lanyon, J. M., Clark, N., Blair, D., Marsh, H., Wolanski, E., & Seddon, J. M. (2023). Cryptic marine barriers to gene flow in a vulnerable coastal species, the dugong (Dugong dugon). *Marine Mammal Science*

McIlroy, J. (1982). The sensitivity of Australian animals to 1080 poison. III. Marsupial and eutherian herbivores. *Wildlife research, 9*(3), 487-503.

Morin, P. A., & McCarthy, M. (2007). Highly accurate SNP genotyping from historical and low-quality samples. *Molecular ecology notes, 7*(6), 937-946.

Murray, K. D., & Borevitz, J. O. (2018). Axe: rapid, competitive sequence read demultiplexing using a trie. *Bioinformatics, 34*(22), 3924-3925.

O'Callaghan, & Moore. (1986). Parasites and serological survey of the common brushtail possum (*Trichosurus vulpecula*) from Kangaroo Island, South Australia. *Journal of Wildlife Diseases, 22*(4), 589-591.

Pattabiraman, N., Morgan-Richards, M., Powlesland, R., & Trewick, S. A. (2022). Unrestricted gene flow between two subspecies of translocated brushtail possums (*Trichosurus vulpecula*) in Aotearoa New Zealand. *Biological Invasions, 24*(1), 247-260.

Pearson, R. G., Raxworthy, C. J., Nakamura, M., & Townsend Peterson, A. (2007). Predicting species distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. *Journal of Biogeography, 34*(1), 102-117.

Pracy, L. T. (1962). *Introduction and liberation of the opossum (Trichosurus vulpecula) into New Zealand*: New Zealand Forest Service.

Ramsay, E. (1888). *Notes on the fauna of the Bellenden-Ker Ranges.* Paper presented at the Proceedings of the Linnaean Society of New South Wales, Second Series.

Riccioli, F. (2020). *Population genetics and conservation of the endemic Mus cypriacus.* Bournemouth University,

Rollins, L. A., Woolnough, A. P., & Sherwin, W. B. (2006). Population genetic tools for pest management: a review. *Wildlife research, 33*(4), 251-261.

Sarre, S. D., Aitken, N., Adamack, A. T., MacDonald, A. J., Gruber, B., & Cowan, P. (2014). Creating new evolutionary pathways through bioinvasion: the population genetics of brushtail possums in N ew Z ealand. *Molecular ecology, 23*(14), 3419-3433.

Smith, A. L., Hodkinson, T. R., Villellas, J., Catford, J. A., Csergő, A. M., Blomberg, S. P., . . . Laine, A.-L. (2020). Global gene flow releases invasive plants from environmental constraints on genetic diversity. *Proceedings of the National Academy of Sciences, 117*(8), 4218-4227.

Taylor, A., Cowan, P., Fricke, B., Geddes, S., Hansen, B., Lam, M., & Cooper, D. (2004). High microsatellite diversity and differential structuring among populations of the introduced common brushtail possum, *Trichosurus vulpecula*, in New Zealand. *Genetics Research, 83*(2), 101-111.

Triggs, & Green. (1989). Geographic patterns of genetic variation in brushtail possums *Trichosurus vulpecula* and implications for pest control. *New Zealand Journal of Ecology*, 1-10.

Urvois, T., Perrier, C., Roques, A., Sauné, L., Courtin, C., Li, Y., . . . Kerdelhué, C. (2022). A first inference of the phylogeography of the worldwide invader Xylosandrus compactus. *Journal of Pest Science, 95*(3), 1217-1231.

Vaux, F., Dutoit, L., Fraser, C. I., & Waters, J. M. (2023). Genotyping-by-sequencing for biogeography. *Journal of Biogeography, 50*(2), 262-281.

Veale, A. J., & Etherington, T. R. (2023). Assessing possum dispersal and the Predator Free Taranaki trapping program me using population genomics. Manaaki Whenua – Landcare Research.

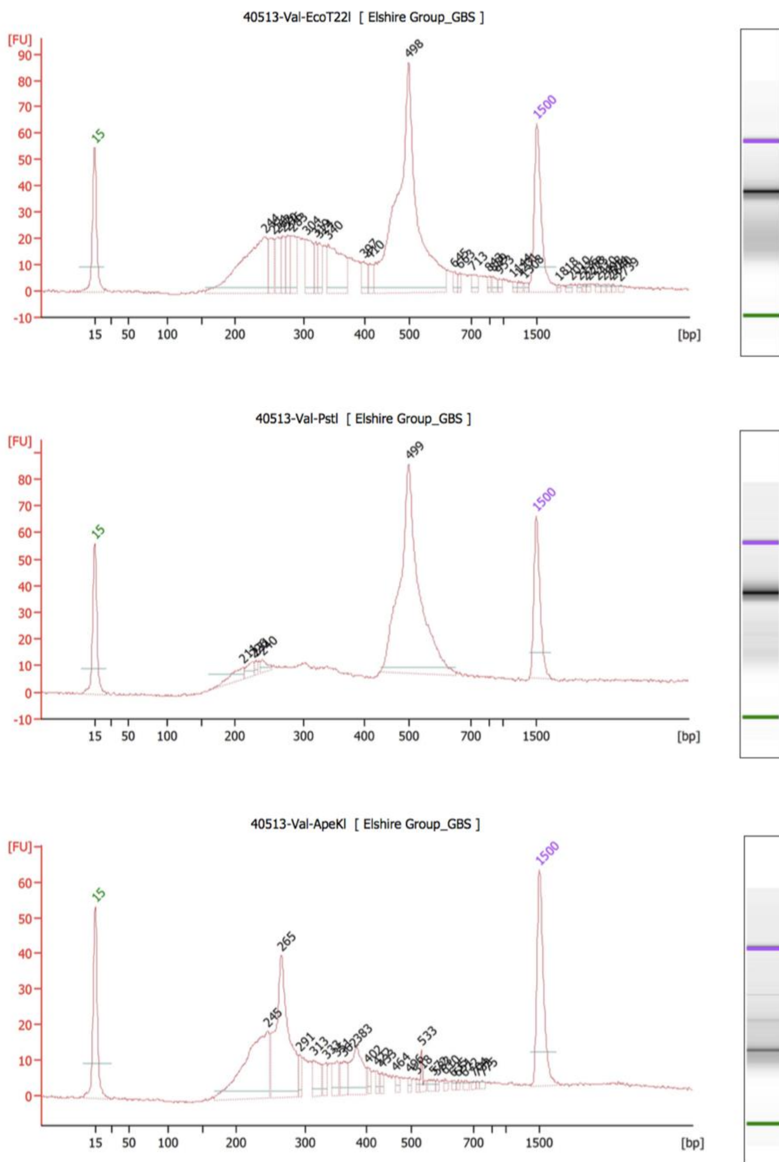
Zalewski, A., Piertney, S. B., Zalewska, H., & Lambin, X. (2009). Landscape barriers reduce gene flow in an invasive carnivore: geographical and local genetic structure of American mink in Scotland. *Molecular ecology, 18*(8), 1601-1615.

SUPPLEMENTARY MATERIAL FOR CHAPTER FOUR:

Comparison of the genetic structure of native and invasive populations of brushtail possum (*Trichosurus vulpecula*) using genotyping-by-sequencing.

