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Origins and diversity of invasive brushtail possums (*Trichosurus vulpecula*) in New Zealand surveyed with mtDNA haplotype and nuclear microsatellite data

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ABSTRACT

The brushtail possum *Trichosurus vulpecula* is native to Australia where six subspecies exist in distinct regions. A composite invasive population is established in Aotearoa New Zealand, which has since been subject to localised bouts of culling. We surveyed population genetic structure across New Zealand to identify the scale of genetic diversity introduced to New Zealand and the resulting pattern of admixture. Australian brushtail possums have high mitochondrial diversity (17%) and prominent spatial structure. Thirty-eight haplotypes among 25 New Zealand population samples (n = 465) were closely related to 45 haplotypes sampled from Victoria and Tasmania in Australia (n = 120), but just one was shared. High haplotype diversity is consistent with multiple successful introductions and rapid population expansion in New Zealand. Nuclear diversity of microsatellite loci screened in 18 New Zealand population samples (n = 374) comprised five genotypic clusters (K = 5), but these groups did not correlate with geography. An overall signal of genetic partitioning within the invasive population suggests limited mixing but ongoing management towards eradication will influence patterns of population recovery, migration and evolution of traits including toxin resistance. We consider the implications of mixed ancestry of the invasive population in terms of variation in toxin tolerance detected in New Zealand.

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Introduction

The introduction of Australian brushtail possums (*Trichosurus vulpecula*) (hereafter 'possums') to Aotearoa New Zealand to establish a fur trade began in 1837 (Clout 2006). Rapid population growth reached an estimated 48 million possums occupying native forest and exotic pastureland throughout New Zealand (Warburton et al. 2009). Their negative impact on native plants (Cowan 1989; Cowan 1990; Sweetapple et al. 2002), birds and their eggs (Brown et al. 1996; McLennan et al. 1996; Clout 2006) and invertebrates (Cowan and Moeed 1987; Payton 2000; Sadlier 2000) have been

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documented. Furthermore, possums are vectors of the notifiable agricultural disease Bovine Tuberculosis (TB) among cattle (Karlson and Lessel 1970; Julian 1981; De Lisle 1993; Coleman and Caley 2000; Livingstone et al. 2015) that has significant economic impact (MPI 2020; Bodey et al. 2022).

In New Zealand, management of troublesome pest-mammals is well developed (Brown and Sherley 2002; Veitch and Clout 2002; Clout and Russell 2006) and the resulting benefits for native fauna and flora are well demonstrated (O'Donnell and Hoare 2012; Byrom et al. 2016; Elliott and Kemp 2016). However, eradication of mammal pests has only been achieved in relatively small, isolated landscapes, primarily offshore islands. As predator-proof fences that reduce reinvasion are effective only on limited spatial scale (Day and MacGibbon 2007), mainland management strategies generally aim to reduce local impacts of pests by suppressing numbers rather than eliminating species (Speedy et al. 2007; Bell et al. 2019; Mill et al. 2020; Innes et al. 2023). Nevertheless, the New Zealand government's proposal to eradicate five invasive mammal species by 2050 (Tompkins 2018) acknowledged the ongoing costs of their management (Owens 2017; Bodey et al. 2022) and raised the stakes for conservationists. The challenges of exterminating invasive mammals from New Zealand are profound and require the full engagement of biologists and landowners (Owens 2017; Linklater and Steer 2018). Eradication will demand novel and dynamic approaches that integrate sensitive population monitoring with control measures, and genetic data can provide critical information about population size and connectivity (Abdelkrim et al. 2005; Rollins et al. 2006; Piertney et al. 2016; Desvars-Larrive et al. 2018; Combs et al. 2019; Burgess et al. 2021). Understanding the effectiveness of culling and detecting re-invasion pathways all benefit from genetic data prior to and during eradication programs (Synnott et al. 2023).

Brushtail possums occur naturally across the Australian continent, where distinct subspecies (Kerle et al. 1991) occupy geographically distinct habitat types ranging from central arid woodlands to wet sclerophyll forests (Kerle 1984; Carmelet-Rescan et al. 2022). At least two of these subspecies of *Trichosurus vulpecula* were introduced to Aotearoa New Zealand (Pracy 1974) and fur colour traits typical of these can still be seen in New Zealand today. However, as they freely interbreed where they co-occur (Pattabiraman et al. 2022) fur colour, that is controlled by just a few genes (Bond et al. 2024), has become a less reliable indicator of ancestry among the invasive population.

More than 300 separate documented introductions between 1838 and the 1950s sourced possums from Tasmania and Australia (putatively the state of Victoria) (Pracy 1974). The major importation phase during the 1890s including black possums from Tasmania and grey possums from Australia that were released in relatively few locations across the main islands of New Zealand (Figure 1 inset). Of these, the majority of the black Tasmanian possums were released in West Coast, South Island. Liberations across the country peaked again in the 1920s with newly imported possums and those dispersed from New Zealand stock (Figure 1). Data are far from complete, but most of the documented possum liberations were by private individuals and formal Acclimatisation Societies (Pracy 1974).

The large number of possum introductions and rapid population growth resulted in high allelic diversity and novel genotypes in New Zealand (Triggs and Green 1989; Taylor et al. 2004; Pattabiraman et al. 2022), but the exact ancestry of the extant population is not known. Genetic data from possums in two New Zealand locations (Hawkes Bay, Dunedin) indicate admixture of possums from Tasmania and mainland southern