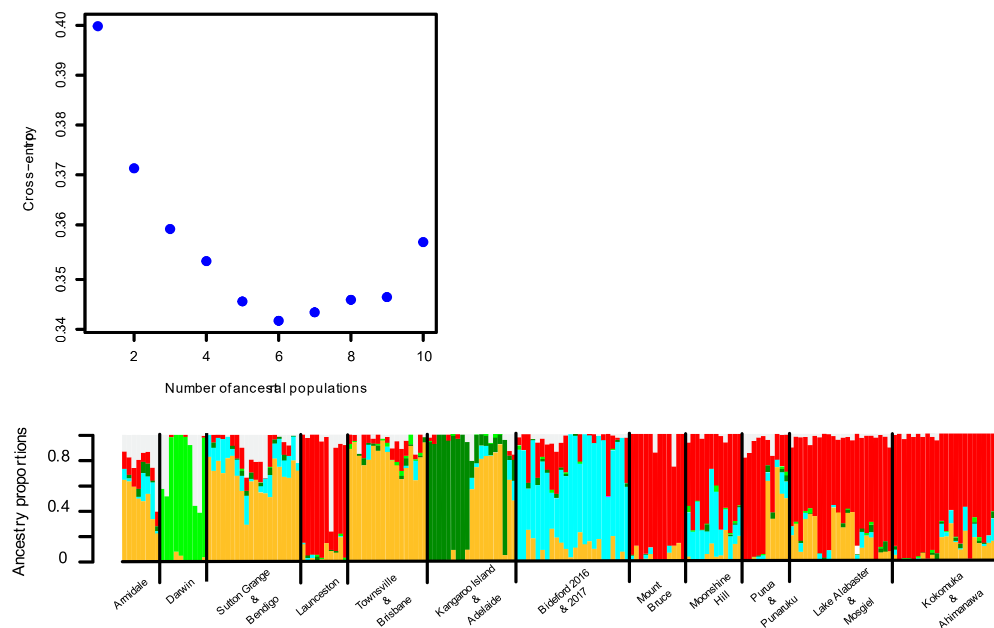
Supplementary figure 4.1

Library validation test results for the *ApeKI*, *PstI*, and *EcoT22i* restriction enzymes, using an Agilent 2100 BioAnalyzer instrument. Libraries made with *PstI* and *EcoT22i* exhibited a spike in DNA fragments around 500 bp, indicating the amplification of repetitive elements. *ApeKI* exhibited smaller spikes at 265 bp. Validation tests conducted by The Elshire Group.

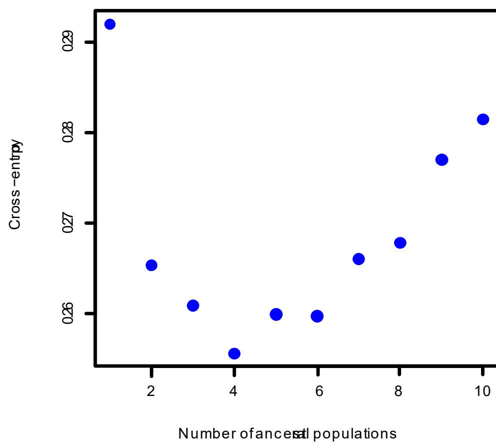


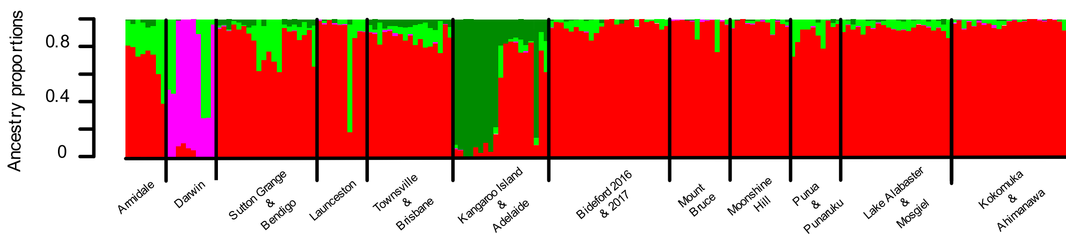
Supplementary figure 4.2 a.

Genotypic variation of native and invasive populations of brushtail possums, *Trichosurus vulpecula* using genotyping-by-sequencing. Ancestry matrix generated by LEA showing Australian and Aotearoa New Zealand samples pooled together (n =188) as a single sample group (r 0.9, p 1, **MAF 0.05**), with 766 variants (7.8% missing data) under the optimal model K = 6. Cross-entropy criterion graph above, and bar plot representing genotypic clusters below. Individuals from Darwin and Kangaroo Island (light green and dark green clusters, respectively) each clustered uniquely. The cluster most common among Aotearoa New Zealand samples was shared with Launceston samples (red cluster), while Bideford 2016 and 2017 indicated the presence of genotypes unique to Aotearoa New Zealand.



b. Genotypic variation of native and invasive populations of brushtail possums, *Trichosurus vulpecula* using genotyping-by-sequencing. Ancestry matrix generated by LEA showing Australian and New Zealand samples pooled together (n =188) as a single sample group (r 0.9, p 1, **MAF 0.01**), with 5,679 variants (7.91% missing data) under the optimal model K = 4. Cross-entropy criterion graph above, and bar plot representing genotypic clusters below. Samples from Darwin (magenta) and Kangaroo Island (dark green) clustered uniquely, leaving two clusters (red and lime green) found throughout the rest of Australia and Aotearoa New Zealand.





Supplementary table 4.1

a. Table showing all the STACKS runs conducted for this study. The table lists the number of samples (n), native/invasive population to which the dataset belongs, number of groups (i.e., sample regions using in each population map), populations settings (-p, -r), and number of loci excluded and retained for each data set after applying filters. The next column lists the mean missing data per sample for each data set, as estimated by VCFR v1.12.0. The final column identifies K, the optimal number of genotypic clusters for the dataset, estimated by LEA v 3.8.0. *Datasets with MAF 0.01. Datasets A, B, and C are shaded in grey. Lower North Island samples include Bideford (2016,2017), Mount Bruce, and Moonshine Hill.

n	Dataset	# Total groups	(-p)	(-r)	# Total loci	# Total variant loci (i.e. SNPs)	Missing data (%)	# Genotypic clusters (k)
188	Combined	1	1	0.9	510,199	776	7.83	6
188	Combined	2	1	0.9	764,222	4,864	47.18	3
84	Aus/Native	1	1	0.9	360,652	586	7.81	4
84	Aus/Native	6	1	0.9	1,226,330	27,813	76.19	6
104	Aotearoa/Invasive	1	1	0.9	745,575	3,756	7.7	3
104	Aotearoa/Invasive	4	1	0.9	1,186,372	37,053	71.19	4
188	Combined *	1	1	0.9	510,199	5,679	7.91%	4
188	Combined *	2	1	0.9	764,222	20,421	45.88%	2
84	Aus/Native *	1	1	0.9	360,652	3,030	7.73%	4
84	Aus/Native *	6	1	0.9	1,226,330	63,875	72.47%	6
104	Aotearoa/Invasive *	1	1	0.9	745,575	3,756	7.70%	2/4
104	Aotearoa/Invasive *	4	1	0.9	1,186,372	37,053	71.19%	4
188	Combined	2	2	0.8	750,804	5,996	13.66%	6
84	Aus/Native	6	2	0.8	1,198,415	62,438	57.53%	6
104	Aotearoa/Invasive	4	2	0.8	1,300,387	55,681	40.78%	4
84	Aus/Native	6	3	0.8	963,063	30,601	44.55%	6
104	Aotearoa/Invasive	4	3	0.8	1,104,626	25,591	24.61%	6
169	Combined	2	1	0.9	769,132	4,280	41.81%	3

151	Combined	8	1	0.9	1,431,281	49,296	80.28%	8
117	Combined	6	1	0.9	1,328,210	29,330	76.46%	6
48	Lower NI	1	1	0.9	820,482	9,396		
48	Lower NI	3	1	0.9	1,429,544	74,775	63.54%	3
84	Aus/Native	6	6	0.3	1,711,252	260,968	38.71%	4
84	Aus/Native	6	1	0.5	2,427,363	650,404	63.87%	
84	Aus/Native	6	6	0.5	1,235,602	78,930	28.27%	4
84	Aus/Native	6	6	0.9	62,137	39	2.14%	8
104	Aotearoa/Invasive	1	1	0.5	2,044,865	391,684	36.70%	4
104	Aotearoa/Invasive	4	4	0.5	1,695,800	187,655	29.11%	4
104	Aotearoa/Invasive	4	3	0.6	1,682,536	176,517	32.55%	4
168	Combined	1	1	0.9	551,693	812	7.58%	4
168	Combined	8	8	0.6	1,190,097	40,163	21.55%	4/3
104	Aotearoa/Invasive	4	4	0.9	444,921	772	5.15%	3
134	Combined	6	5	0.6	1,512,593	104,911	29.00%	5/6