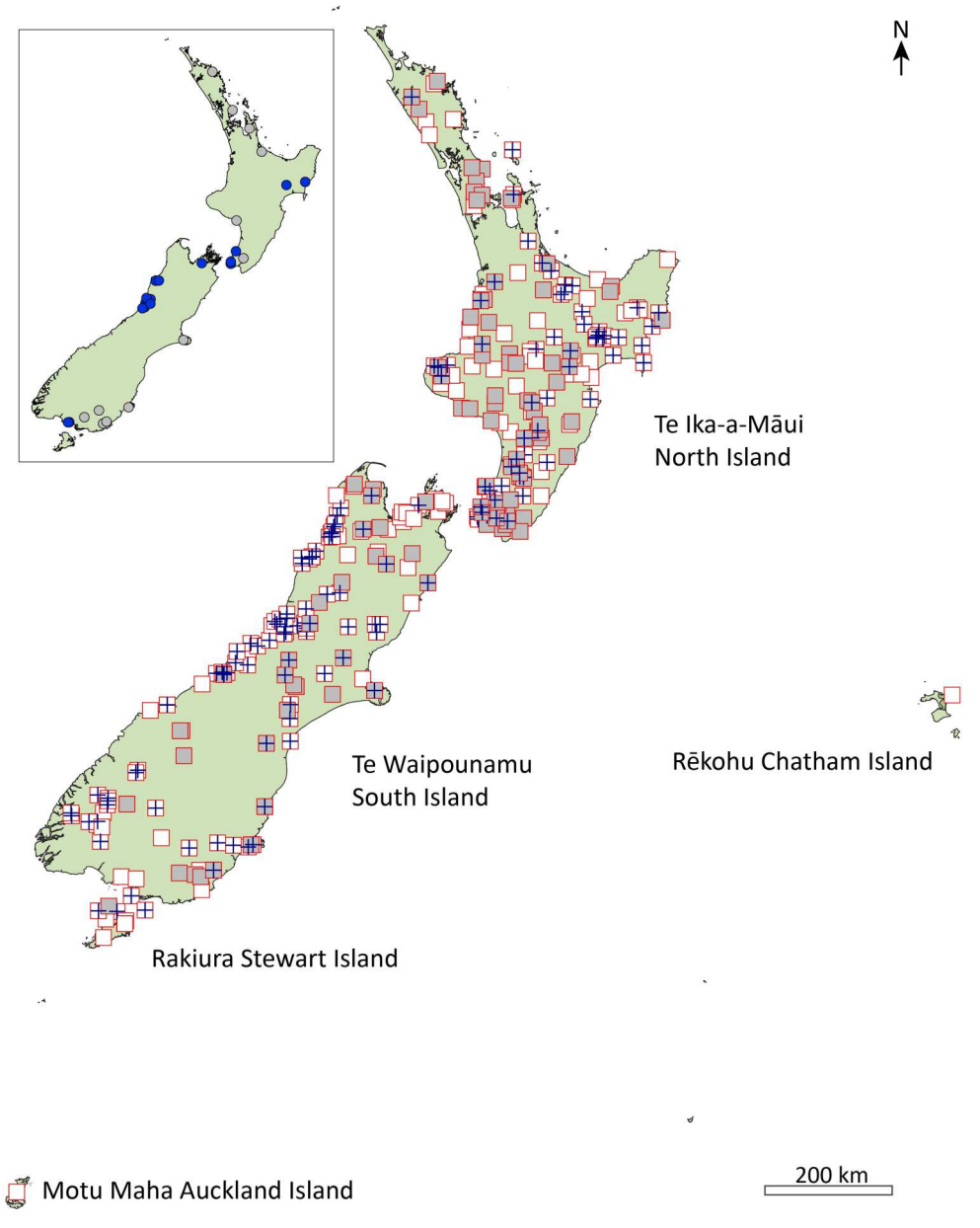

Figure 1. Documented release sites (red squares) of brushtail possums in New Zealand between 1865 and 1952 (data from Pracy 1974). Black possums (dark blue cross) and grey possums (grey fill) are indicated where known. Inset map shows locations of first introductions from Tasmanian (dark blue) and Australian (grey) importations made between 1837 and 1898.

Australia (Campbell et al. 2021; Bond et al. 2023), but the pattern of mixing across New Zealand varies (Taylor et al. 2004) (Figure 2).

Not only is information about gene flow critical for understanding the spatial density and response to regional population control measures, but genotypic variation may have major implications for adaptation (Prentis et al. 2008; Biedrzycka et al. 2022). Notably,

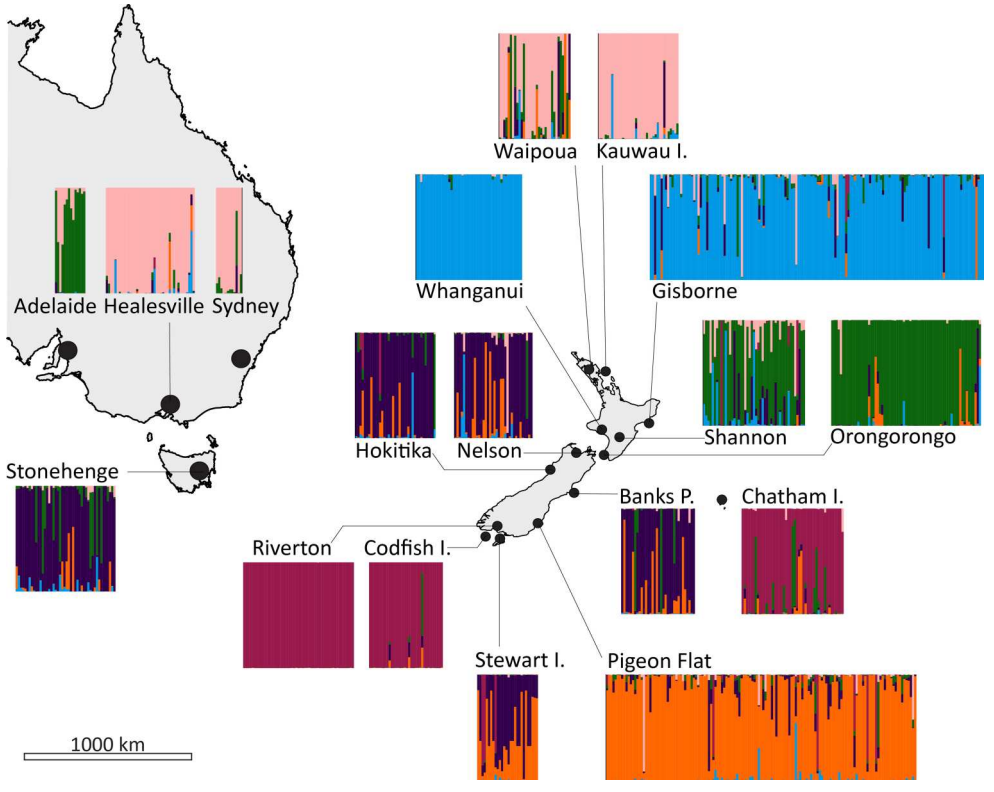

Figure 2. Novel naïve Bayesian analysis of genetic structure of *Trichosurus vulpecula* possums sampled in New Zealand and southeast Australia, using four microsatellite loci from data originally produced by Taylor et al. (2004).

possum populations in Australia display variation in their tolerance to 1080 (sodium fluoroacetate), the poison most widely used in New Zealand to kill possums (Table 1). Western Australian possums experience natural high levels of 1080 in plants they eat and have high tolerance to the poison, but even small differences could be biologically significant. For example, LD_{50} of 0.92 mg/kg in Tasmanian possums is lower than Western Australian populations, but might be significantly higher than other sources of invasive possums such as New South Wales (0.62 mg/kg; McIlroy 1981, 1983). Thus, the sources of possums have significant management implications, because the invasive population is exposed to intense selective management practices in New Zealand.