b. Table showing all the STACKS runs conducted for datasets A2,B2 and C2. The table lists the minimum coverage depth per locus (minDP), maximum missingness allowed per locus (max missingness), minimum minor allele frequency (MAF), and the number of loci retained. The next three columns represent the number of individuals retained after filtering for missingness per individual at 50%, 60% and 70% respectively. This is followed by the mean coverage depth of each dataset. The final column identifies K, the optimal number of genotypic clusters for the dataset. Datasets A2, B2, and C2 are shaded in grey

minDP	**Max missingness (loci)**	**MAF**	**Retained loci**	**50% md (ind)**	**60% md (ind)**	**70% Md (ind)**	**Cov depth**	**k**
			All populations (n = 188)					
6	0.3	0.005	13,867	52				
5	0.4	0.005	13,018	87	126		7.9x(4.9)	
5	0.5	0.005	3,335	139			7.9x	
4	0.5	0.005	16,918	127	153		6.8x(4.2)	
6	0.5	0.005	1,193	162			22x(14)	5
4	0.3	0.005	186,064	51				
4	0.2	0.005	435,000	20				
4	0.5	0.005	16,918					5
6	0.6	0.005	620	178			23x (15)	6
6	0.6	0.008	563	177			26.5x(18)	
5	0.7	0.008	470	181			28x(19)	5
5	0.5	0.008	2,864	140			13x(8)	
5	0.6	0.008	929	173			19x(13)	
4	0.7	0.008	833	181			19x(13)	
4	0.9	0.008	178	185			49x(34)	
4	0.5	0.008	14,186	126			7x(4)	
6	0.5	0.008	1,051	162			22x(10)	5
6	0.5	0.01	961	163			20x(12)	5
5	0.5	0.01	2,573	141			13x(8)	
6	0.6	0.01	524	178			26x(18)	
5	0.4	0.01	9905	89				4

5	0.4	0.01	9905			155		5
6	0.3	0.01	10,654	51				3
6	0.3	0.01	10,654			124		4
Australia (n = 84)								
6	0.4	0.005	2,106	49			16x(9)	3
6	0.5	0.005	955	70			22x(14)	4
5	0.6	0.005	779	77			19x(13)	4
5	0.5	0.005	1958	61			15x(9)	3
4	0.7	0.005	723					
4	0.5	0.005	8944	55				
4	0.5	0.005	8944		65		7.5x(4)	3
6	0.7	0.01	303	78			32.77x(21)	4
6	0.5	0.01	808	70			23.1(14)	5
5	0.7	0.01	408					
5	0.5	0.01	1582	65	73		15x(9)	
4	0.5	0.01	6888	65			7.9(4)	
6	0.5	0.05	468	71			23x(14)	
5	0.5	0.05	856	65	73		16x(10)	
5	0.3	0.05	2223	65			11x(7)	
4	0.5	0.05	3498	56				
Aotearoa New Zealand (n = 104)								
6	0.5	0.005	1884	83			14x(8)	2
5	0.7	0.005	659	102			20x(13)	4
5	0.5	0.005	7387	69			8.8x(5)	2
6	0.6	0.01	600	101			20x(13)	5
6	0.5	0.01	1,500	87			14x(8)	2
5	0.7	0.01	577	102			20.5x(13)	3
5	0.4	0.01	8078			91	8.3x(4)	2
5	0.5	0.01	5361	72			9x(5.2)	2
6	0.7	0.05	270	104			27x(18)	2
6	0.5	0.05	934	91			16x(9)	2
5	0.7	0.05	388	103			22x(14)	2
5	0.5	0.05	299	76			10x(5)	2

4	0.9	0.05	102	104		37x(23)	2
4	0.5	0.05	3849	92		8x(5)	2
			Combined (n = 168)				
5	0.6	0.016	882	157		18x(12)	3
5	0.4	0.016	5833		139	8.8x(5.6)	3
5	0.6	0.01	811	157		19.3x(13.6)	3
5	0.4	0.01	5184		140	10.23(5.9)	3

Supplementary table 4.2

Pairwise genetic differentiation (F_{ST}) between populations of brushtail possums in the three datasets (A, B, and C) used in this study calculated using HIERFSTAT v0.5-11 (Goudet et al. 2015) in R v4.2.0. Values in grey are non-significant from zero. Observed and expected heterozygosity estimates for each dataset within tables.

a) Dataset A- Australia/ native population; n = 84, -r 0.5, p 6, 78,930 SNPs. SG&B- Sutton Grange and Bendigo, B&T- Brisbane and Townsville.

	Armidale	Darwin	SG&B	Launceston	B&T	Kangaroo	Ho	He
Armidale							0.0423	0.0789
Darwin	0.695						0.037	0.096
SG&B	0.0569	0.661					0.068	0.111
Launceston	0.278	0.742	0.214				0.039	0.059
B&T	0.084	0.658	0.061	0.244			0.062	0.108
Kangaroo	0.411	0.684	0.371	0.492	0.386		0.109	0.159
Adelaide	0.108	0.645	0.081	0.264	0.090	0.348	0.076	0.127

b) Dataset B- Aotearoa New Zealand/ Invasive population; n = 104, -r 0.9, p 4, 772 SNPs. Bid-Bideford, MBr-Mount Bruce, MH- Moonshine Hill, Pur- Purua, Pun- Punaruku, Mos- Mosgiel,LAl- Lake Alabaster, Kok- Kokomuka, Ahi- Ahimanawa.

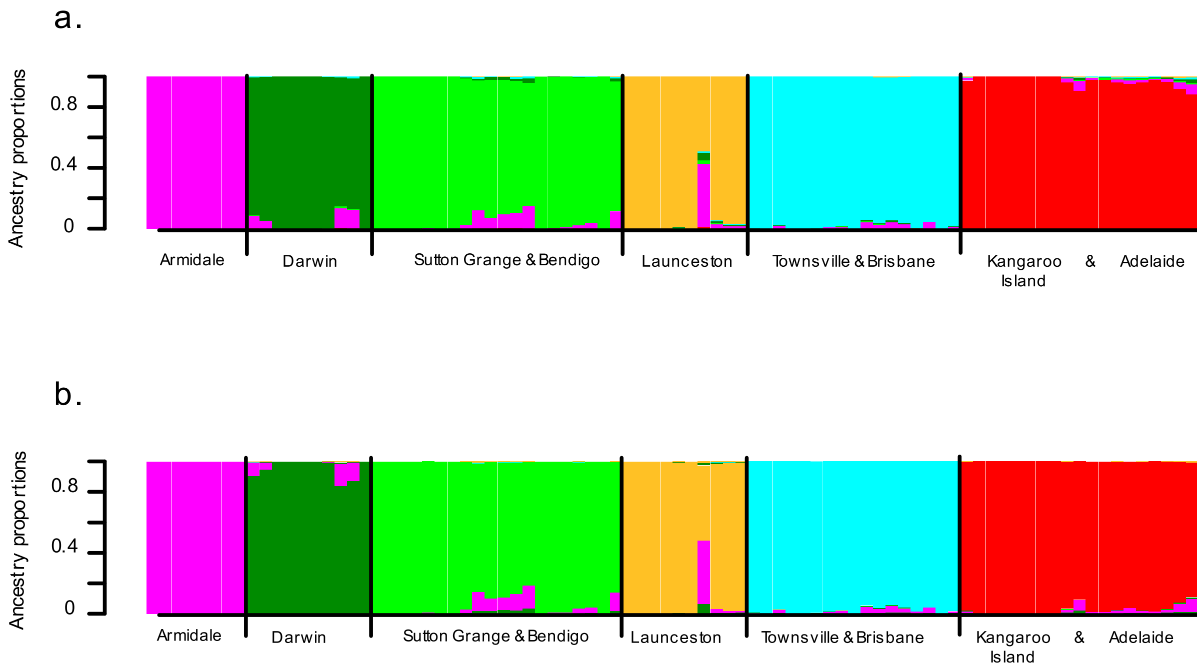
	Bid16	Bid17	MBr	MH	Pur	Pun	Mos	LAl	Kok	Ho	He
Bid16										0.204	0.209
Bid17	0.004									0.237	0.232
MBr	0.107	0.128								0.145	0.123
MH	0.048	0.067	0.049							0.175	0.158
Pur	0.083	0.105	0.047	0.025						0.132	0.105
Pun	0.079	0.090	0.124	0.083	0.096					0.165	0.165
Mos	0.090	0.113	0.061	0.047	0.054	0.075				0.141	0.122
LAl	0.095	0.122	0.456	0.040	0.036	0.111	0.041			0.131	0.111
Kok	0.118	0.144	0.050	0.058	0.046	0.150	0.069	0.039		0.118	0.092
Ahi	0.091	0.106	0.056	0.045	0.039	0.075	0.047	0.052	0.073	0.164	0.150

c) Dataset C- combined; n = 134, -r 0.6, p 5, 104,911 SNPs. Laun- Launceston, S.Isl- South Island (Lake Alabaster & Mosgiel)