Identifying the scale and spatial distribution of variation at neutral genetic loci is a first step towards integrated monitoring, modelling and eradication (Taylor et al. 2004; Adams et al. 2014; Browett et al. 2020; Pattabiraman et al. 2022; Yarita et al. 2023). We used mitochondrial haplotype data for the first time to determine how many distinct matrilines are established in New Zealand and to infer their geographic origins in Australia. Inclusion of population samples from several locations in Australia meant the origins of the invasive population could be tested and sampling of possums across New Zealand provides an improved view of the structure of the invasive population. Using a combination of mitochondrial and nuclear markers we examined the

Table 1. Toxicity of compound 1080 (sodium fluoroacetate) to *Trichosurus vulpecula* possums in various geographic populations. Oral dosing involve animal restraint. IP intraperitoneal injection; † self-administered free-living population; j Immature.

Location			Treatment	N	LD_{50} mg/kg	95% confidence	Reference
Australia	WA			3	>125.0		King et al. 1978
	SA			3	0.75		Twigg and King 1991
	Tasmania		oral	12	0.92	0.79–1.20	McIlroy 1983
	NSW	Bombala	oral	12	0.42	0.29–0.6	McIlroy 1983
	NSW	Canberra	oral	5	0.68	0.59–0.78	McIlroy 1981
	NSW	Bombala	IP	5	0.57	0.51–0.64	McIlroy 1981
	NSW	Bombala	oral	5	0.67	0.49–0.92	McIlroy 1981
	NSW	Bombala	oral	5	0.62	0.55–0.71	McIlroy 1981
	NSW	Bombala	oral	3	0.58	0.40–0.84	McIlroy 1981
	NSW	Bombala	oral	3	0.58	0.40–0.84	McIlroy 1981
	NSW	Bombala	oral	5	0.86	0.67–1.09	McIlroy 1981
	NSW	Bombala	oral	20j	0.86	0.67–1.009	McIlroy 1982
	NSW	Bombala	oral	144	0.47–0.79	0.34–1.03	McIlroy 1982
New Zealand	North Island	Whanganui & Upper Hutt	oral	35	0.78	0.69–0.91	Bell 1972
	South Island	Rangiora	oral		1.5		Anon. 1979
	South Island & North Island	Greymouth & Whanganui	oral oral		1.3–2.1		Anon. 1979
	South Island	Haupiri	oral†		1.48		Anon. 1979

scale and spatial distribution of genetic diversity among possums in New Zealand. MtDNA and microstatellite markers are a convenient way to build datasets that incorporate various types of sampling over many years of pest management (Synnott et al. 2023), ultimately enabling analysis of re-invasion pathways and the outcome of population management operations (Velando et al. 2017). Although multilocus markers provide a way to examine patterns of gene flow among established populations, non-recombining mtDNA can provide clear genealogical information because maximum retention of founding diversity is expected in an expanding population (Excoffier et al. 2009).

Materials and methods

Brushtail possum samples were obtained from their native range in Australia (n = 120) and from the invasive range in Aotearoa New Zealand (n = 465; Figure 3; Table 2). Muscle/liver tissue samples of Australian possums were provided by the South Australian Museum, Adelaide. These represent five subspecies (Kerle et al. 1991) sampled near Darwin (Northern Territory), Balranald (New South Wales), Kangaroo Island and Adelaide (South Australia), Launceston (Tasmania), Townsville and Brisbane (Queensland), and Sutton Grange and Bendigo (Victoria) (Table 2). Ear clip samples of New Zealand brushtail possums were collected by Government and private pest management practitioners in 2003, 2016 and 2019, at 26 locations across the country (Table 2, Figure 3).

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).

Table 2. Locations in Aotearoa New Zealand and Australia (subspecies names provided) where population samples of brushtail possum (*Trichosurus vulpecula*) were collected. mtDNA = 572 base pairs of D-loop, Micro = 7 nuclear microstatellite loci, n = sample size.

Location name	Code	Subspecies or NZ region	Latitude	Longitude	mtDNA n	Micro n
Aropaoanui	ARO	Hawkes Bay, NI	−39.1547	176.5903		16
Bideford	BID	Wairarapa, NI	−40.907857	175.925866	32	50
Kaitake	KAI	Taranaki, NI	−39.16597	173.95983	17	34
Kingma Peak	KIN	Hawkes Bay, NI	−39.023796	176.790385	10	
Kokomuka	KOK	East Cape, NI	−37.68637	178.17109	13	16
Mahia	MAH	Hawkes Bay, NI	−39.126731	177.880154	10	
Moonshine Hill	MOO	Upper Hutt, NI	−41.108062	175.012184	14	
Pukewharaiki	PUK	Northland, NI	−35.0604	173.4157	13	16
Punaruku	PUN	Northland, NI	−35.37199	174.31295	5	
Purua	PUR	Northland, NI	−35.63253	174.10609	6	16
Tapora	TAP	Northland, NI	−36.2116	174.1829	12	16
Turitea	TUR	Manawatu, NI	−40.40853	175.65881	15	12
Waiorongomai	WAI	Te Aroha, NI	−37.557098	175.757904	10	
Whangara	GIS	Hawkes Bay, NI	−38.56918	178.22965	10	16
Copland Valley	COP	West Coast, SI	−43.612962	169.860306	14	
Fox Valley	FOX	West Coast, SI	−43.476591	170.017033	28	16
Haast Plain	HAA	West Coast, SI	−43.875623	169.032726	9	10
Kahurangi Point	KAH	Tasman, SI	−40.777552	172.220478	20	
Kenepuru	KEN	Marlborough Sounds, SI	−41.09844	173.87163	112	40
Lake Alabaster	ALA	Southland, SI	−44.489158	168.185577	37	40
Mosgiel	MOS	Otago, SI	−45.784045	170.433655	17	24
Robinson's Bay	ROB	Banks Peninsula, SI	−43.86492	172.77970	6	6
Skyfarm	SKY	Tasman, SI	−40.92777	172.8569	10	10
Whariwharangi	WHA	Tasman, SI	−40.78756	172.977247	12	16
Ryans Creek	RYA	Rakiura, Stewart I	−46.900554	168.095798	19	20
Rēkohu	REK	Chatham Is	−43.992035	176.523806	14	
Darwin, NT		*arnhemensis*	−12.3805	130.9868	11	
Townsville, QLD		*johnstonii*	−19.2213	146.7620	8	
Moggil, Brisbane,QLD		*vulpecula*	−27.571500	152.873154	7	
Yanga, NSW		*vulpecula*	−34.723810	143.597336	6	
Sutton Grange, VIC		*vulpecula*	−36.989147	144.358755	9	
Tang Tang, Bendigo, VIC		*vulpecula*	−36.36365	144.294808	10	
Launceston, Tasmania		*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		*hypoleucus*	−31.9754	115.8526	23	
Perth, WA		*hypoleucus*	−31.975049	115.854248	19	
Total					585	374