	SG&B	Laun	Bid16	Bid17	MBr	MH	Pur	Pun	S.Isl	Kok	Ho	He
SG&B											0.091	0.141
Laun	0.216										0.093	0.130
Bid16	0.089	0.146									0.122	0.189
Bid17	0.097	0.162	0.0029								0.151	0.215
MBr	0.215	0.061	0.143	0.154							0.115	0.156
MH	0.136	0.081	0.055	0.068	0.072						0.124	0.173
Pur	0.194	0.069	0.107	0.122	0.066	0.037					0.084	0.119
Pun	0.102	0.245	0.117	0.117	0.225	0.150	0.214				0.095	0.149
S.Isl	0.133	0.074	0.097	0.118	0.066	0.038	0.040	0.165			0.091	0.134
Kok	0.263	0.082	0.179	0.189	0.085	0.100	0.092	0.280	0.089		0.093	0.128
Ahi	0.137	0.075	0.090	0.103	0.072	0.046	0.047	0.141	0.047	0.103	0.138	0.186

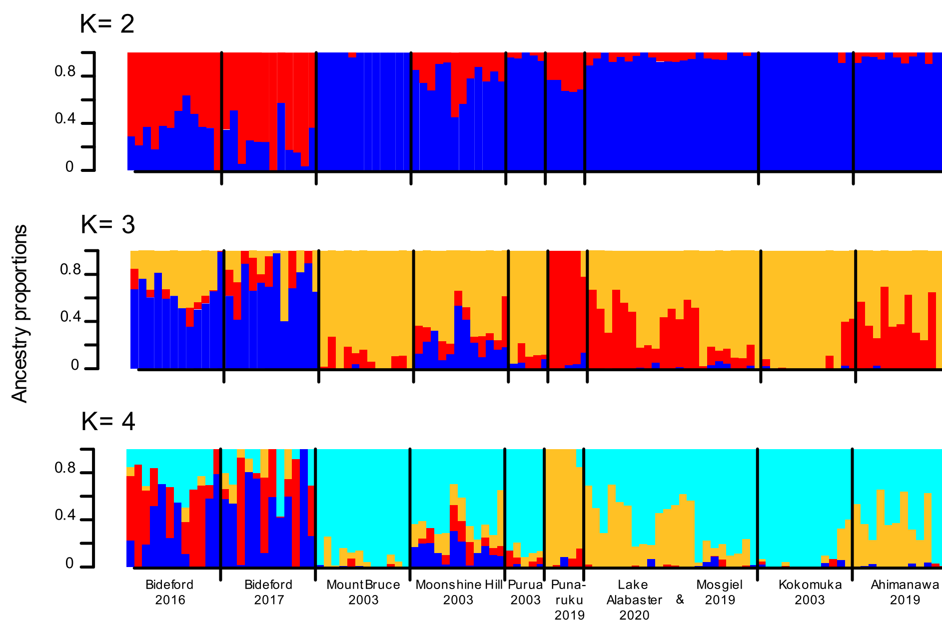
Supplementary figure 4.3

Ancestry matrices generated by LEA showing native Australian samples (n =84) under two conditions. **a.** r 0.9, p 1, MAF 0.05 with 27,813 variants (76.19% missing data) under the optimal model K = 6. **b.** r 0.9, p 1, MAF 0.01 with 63,875 variants (72.47% missing data) under the optimal model K = 6. Both datasets show very similar genotypic cluster distributions.



Supplementary figure 4.4

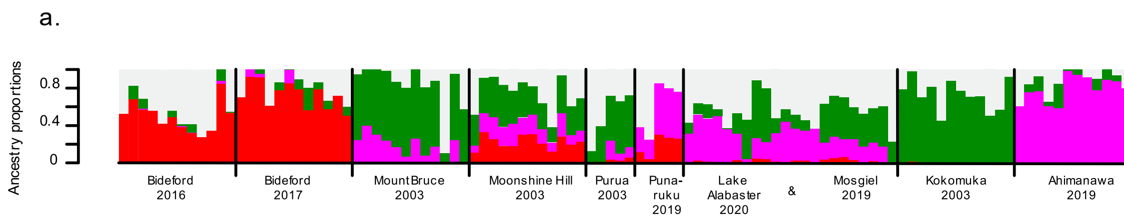
Genotypic variation of invasive populations of brushtail possums, *Trichosurus vulpecula* across Aotearoa New Zealand. Ancestry matrices generated by LEA under the conditions r 0.9 and p 4 with 772 variants (5.15% md) which resulted in in K = 3 optimal clusters. Bideford and Moonshine Hill share a unique cluster while the other two clusters are shared among all Aotearoa New Zealand possums. K= 2 and K = 4 while showing similar pattern of population clustering, show lower and higher rates of admixture, respectively, within Bideford and Moonshine Hill populations.



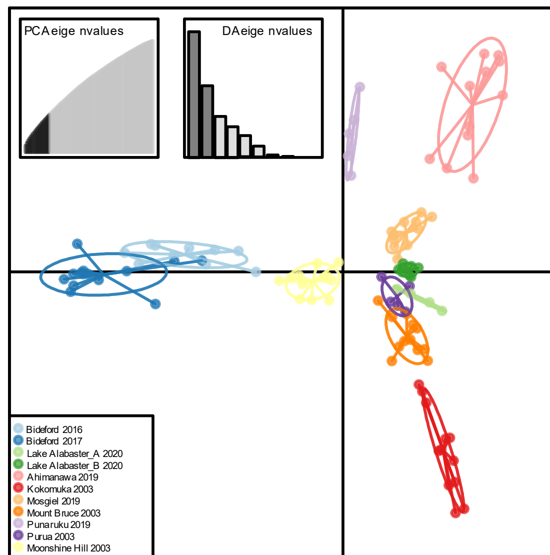
Supplementary figure 4.5

Genotypic variation of invasive populations of brushtail possums, *Trichosurus vulpecula* across Aotearoa New Zealand. Ancestry matrix generated by LEA showing four Aotearoa New Zealand population groups under r 0.5 and p 4 with 187,655 variants (29.11% md), resulting in **a.** K =4 optimal clusters, with one cluster (grey) representing missing data. **b.** Discriminant Principal components analysis plot with the same data.

a.

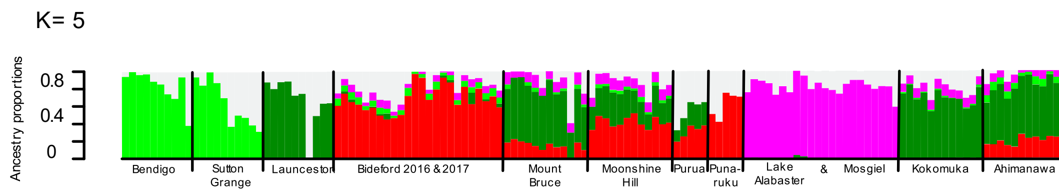


b.



Supplementary figure 4.6

Genotypic variation of invasive populations of brushtail possums, *Trichosurus vulpecula* in Sutton Grange and Bendigo, Launceston, and Aotearoa New Zealand. Ancestry matrix generated by LEA showing four New Zealand population groups under r 0.6 and p 5 with 104,911 variants (29% missing data), here representing K=5 optimal clusters, with one cluster (grey) representing missing data. This figure emphasises that the difference between K =5 and K= 6 is the levels of admixture that exist in Mount Bruce, Moonshine Hill, and Ahimanawa populations are higher.