Mitochondrial haplotype diversity

Polymerase Chain Reaction (PCR) primers specifically designed from whole mtDNA of short-eared (*Trichosurus canninus*) and brushtail possum (*Trichosurus vulpecula*) were used to target partial mtDNA D-loop (Carmelet-Rescan et al. 2022). Consistent amplification was obtained from *T. vulpecula* using the primer combination Tcan_218f (AAGGCAACAACACCTCACCA) and Tvul_1023r (TCCCGCCCAGTTGATAAACC) in 20µl volumes with final concentrations of 1x DreamTaq buffer (ThermoFisher Scientific), 200 µM each deoxy-nucleotide phosphates (dNTP), 2.5 mM Magnesium Chloride (additional 0.4 µl of 25 mM $MgCl_2$), 0.05U DreamTaq DNA polymerase, and 0.25 µM each primer. Thermocycling consisted of an initial denaturation step at 95°C for 90 s followed by 36 cycles of 94°C for 20 s, 51°C for 20 s, and 72°C for 1 min; 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

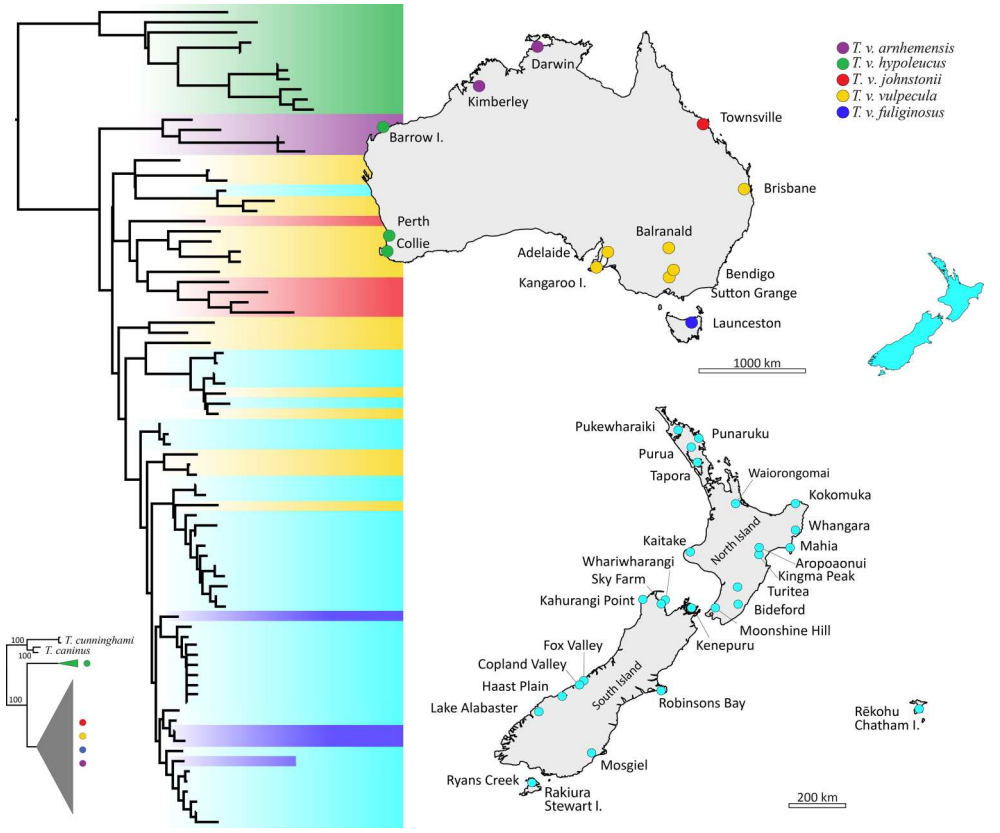

Figure 3. Haplotypic diversity of *Trichosurus vulpecula* possums sampled in Aotearoa New Zealand nest within the diversity from the natural range in Australia (sampling locations shown on maps). Neighbour-joining phylogeny of mtDNA D-loop (572 bp) haplotypes from Australia (n = 120) and Aotearoa New Zealand (n = 465). Colours correspond to regional and subspecies sampling. Inset ML phylogeny showing relationship of *T. vulpecula* diversity to *T. cunninghami* and *T. caninus* with result of 1000 bootstrap resamples.

analyser (Macrogen Inc). Sequences were edited using the software GENEIOUS 11.5 (Kearse et al. 2012) and aligned with Clustal Omega (Sievers and Higgins 2018) via the EBI-EMBL dispatcher (https://www.ebi.ac.uk/jdispatcher/msa/clustalo).

Population sample statistics of haplotype number (H), haplotype diversity (Hd) and nucleotide diversity (π), and DNA polymorphism analysis were conducted using the software DNASP v 6.12.01. Pairwise population differentiation (F_{ST} and Φ_{ST}) was calculated and tested for significant departures from zero using 1000 resampling permutations in ARLEQUIN v3.5.2.2 (Excoffier and Lischer 2010). In this case where substantial haplotype divergence in possums predates their introduction to New Zealand (Carmelet-Rescan et al. 2022; Pattabiraman et al. 2022), it is expected that regional diversity will initially be dominated by the pattern of history of translocations. To limit over-emphasis of deep lineage diversity on population differentiation in New Zealand we focus on estimates of F_{ST} which do not incorporate the level of sequence divergence among haplotypes. Haplotype diversity was visualised using a neighbour-joining phylogeny for the full dataset and median-joining networks (Bandelt et al. 1999) of a trimmed (520 bp)

alignment inferred using POPART (Leigh and Bryant 2015). Maximum-likelihood phylogenetic analysis (Best-fit model: HKY + F + I + R2) using an alignment of sequence variants from the *Trichosurus vulpecula* data set plus an outgroup comprising representative sequences of *T. cunninghami* and *T. canninus* implemented in iQ-Tree2 through IQ-Tree tools (Trifinopoulos et al. 2016; Minh et al. 2020) using model selection (Kalyaanamoorthy et al. 2017) and 1000 replicates of ultrafast bootstrapping (Hoang et al. 2018).

Nuclear microsatellite analyses

An optimal set of seven microsatellite loci (Pattabiraman et al. 2022) were used to genotype possums using two multiplexes (1: Tv_16, Tv_19, Tv_58, Tv_53, and 2: Tv_64, Tv_PnMs16, Tv_M1) of PCR primers (Taylor and Cooper 1998; Lam et al. 2000; Sarre et al. 2014). 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) with the inclusion of 500 LIZ size standard (GeneScan). Allele lengths 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). We tested for linkage disequilibrium using pairwise tests with the software GENEPOP on the Web (Rousset 2008). Mean number of alleles in the population sample (A), observed heterozygosity (H_o), expected heterozygosity (H_e), and the Garza-Williamson index (M) were calculated using R packages *adegenet v 2.1.10* (Jombart and Ahmed 2011) and *poppr v.2.9.6* (Kamvar et al. 2014).

The Garza-Williamson index (Garza and Williamson 2001) was used to seek evidence for population bottlenecks (Excoffier and 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 and Lischer 2010).

To identify and assign individuals to genotypic clusters based on allele frequencies, we 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 unlinked (Pritchard et al. 2000). Bayesian Markov-Chain Monte Carlo (MCMC) clustering of the individual genotypes was run with a 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 1–10 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 (Evanno et al. 2005) implemented and graphs generated in StructureSelector (Li and Liu 2018).

We applied the same approaches to reanalyse previously published (Taylor et al. 2004) microsatellite data for brushtails possums that included samples (n = 912) from 14

locations in New Zealand and four southwest Australia. These data were obtained for a subset of the microsatellite loci used in the present study (Five loci: Tv16, Tv19, Tv27, Tv58, Tv64), but could not be merged because they had been scored using different tools and cross-validation was not possible. We previously demonstrated that two of these loci (Tv16, Tv27) are linked and thus not independently inherited (Pattabiraman et al. 2022) so Taylor et al. (2004) data were reduced to four loci for naïve Bayesian clustering. STRUCTURE analyses were run with and without admixture but the same optimal number of clusters (K = 6) was found and pattern of assignment near identical (Figure 2).

Results

Phylogeography with mitochondrial DNA

Mitochondrial D-loop DNA sequences (572 bp) were obtained and aligned from 585 individuals. Among these we identified 79 haplotypes of which 45 were unique to possums sampled in Australia and 38 to possums in New Zealand (Table 3). One haplotype was encountered in samples from both countries. The population samples from Northern Territory (*T. v. arnhemensis*) and Western Australia (*T. v. hypoleucus*) had distinct mtDNA haplotypes that formed separate monophyletic clades in the phylogenetic analysis (Figure 3), however the possums sampled from east and southeast Australia were polyphyletic with respect to the three subspecies. Haplotypes from putative *T. v. johnstonii* and *T. v. fuliginosus* populations nested among the genetic diversity of *T. v. vulpecula* samples (Figure 3).

A total of 465 brushtail possums were screened from 25 locations across New Zealand. We identified 38 unique D-loop haplotypes that nested among the sampled diversity of *T. v. vulpecula* from sites in New South Wales, South Australia, Victoria and Brisbane and *T. v. fuliginosus* in Tasmania. In our sample only one haplotype was encountered in both Australia and New Zealand: Launceston (Tasmania, n = 3/11) and West Coast, NZ (HAA n = 3/9, FOX n = 10/28, COP n = 14/14) (Figure 4A). In New Zealand about half of the individuals sampled (54%) had one of two common haplotypes, and the most common haplotype was represented throughout New Zealand except the north (Figure 4B). Highest haplotype diversity detected was in the Northland population samples of Punaruku (PUN, Hd = 0.9) and Tapora (TAP, Hd = 0.88) and lowest at Lake Alabaster in Southland (ALA, Hd = 0.054; Table 3).

Most population samples were genetically distinct from one another with about 77% of pairwise F_{ST} estimates significantly greater than zero ($p < 0.001$), implying restricted gene flow across the New Zealand landscape (Table 4). Some samples from nearby locations had significantly high F_{ST} such as Lake Alabaster (ALA) and Haast Plain (HAA) in southwest South Island ($F_{ST} = 0.846$). However, some pairwise comparisons did not deviate significantly from zero including samples from locations that were far apart, such as Lake Alabaster (ALA) in southern South Island and North Island samples 380 km away at Kaitake (KAI) and Kingma (KIN) (Table 4). In terms of variance among population samples, similar results were obtained from estimates of pairwise Φ_{ST} among population samples.

Table 3. Haplotype diversity at the mtDNA D-loop locus of population samples from Aotearoa New Zealand and Australian brushtail possums. n: Number of individuals, h: number of haplotypes, Hd: Haplotype diversity, π: Nucleotide diversity. Arranged alphabetically by island.

Location name	Code	n	h	Hd	π
Bideford	BID	32	3	0.325	0.009
Kaitake	KAI	17	2	0.221	0.0004
Kingma Peak	KIN	10	2	0.2	0.0003
Kokomuka	KOK	13	3	0.679	0.008
Mahia	MAH	10	2	0.2	0.0008
Moonshine Hill	MOO	14	3	0.275	0.0019
Pukewharaiki	PUK	13	5	0.718	0.007
Punaruku	PUN	5	3	0.8	0.019
Purua	PUR	6	2	0.5	0.001
Tapora	TAP	12	6	0.879	0.009
Turitea	TUR	15	4	0.543	0.011
Waiorongomai	WAI	10	3	0.2	0.0004
Whangara Gisborne	GIS	10	4	0.711	0.007
Copland Valley	COP	14	1	0.0	0.0
Fox Valley	FOX	28	4	0.712	0.0061
Haast Plain	HAA	9	2	0.5	0.0125
Kahurangi Point	KAH	20	2	0.1	0.0028
Kenepuru	KEN	112	4	0.521	0.014
Lake Alabaster	ALA	37	2	0.054	0.0001
Mosgiel	MOS	17	3	0.618	0.0155
Robinson's Bay	ROB	6	2	0.533	0.0082
Sky Farm	SKY	10	3	0.533	0.0033
Whariwharangi	WHA	12	4	0.439	0.0012
Ryans Creek	RYA	19	2	0.491	0.0047
Chatham Island	REK	14	1	0.0	0.0
Darwin, NT		11	5	0.855	0.0162
Townsville, QLD		7	5	0.857	0.0247
Moggil, Brisbane, QLD		8	4	0.750	0.0168
Yanga, Balranald, NSW		6	2	0.333	0.0141
Sutton Grange, VIC		9	3	0.639	0.0084
Tang Tang, Bendigo, VIC		10	4	0.800	0.0229
Launceston, TAS		10	6	0.891	0.0097
Kangaroo Island, SA		9	2	0.389	0.0015
Adelaide, SA		8	5	0.786	0.0137
Western Australia		23	12	0.846	0.0349
Perth, WA		19	8	0.772	0.0287