Chapter Five

Thesis Discussion

The invasion of a species is not just an introduction into a non-native range, but the success with which a species spreads, competes for and consumes natural resources, and negatively impact upon the affected ecosystem. Only recently has the purview of genetics been considered for invasive pest management (Barrett 2016; Burgess et al. 2021). While the introduction of a subset of genetic diversity from a source population could result in founder effects in establishing populations (Nei et al. 1975), there is plenty of evidence to support invasion species' success in a novel environment (see reviews- Dlugosch & Parker 2008; Roman & Darling 2007; Simberloff 2009). This success could result from a number of factors including the scale of invasions, the multiple complex routes of invasions and the resulting patterns of range expansion that contrast with their those of their native landscapes (Michaelides et al. 2018; Sherpa & Després 2021; Shirk et al. 2014; Suarez & Tsutsui 2008). There is value in contrasting genetic diversity of a species in their native and invasive ranges, especially when historical native samples are available, as this could provide context to interpreting the evolutionary potential of a species in its invasive habitat as well as assessing patterns of gene flow and determining source populations for establishing populations (Cristescu 2016; Gatto-Almeida et al. 2022; Hagenblad et al. 2015; McCann et al. 2018; Michaelides et al. 2018; Prentis et al. 2008; Puillandre et al. 2008; Sakai et al. 2001).

The three data chapters in this thesis focused on understanding the population genetic structure of brushtail possums after more than 150 years since deliberate introductions from Australia into Aotearoa New Zealand. Brushtail possums (*Trichosurus vulpecula*) in Aotearoa New Zealand provide a major *in natura* experiment, as there is a well-recorded history of introductions and further translocations within a country with no natural predators and abundant potential for foraging.

What has become of this generalist marsupial species under these conditions, especially when we consider their declining source populations in Australia, where they are endangered and protected. I included three different geographic and temporal scales to address this question.

Main Conclusions

In chapter two, I aimed to test reproductive segregation among possums displaying the phenotypic traits of recognised subspecies, by surveying gene flow in restricted populations on the Kenepuru Peninsula (400km^2). I used genetic markers- seven microsatellite markers and the control region of the mtDNA- on samples from 132 individuals to determine if there was any assortative mating based on fur, time or geographic differences. Evidence from both nDNA and mtDNA diversity estimates indicated high levels of genetic variation, and pairwise differentiation tests using both markers showed no evidence of genetic partitioning. mtDNA sequences showed presence of two major haplogroups Additionally, morphological evidence determined that while there are predominantly two colour morphs (suggested to be from the two source populations that possums were introduced from), possums on the island are one freely interbreeding population for the purposes of efficient pest management towards eradication. Furthermore, there was a lack of partitioning linked to geography, time or morphology. Since my work was published (Pattabiraman et al. 2021), a new study of possums in the Taranaki-Egmont Ring Plain, New Zealand attempted to associate spatial distribution of possums to the different coat colours (Veale & Etherington 2023). The Genome Wide Association Study (GWAS) that identified the expected Agouti Signalling Protein gene (ASIP) on Chromosome 3 as responsible for coat colour expression in possums, as is the case in many other mammalian species (Cieslak et al. 2011). The Taranaki study using a large number of nuclear loci found no spatial pattern in the distribution of possum coat colour, in agreement with the findings on Kenepuru using seven nuclear microsatellite markers and one mitochondrial marker (Pattabiraman et al. 2022)

Kenepuru did not have any pronounced landscape features that might have hindered the movement of possums across the island. While the Kenepuru study area had little connection to the mainland, the Taranaki study was conducted in a very different landscape with potential barriers to dispersal (Veale & Etherington 2023). The authors note that forest patches appear to maintain metapopulations intersected by open pastureland suggesting that human land use has a significant role in partitioning possums. Similar genetic studies of the invasive pest American mink (*Neovison vison*) in Scotland identified management units for directing eradication efforts (Fraser et al. 2013), however, after culling genetic data revealed an increase in long-distance compensatory immigration (Oliver et al. 2016). Thus, ongoing refinement of management models using population genetics data is required for pest eradication.

In chapter three, I set out to determine the pattern of population genetic structure and evidence of admixture among brushtail possums across their invasive range in New Zealand after ~110 generations of introduction from their native Australian source populations. For this study, I used the seven microsatellite markers on Aotearoa possum samples and the mtDNA marker on both native and invasive populations. Haplotypic mtDNA variation showed that New Zealand possums were nested within diversity of southeast Australian possums, and they were most closely related to source populations from Victoria and Tasmania. Additionally, multiple haplotypes existed only in New Zealand (high haplotype diversity), suggests that a large subset of source population genetic diversity was introduced to New Zealand and rapid population growth meant the bottleneck was very short. In contrast to the genetic homogeneity found on a geographically small scale (Chapter Two), there was a signal of regional partitioning indicated by presence of four genotype clusters, but little spatial corelation of these clusters. Genetic structure among populations could serve to identify eradication units to target management effort as seen in other invasive species (Browett et al. 2020). For example, invasive swine populations in USA showed heterogeneity in genetic partitioning on a regional scale, and also showed a hierarchical genetic structure that points to source populations. This information could be used to inform management for isolation and targeting of populations (McCann et al. 2018). However the evidence at this scale for brushtail possums suggests a pronounced spatial structure and lack of homogeneity at a regional level. This type of structure has been reported in other invasive species such as feral pigs in USA and Australia, invasive plants (*Lupinus polyphyllus* and *Heracleum sosnowskyi* sp.) in Russia and Ukraine, gammarids in Europe, and stone martens in Poland (Baltazar-Soares et al. 2017; Hampton et al. 2004; McCann et al. 2018; Osipova et al. 2021; Wereszczuk et al. 2017). Although mtDNA data are not always perfect for identifying the origin of mammal invasions because stochastic lineage sorting and hybridisation can obscure the historical signal (Browett et al. 2020), the spatial separation of mtDNA lineages in Australia suggests my mtDNA data accurately aligns with historical records about the source populations of possums translocated to New Zealand (Carmelet-Rescan et al. 2022). The indications about the genetic potential of invasive individuals to tolerate poison applied during management or eradication is valuable information (Carmelet-Rescan et al. 2022; Morgan et al. 2018).

The results from Chapter Two and Chapter Three provide foundational insight of the genetic distribution of brushtail possums that has not been previously observed at this scale. However, it was apparent that a more richly sampled genomic data set would provide more detailed tests of genetic diversity.

Chapter Four

Individual SNP loci may have lower mutation rates than microsatellite markers and so are less informative, but the modern capacity to generate data for very large numbers of SNPs can outweigh this limitation. There is evidence that supports this for non-model organisms with low sample sizes, where more SNPs can be generated (Andrews et al. 2016; Morin et al. 2004; Morin & McCarthy 2007). On comparing the performance of the two types of markers, one study found RAD-seq data to be able to discern fine-scale population genetic structure compared to microsatellites, with fewer samples (Sunde et al. 2020). Another study that determined that no greater than eight samples were required to generate thousands of SNPs for a non-model Amazonian plant species that had no reference genome (Nazareno et al. 2017). Furthermore, a study comparing genetic diversity estimates (allele frequencies, expected heterozygosities) between four microsatellite datasets from different species and variable sample sizes, recommended using 25-30 individuals per population (Hale et al. 2012). Nevertheless, microsatellite markers do have their advantage for parentage analyses and are able to resolve population-level structure in species with moderate genetic diversity estimates (Haasl & Payseur 2011; Hauser et al. 2021; Lemopoulos et al. 2019).

I resolved the underlying genetic structure of brushtail possums in their native and invasive ranges by using high-resolution genomic markers. I generated SNP data using GBS with possum samples from their native range in Australia (n = 84) and their invasive range in Aotearoa New Zealand (n=104) and conducted detailed population genomics analyses on three datasets that included various permutations of those sample groups.

The first dataset produced diversity indices and genetic differentiation that showed samples from Darwin (Northern Territory) and Kangaroo Island (South Australia) to cluster uniquely, with two clusters running through southeast Australia and another cluster predominantly through Tasmania (not found in Chapter Three). New Zealand populations showed less admixture compared to the results found with seven microsatellite markers (Chapter Three), with a unique cluster belonging to the Bideford and other parts of the lower North Island. This unique cluster continues to be reflected by the third dataset, and additionally indicating that the cluster possessed by Tasmanian possums is more widespread among Aotearoa New Zealand possums. Clusters belonging to Victorian possums had a very minor presence in New Zealand samples. The detection of greater levels of diversity and structure using SNPs compared to mtDNA was also demonstrated in the study of invasive racoons in Europe that discovered that historical records were misleading (Fischer et al. 2015).