Population structure using nuclear genotypes

Multilocus genotypes were obtained for 374 individuals from the invasive possum population in New Zealand using seven microsatellite loci. In our sample the highest average number of alleles per locus was observed in our largest population sample (BID, 9.57 ± 2.76; n = 50) from southern North Island. High allelic diversity was also indicated by observed heterozygosity which ranged from 0.55 in the south (RYA, ROB) to 0.83 Pukewharaiki (PUK) in Northland (Table 5). Mean observed heterozygosity levels did not differ significantly from expected heterozygosity (Table 5). Garza-Williamson statistic (M) values were smaller than the critical value ($M < 0.68$) as the number of alleles reduced more than the allelic range, suggesting recent bottleneck effects (Table 5) (Excoffier and Lischer 2010).

From 15 iterations of naïve Bayesian genotype assignment for 374 brushtail possums an optimal number of clusters (K) of 5 was inferred. In this naïve approach, the K value reflects the most likely number of hypothetical population clusters within the data (Verity and Nichols 2016) rather than precisely representing biological clusters, and

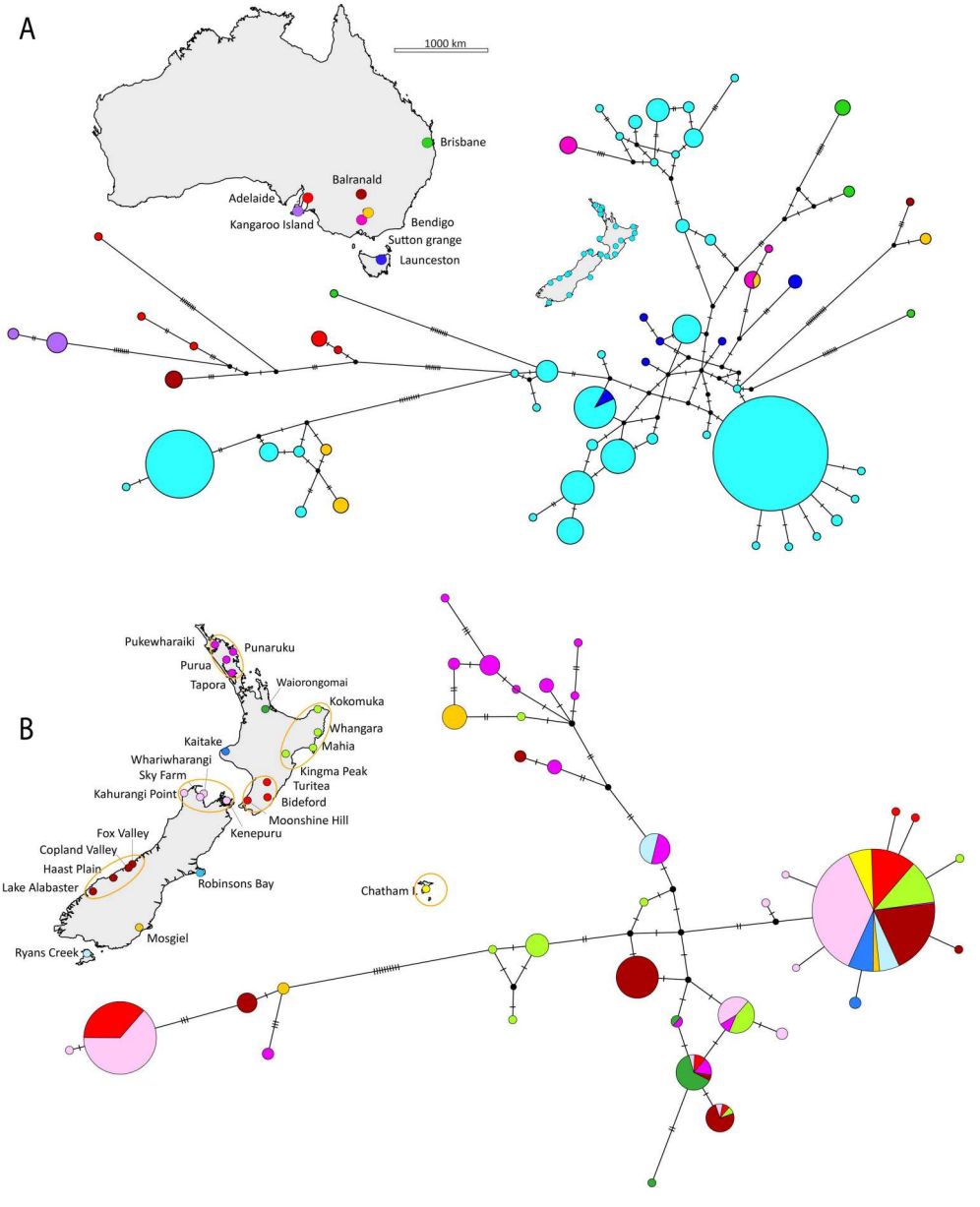

Figure 4. Mitochondrial D-loop haplotype (520 bp) diversity among 525 brushtail possums (*Trichosurus vulpecula*) sampled at 25 New Zealand and seven Australian locations. Nodes on the median-joining network are proportional to sample sizes. Colours indicate regional population sampling in (A) southeast Australia, Tasmania and New Zealand, and (B) Sites in North Island, South Island, Stewart Island and Chatham Island. Hashmarks on edges indicate number of nucleotide differences between haplotype sequences.

consistent population structure was apparent for other values of K. However, the genotypic clusters did not, in general, reflect geographical proximity of population samples (Figure 5). Individuals from two of the three Northland population samples with high

Table 4. Pairwise F_{ST} among population samples of brushtail possums in New Zealand from mtDNA sequences (D-loop 520 bp). Values that differ significantly from zero are in bold ($p < 0.001$). Location codes as in Table 2, n = sample size.

	n	BID	KAI	KIN	KOK	MAH	MOO	PUK	PUN	PUR	TAP	TUR	WAI	GIS	COP	FOX	HAA	KAH	KEN	ALA	MOS	ROB	SKY	WHA	RYA
Bideford BID	32																								
Kaitake KAI	17	**0.670**																							
Kingma Peak KIN	10	**0.667**	-0.023																						
Kokomuka KOK	13	**0.534**	**0.314**	0.282																					
Mahia MAH	10	**0.711**	**0.787**	**0.800**	**0.573**																				
Moonshine Hill MOO	14	**0.646**	-0.015	-0.042	**0.268**	**0.741**																			
Pukewharaiki PUK	13	**0.547**	**0.548**	**0.516**	**0.334**	**0.552**	**0.503**																		
Punaruku PUN	5	**0.558**	**0.613**	**0.581**	**0.315**	**0.581**	**0.556**	**0.281**																	
Purua PUR	6	**0.673**	**0.747**	**0.749**	**0.490**	-0.051	**0.687**	**0.463**	**0.450**																
Tapora TAP	12	**0.485**	**0.505**	**0.463**	**0.269**	**0.463**	**0.456**	**0.165**	**0.168**	**0.361**															
Turitea TUR	15	**0.436**	0.078	0.052	0.165	**0.579**	0.034	**0.363**	**0.366**	**0.504**	**0.312**														
Waiorongomai WAI	10	**0.693**	**0.755**	**0.757**	**0.559**	-0.042	**0.707**	**0.539**	**0.556**	-0.103	**0.456**	**0.563**													
Whangara Gisborne GIS	10	**0.504**	**0.207**	0.172	-0.050	**0.544**	0.158	**0.282**	**0.252**	**0.446**	**0.216**	0.065	**0.531**												
Copland Valley COP	14	**0.784**	**0.880**	**0.917**	**0.701**	**0.917**	**0.863**	**0.687**	**0.777**	**0.902**	**0.610**	**0.721**	**0.863**	**0.692**											
Fox Valley FOX	28	**0.459**	**0.343**	**0.316**	**0.229**	**0.442**	**0.306**	**0.290**	**0.258**	**0.393**	**0.228**	**0.213**	**0.451**	**0.170**	**0.363**										
Haast Plain HAA	9	**0.623**	**0.674**	**0.656**	**0.436**	**0.656**	**0.631**	**0.410**	**0.378**	**0.570**	**0.314**	**0.475**	**0.631**	**0.391**	**0.692**	**0.287**									
Kahurangi Point KAH	20	**0.714**	0.013	-0.019	**0.413**	**0.866**	0.002	**0.640**	**0.732**	**0.843**	**0.599**	0.119	**0.827**	**0.313**	**0.941**	**0.409**	**0.765**								
Kenepuru KEN	112	**0.197**	**0.254**	**0.242**	**0.270**	**0.554**	**0.232**	**0.408**	**0.408**	**0.518**	**0.372**	0.054	**0.542**	**0.200**	**0.608**	**0.298**	**0.484**	**0.265**							
Lake Alabaster ALA	37	**0.786**	0.061	0.032	**0.543**	**0.913**	0.056	**0.745**	**0.832**	**0.904**	**0.715**	**0.247**	**0.882**	**0.456**	**0.961**	**0.505**	**0.846**	-0.012	**0.335**						
Mosgiel MOS	17	**0.530**	**0.391**	**0.362**	**0.263**	**0.555**	**0.349**	**0.340**	**0.318**	**0.480**	**0.274**	**0.240**	**0.543**	0.198	**0.668**	**0.256**	**0.431**	**0.478**	**0.319**	**0.592**					
Robinson's Bay ROB	6	**0.577**	0.151	0.124	0.162	**0.669**	0.098	**0.351**	**0.342**	**0.567**	**0.282**	0.030	**0.637**	0.056	**0.837**	0.204	**0.486**	0.286	0.197	0.450	0.236				
Sky Farm SKY	10	**0.558**	0.035	0.009	0.149	**0.597**	-0.020	**0.359**	**0.361**	**0.508**	**0.302**	-0.022	**0.575**	0.043	**0.773**	**0.201**	**0.483**	0.110	0.174	0.223	**0.230**	0.000			
Whariwharangi WHA	12	**0.626**	**0.674**	**0.658**	**0.452**	-0.004	**0.615**	**0.428**	**0.403**	-0.036	**0.336**	**0.463**	0.013	**0.411**	**0.789**	**0.348**	**0.515**	**0.759**	**0.489**	**0.838**	**0.446**	**0.503**	**0.458**		
Ryans Creek RYA	19	**0.562**	0.191	0.167	0.225	**0.622**	0.155	0.219	**0.409**	**0.559**	**0.350**	0.108	**0.606**	0.135	**0.724**	**0.257**	**0.505**	**0.265**	**0.243**	**0.372**	**0.288**	0.121	0.085	**0.516**	
Rēkohu REK	14	**0.747**	0.046	0.035	**0.440**	**0.917**	0.038	**0.658**	**0.777**	**0.902**	**0.610**	0.177	**0.863**	**0.348**	**1.000**	**0.418**	**0.800**	-0.019	**0.306**	-0.032	**0.495**	0.387	0.159	**0.789**	0.293

Table 5. Genetic diversity at seven microsatellite loci among 18 New Zealand population samples of brushtail possums. Location codes as in Table 2. n: Number of individuals, A: Average number of alleles in the population sample, H_o: Mean observed heterozygosity, H_e: Mean expected heterozygosity, M: Garza-Williamson index.