Considerations for future research

This work has put the invasion of brushtail possums into context with their spatial (regional) and temporal pattern of genetic structure and juxtaposed it with historical source samples. It has utilised a variety of available sampling and established the suitability of different markers for the analysis of an exceptional invasion system containing high levels of genetic variation. While we have used genetic differentiation of neutral loci as an indirect indicator of gene flow, further work needs to explore the process of gene flow in relation to spatial and environmental factors (eg- Peakall et al. 2003; Spear et al. 2010) and in particular in response to the intense local population control measures that are applied (Rollins et al. 2006; Slatkin 1987). Critically, careful experimental design is needed that achieves appropriate spatial and temporal sampling that takes into consideration the generation time, landform and habitat quality, plus external manipulations.

In a preliminary examination of fine scale analysis of gene flow in brushtail possums, I analysed data from the southern North Island samples included in my current GBS dataset (Chapter four) which shows much promise with high estimates of genetic diversity as shown by nuclear data (microsatellite and GBS) results (Chapter three and chapter four). I extracted data for 48 possums collected from Mount Bruce (2003), Moonshine Hill (2003) and Bideford-Masterton area (2016 and 2017). This sampling spans an area of about 1881.476 km^2 with linear distances between sites of 157.91 km with a mix of vegetation types including pasture and native bush. This area (Wellington Region) has been subject to persistent possum management to reduce their numbers as part of the national TB control programme which has completed eight operations across New Zealand, three in West Coast of the South Island (OSPRI 2023). By altering my Stacks settings to discern population genetic structure at a regional scale, I observed two scenarios:

a. The first considered four population groups (n=48) and required loci to be present in at least two populations (-p 2) and at least 90 percentage of individuals (-r 0.9). This setting produced 50,127 variants (44.2% missing data) and produced the outcome as shown by Figure 5.1.

While this setting proved that there were four different clusters and genetic distribution of clusters was restricted between the populations, this dataset also had a high percentage of missing data.

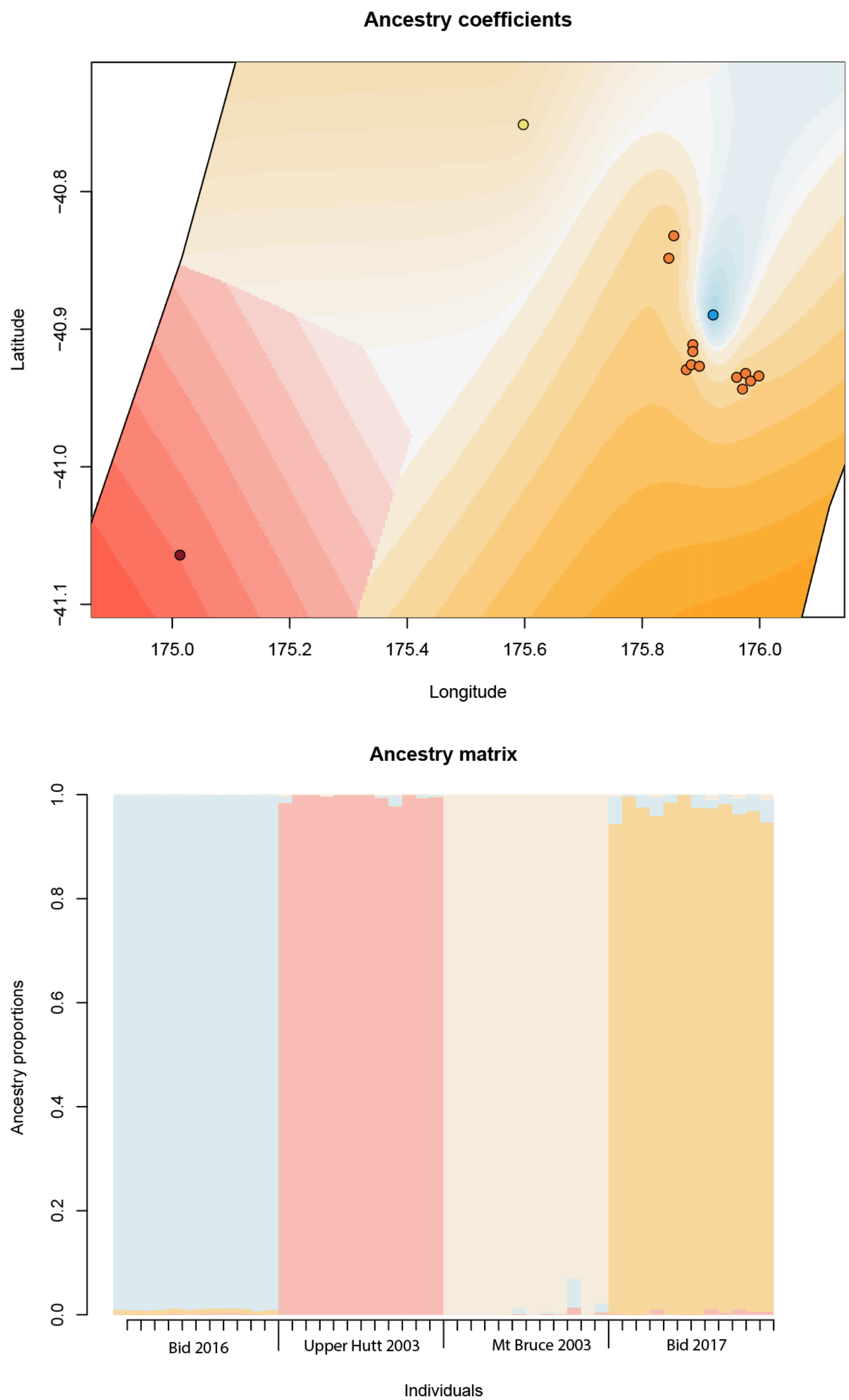


Figure 5.1 a Genotypic variation of invasive populations of brushtail possums, *Trichosurus vulpecula* across the Tararua region. Ancestry matrices generated by Tess3r showing four groups (based on location and time of collecting) under -r 0.9 and -p 4 with 50,127 variants (44.2% md), also resulting in **b.** K = 4 optimal clusters. Genetic structure seems to completely separate based on four different population groups.

b. When samples from 2016 and 2017 just in the Bideford area (640.53 km^2) were considered as a sample group (n=24), our Stacks setting required all the loci to be present in that one group (-p 1) and in at least 90 percentage of individuals of the group (-r 0.9). This resulted in 71,531 variants (13.5% missing data) that provided two main clusters differentiating the 2016 from the 2017 samples (Figure 5.2).

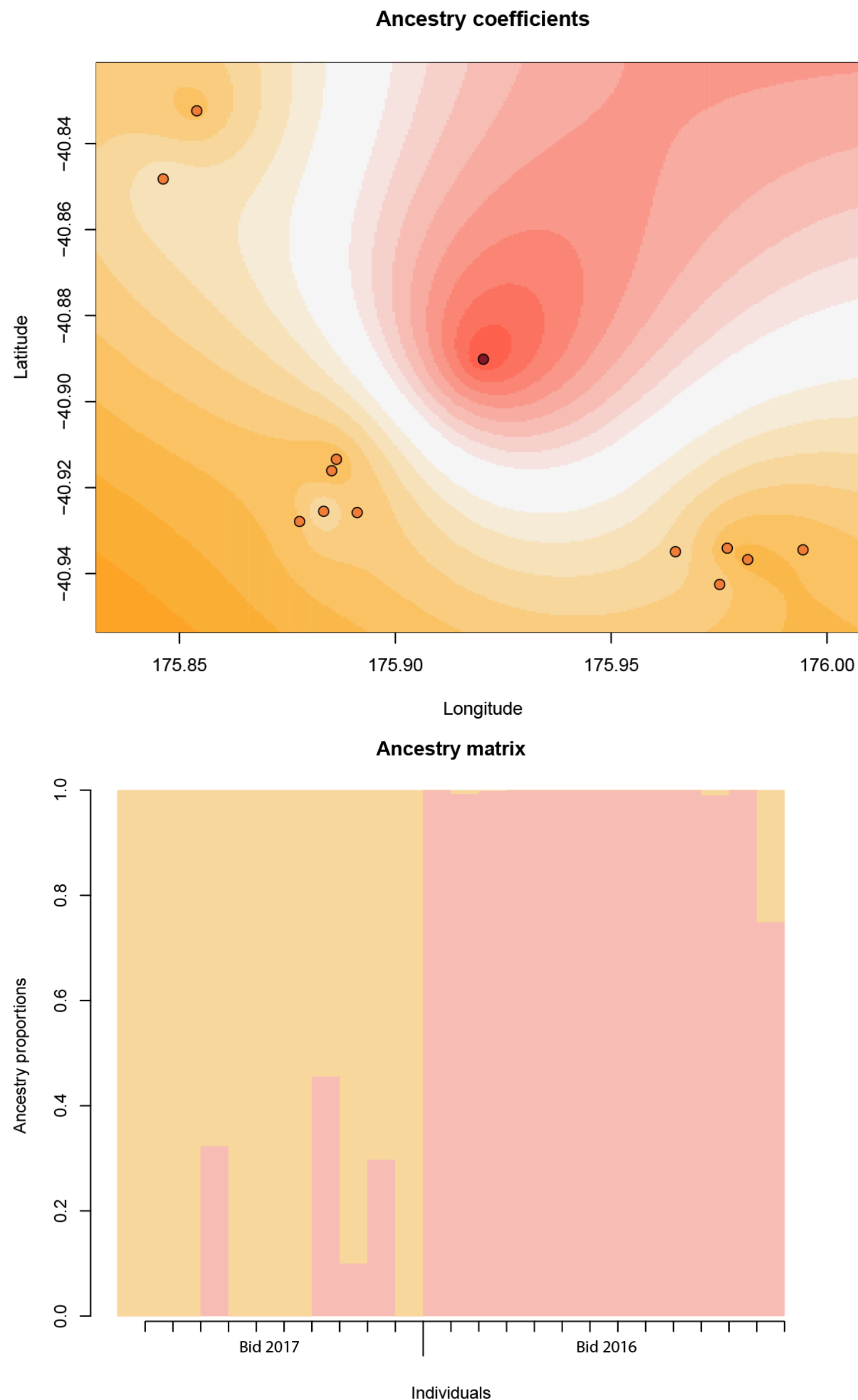


Figure 5.2 b Genotypic variation of invasive populations of brushtail possums, *Trichosurus vulpecula* across the Bideford-Masterton area. Ancestry matrices generated by Tess3r showing two groups (based on location and time of collecting) under -r 0.9 and -p 1 with 71,531 variants (13.5% md), also resulting in **b.** K = 2 optimal clusters.

While we see that individual location points for 2017 samples gives us a more accurate image of genetic population structure in the area, the various layers indicating grouping of 2016 samples makes it difficult to identify the actual distribution of genetic diversity and gene flow between various sample sites. This level of sampling and analysis, while providing an outline of how genetic diversity is distributed, does not provide information that could directly impact management decisions. As population structure is affected by dispersal and gene flow among these populations (Lawson Handley et al. 2011), it would be beneficial to conduct experiments which involve carefully managed sampling in space and time to test the relative contributions to population recovery from local reproduction vs migration. A useful addition to this current dataset could be to extend sample collections on either side of the Cook Strait to fill the gaps in information about movement of brushtail possums between the two main New Zealand islands. Moreover, increasing sample collection in the South Island will enhance our understanding of overall diversity indices.

The presence of high genetic diversity among the populations at a regional level could aid in the development of local adaptation (Sakai et al. 2001). For example, earlier studies of brushtail possums in Australia found physiological differences with respect to processing plant metabolites and showing higher tolerance to plant toxins between possums of the same species from geographically separated populations (DeGabriel et al. 2009; King et al. 1978; McIllroy et al. 1983), which has implications for control efforts in New Zealand. High diversity in a extensively dispersive species could mean more gene flow between populations (Sakai et al. 2001). However, the rate and extent of this gene flow could be better measured by detailing migration patterns and effective gene immigration into new populations (Kawai & Ebert 2004). For the future of this project, it would mean non-lethal collecting of genetic information for metapopulations before control measures and monitoring the area for changes in genetic diversity during and after control efforts. Ultimately, this could be used to determine the cause of successful eradications or reinvasion pathways from failed eradication efforts (Burgess et al. 2021).

For brushtail possums in New Zealand, there is an advantage of having a reference genome as well as samples from both native and invasive ranges, with a well-recorded introduction history. This creates opportunities to delve into the area of invasion genomics by identifying functional aspects of the genome that contribute to phenotypic differences between sample groups and confirming the role of heterozygosity in invasion success (Carmelet-Rescan 2023; Biedrzycka et al. 2022; Matheson & McGaughran 2022; Prentis et al. 2008). Additionally, a genetically informed understanding of patterns of invasion with respect to the environment and demographic modelling

could provide support for well-informed management decisions (North et al. 2021; Selechnik et al. 2019; Sillero et al. 2020).

References

Andrews, K. R., Good, J. M., Miller, M. R., Luikart, G., & Hohenlohe, P. A. (2016). Harnessing the power of RADseq for ecological and evolutionary genomics. *Nature Reviews Genetics, 17*(2), 81-92.

Baltazar-Soares, M., Paiva, F., Chen, Y., Zhan, A., & Briski, E. (2017). Diversity and distribution of genetic variation in gammarids: Comparing patterns between invasive and non-invasive species. *Ecology and Evolution, 7*(19), 7687-7698.

Barrett, S. C. (2016). Foundations of invasion genetics: the Baker and Stebbins legacy. *Invasion genetics: the Baker and Stebbins legacy*, 1-18.

Biedrzycka, A., Fijarczyk, A., Kloch, A., & Porth, I. M. (2022). Genomic basis of adaptations to new environments in expansive and invasive species. In (Vol. 10): Frontiers Media Sa Avenue Du Tribunal Federal 34, Lausanne, Ch-1015, Switzerland.

Browett, S. S., O'Meara, D. B., & McDevitt, A. D. (2020). Genetic tools in the management of invasive mammals: recent trends and future perspectives. *Mammal Review, 50*(2), 200-210.

Burgess, B. T., Irvine, R. L., Howald, G. R., & Russello, M. A. (2021). The promise of genetics and genomics for improving invasive mammal management on islands. *Frontiers in Ecology and Evolution, 9*, 704809.

Carlmelet-Rescan, D. (2023). Genomic differentiation of brushtail possum (*Trichosurus vulpecula*) populations [Unpublished doctoral dissertation]. Massey University, New Zealand.

Carmelet-Rescan, D., Morgan-Richards, M., Pattabiraman, N., & Trewick, S. A. (2022). Time-calibrated phylogeny and ecological niche models indicate Pliocene aridification drove intraspecific diversification of brushtail possums in Australia. *Ecology and Evolution, 12*(12), e9633.

Cieslak, M., Reissmann, M., Hofreiter, M., & Ludwig, A. (2011). Colours of domestication. *Biological reviews, 86*(4), 885-899.

Cristescu, M. E. (2016). Genetic reconstructions of invasion history. *Invasion genetics: the Baker and Stebbins legacy*, 267-282.

Dlugosch, K. M., & Parker, I. M. (2008). Invading populations of an ornamental shrub show rapid life history evolution despite genetic bottlenecks. *Ecology Letters, 11*(7), 701-709.

Fischer, M. L., Hochkirch, A., Heddergott, M., Schulze, C., Anheyer-Behmenburg, H. E., Lang, J., . . . Hoffmann, L. (2015). Historical invasion records can be misleading: genetic evidence for multiple introductions of invasive raccoons (Procyon lotor) in Germany. *PloS one, 10*(5), e0125441.

Gatto-Almeida, F., Pichlmueller, F., Bodey, T. W., Samaniego, A., & Russell, J. C. (2022). The tails of two invasive species: genetic responses to acute and chronic bottlenecks. *Biological Invasions, 24*(10), 3263-3273.

Haasl, R. J., & Payseur, B. A. (2011). Multi-locus inference of population structure: a comparison between single nucleotide polymorphisms and microsatellites. *Heredity, 106*(1), 158-171.

Hagenblad, J., Hülskötter, J., Acharya, K. P., Brunet, J., Chabrerie, O., Cousins, S. A., . . . Hermy, M. (2015). Low genetic diversity despite multiple introductions of the invasive plant species Impatiens glandulifera in Europe. *BMC genetics, 16*(1), 1-16.

Hale, M. L., Burg, T. M., & Steeves, T. E. (2012). Sampling for microsatellite-based population genetic studies: 25 to 30 individuals per population is enough to accurately estimate allele frequencies.

Hampton, J. O., Spencer, P. B., Alpers, D. L., Twigg, L. E., Woolnough, A. P., Doust, J., Pluske, J. (2004). Molecular techniques, wildlife management and the importance of genetic population structure and dispersal: a case study with feral pigs. *Journal of Applied Ecology, 41*(4), 735-743.

Hauser, S. S., Athrey, G., & Leberg, P. L. (2021). Waste not, want not: Microsatellites remain an economical and informative technology for conservation genetics. *Ecology and Evolution, 11*(22), 15800-15814.

Lawson Handley, L.-J., Estoup, A., Evans, D., Thomas, C., Lombaert, E., Facon, B., . . . Roy, H. (2011). Ecological genetics of invasive alien species. *BioControl, 56*, 409-428.

Lemopoulos, A., Prokkola, J. M., Uusi-Heikkilä, S., Vasemägi, A., Huusko, A., Hyvärinen, P., . . . Vainikka, A. (2019). Comparing RADseq and microsatellites for estimating genetic diversity and relatedness—Implications for brown trout conservation. *Ecology and Evolution, 9*(4), 2106-2120.

Matheson, P., & McGaughran, A. (2022). Genomic data is missing for many highly invasive species, restricting our preparedness for escalating incursion rates. *Scientific Reports, 12*(1), 13987.

McCann, B. E., Smyser, T. J., Schmit, B. S., Newman, R. A., Piaggio, A. J., Malek, M. J., . . . Simmons, R. B. (2018). Molecular population structure for feral swine in the United States. `*The Journal of Wildlife Management, 82*(4), 821-832.

Michaelides, S. N., Goodman, R. M., Crombie, R. I., & Kolbe, J. J. (2018). Independent introductions and sequential founder events shape genetic differentiation and diversity of the invasive green anole (Anolis carolinensis) on Pacific Islands. *Diversity and Distributions, 24*(5), 666-679.

Morgan, A. P., Didion, J. P., Hughes, J. J., Searle, J. B., Jolley, W. J., Campbell, K. J., . . . Pardo Manuel de Villena, F. (2018). Genetic characterization of invasive house mouse populations on small islands. *BioRxiv*, 332064.

Morin, P. A., Luikart, G., Wayne, R. K., & Group, S. W. (2004). SNPs in ecology, evolution and conservation. *Trends in ecology & evolution, 19*(4), 208-216.

Morin, P. A., & McCarthy, M. (2007). Highly accurate SNP genotyping from historical and low-quality samples. *Molecular ecology notes, 7*(6), 937-946.

Nazareno, A. G., Bemmels, J. B., Dick, C. W., & Lohmann, L. G. (2017). Minimum sample sizes for population genomics: an empirical study from an Amazonian plant species. *Molecular ecology resources, 17*(6), 1136-1147.

Nei, M., Maruyama, T., & Chakraborty, R. (1975). The bottleneck effect and genetic variability in populations. *Evolution*, 1-10.

North, H. L., McGaughran, A., & Jiggins, C. D. (2021). Insights into invasive species from whole-genome resequencing. *Molecular ecology, 30*(23), 6289-6308.

Osipova, E. S., Stepanova, A. Y., Tereshonok, D. V., Gladkov, E. A., & Vysotskaya, O. N. (2021). Genetic Diversity in Invasive Populations of Lupinus polyphyllus Lindl. and Heracleum sosnowskyi Manden. *Biology, 10*(11), 1094.

OSPRI, 2023. *National aerial pest control operations*. https://www.ospri.co.nz/tb-and-pest-control/aerial-operations/

Pattabiraman, N., Morgan-Richards, M., Powlesland, R., & Trewick, S. A. (2022). Unrestricted gene flow between two subspecies of translocated brushtail possums (*Trichosurus vulpecula*) in Aotearoa New Zealand. *Biological Invasions, 24*(1), 247-260.

Peakall, R., Ruibal, M., & Lindenmayer, D. B. (2003). Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat, Rattus fuscipes. *Evolution, 57*(5), 1182-1195.

Prentis, P. J., Wilson, J. R., Dormontt, E. E., Richardson, D. M., & Lowe, A. J. (2008). Adaptive evolution in invasive species. *Trends in plant science, 13*(6), 288-294.

Puillandre, N., Dupas, S., Dangles, O., Zeddam, J.-L., Capdevielle-Dulac, C., Barbin, K., . . . Silvain, J.-F. (2008). Genetic bottleneck in invasive species: the potato tuber moth adds to the list. *Biological Invasions, 10*, 319-333.

Rollins, L. A., Woolnough, A. P., & Sherwin, W. B. (2006). Population genetic tools for pest management: a review. *Wildlife research, 33*(4), 251-261.

Roman, J., & Darling, J. A. (2007). Paradox lost: genetic diversity and the success of aquatic invasions. *Trends in ecology & evolution, 22*(9), 454-464.

Sakai, A. K., Allendorf, F. W., Holt, J. S., Lodge, D. M., Molofsky, J., With, K. A., . . . Ellstrand, N. C. (2001). The population biology of invasive species. *Annual review of ecology and systematics, 32*(1), 305-332.

Selechnik, D., Richardson, M. F., Shine, R., DeVore, J. L., Ducatez, S., & Rollins, L. A. (2019). Increased adaptive variation despite reduced overall genetic diversity in a rapidly adapting invader. *Frontiers in genetics, 10*, 1221.

Sherpa, S., & Després, L. (2021). The evolutionary dynamics of biological invasions: A multi-approach perspective. *Evolutionary Applications, 14*(6), 1463-1484.

Shirk, R., Hamrick, J., Zhang, C., & Qiang, S. (2014). Patterns of genetic diversity reveal multiple introductions and recurrent founder effects during range expansion in invasive populations of Geranium carolinianum (Geraniaceae). *Heredity, 112*(5), 497-507.

Sillero, N., Huey, R. B., Gilchrist, G., Rissler, L., & Pascual, M. (2020). Distribution modelling of an introduced species: do adaptive genetic markers affect potential range? *Proceedings of the Royal Society B, 287*(1935), 20201791.

Simberloff, D. (2009). The role of propagule pressure in biological invasions. *Annual Review of Ecology, Evolution, and Systematics, 40*, 81-102.

Slatkin, M. (1987). Gene flow and the geographic structure of natural populations. *Science, 236*(4803), 787-792.

Spear, S. F., Balkenhol, N., FORTIN, M. J., McRae, B. H., & Scribner, K. (2010). Use of resistance surfaces for landscape genetic studies: considerations for parameterization and analysis. *Molecular ecology, 19*(17), 3576-3591.

Suarez, A. V., & Tsutsui, N. D. (2008). The evolutionary consequences of biological invasions. *Molecular ecology, 17*(1), 351-360.

Sunde, J., Yıldırım, Y., Tibblin, P., & Forsman, A. (2020). Comparing the performance of microsatellites and RADseq in population genetic studies: Analysis of data for pike (Esox lucius) and a synthesis of previous studies. *Frontiers in genetics, 11*, 218.

Veale, A. J., & Etherington, T. R. (2023). Assessing possum dispersal and the Predator Free Taranaki trapping program me using population genomics. Manaaki Whenua – Landcare Research.

Wereszczuk, A., Leblois, R., & Zalewski, A. (2017). Genetic diversity and structure related to expansion history and habitat isolation: stone marten populating rural–urban habitats. *BMC ecology, 17*, 1-16.