Location	n	A	H_o	H_e	M
Aropaoanui ARO	16	6.14 ± 2.6	0.75 ± 0.06	0.73 ± 0.12	0.28 ± 0.06
Bideford BID	50	9.57 ± 2.76	0.72 ± 0.07	0.84 ± 0.04	0.26 ± 0.05
Kaitake KAI	34	5.57 ± 1.72	0.67 ± 0.18	0.65 ± 0.17	0.28 ± 0.18
Kokomuka KOK	16	5.42 ± 1.9	0.66 ± 0.15	0.65 ± 0.17	0.27 ± 0.12
Pukewharaiki PUK	16	7.57 ± 1.13	0.83 ± 0.11	0.82 ± 0.03	0.29 ± 0.09
Purua PUR	16	4.85 ± 1.77	0.56 ± 0.17	0.58 ± 0.17	0.38 ± 0.15
Taparoa TAP	16	6.28 ± 1.38	0.75 ± 0.78	0.75 ± 0.05	0.33 ± 0.12
Turitea TUR	12	5.85 ± 2.67	0.68 ± 0.19	0.73 ± 0.11	0.20 ± 0.05
Whangara GIS	16	4.74 ± 1.79	0.69 ± 0.18	0.66 ± 0.15	0.28 ± 0.11
Fox Valley FOX	16	7.28 ± 3.04	0.63 ± 0.15	0.71 ± 0.17	0.29 ± 0.08
Haast Plain HAA	10	6.81 ± 2.13	0.685 ± 0.04	0.68 ± 0.52	0.26 ± 0.06
Kenepuru KEN	40	6.57 ± 2.22	0.695 ± 0.14	0.696 ± 0.11	0.24 ± 0.06
Lake Alabaster ALA	40	4.43 ± 2.64	0.693 ± 0.18	0.698 ± 0.16	0.40 ± 0.18
Mosgeil MOS	24	6.85 ± 3.48	0.68 ± 0.13	0.73 ± 0.11	0.32 ± 0.09
Robinson's Bay ROB	6	3.85 ± 1.25	0.55 ± 0.23	0.59 ± 0.25	0.19 ± 0.07
Sky Farm SKY	10	3.85 ± 2.61	0.57 ± 0.31	0.58 ± 0.21	0.27 ± 0.11
Whariwharangi WHA	16	5.28 ± 0.95	0.59 ± 0.21	0.69 ± 0.12	0.34 ± 0.16
Ryans Creek RYA	20	4.42 ± 1.27	0.55 ± 0.28	0.57 ± 0.25	0.21 ± 0.09
TOTAL	374	5.85 ± 2.07		0.69 ± 0.16	0.28 ± 0.10

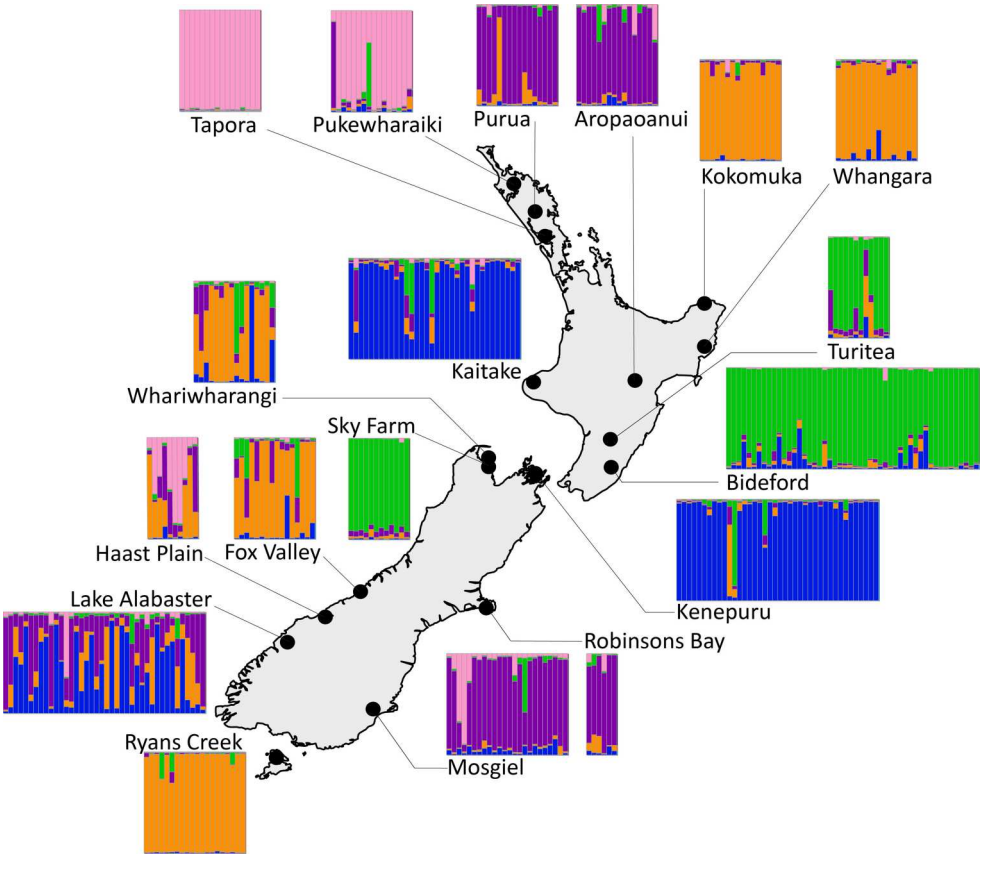

Figure 5. Genetic structure in brushtail possum (*Trichosurus vulpecula*) population samples across Aotearoa New Zealand (n = 374). Assignment probability of each individual for K = 5 genotypic clusters inferred from seven independent neutral nuclear loci.

haplotype diversity (Table 3) had high assignment probability to a distinct genotype cluster (Figure 5). Non-adjacent populations (in North Island, and South Island) were dominated by individuals with high assignment probability to the same genetic clusters. For example, at K = 5, Kokomuka and Whangara near East Cape North Island were assigned to the same genotype cluster as the southernmost sample from Ryans Creek, Stewart Island (Figure 5).

Discussion

A hundred years ago, releasing introduced possums into New Zealand forests was considered an economically beneficial activity: 'We shall be doing a great service to the country in stocking these large areas … with this valuable and harmless animal' (Annual Report Auckland Acc. Soc. 1916–1917; cited by Pracy 1974). However, the meagre benefits from a fur trade did not match the negative ecological impact and damage to agriculture from disease spread resulting from invasive brushtail possums in New Zealand.

Origins and lineages in NZ

In many instances invasive populations are established from few individuals so display low genetic diversity and little spatial structure (Abdelkrim et al. 2005 Lack et al. 2013), although this depends in part on the number of source populations and the diversity within and between them (Abdelkrim et al. 2005; Benvenuto et al. 2012; Rius and Darling 2014), as well as the pattern of establishment and expansion (e.g. Clegg et al. 2002). However, detailed genetic analysis of invasive mammals has been revealing that many invasive populations retain genetic diversity from their historic genetic footprint (Synnott et al. 2023).

Despite a large number of brushtail possum release events in New Zealand (Pracy 1974) the contribution these made to the invasive population was not known. The number of mtDNA lineages in New Zealand depends on what was transferred from Australia (equivalent to biogeographic dispersal) and their establishment success. Theoretically, a rapidly expanding population has potential to retain high genetic diversity from founders (Excoffier et al. 2009) as observed in rabbits introduced to Australia (Zenger et al. 2003). Deep mtDNA diversity exists among native *Trichosurus vulpecula* populations across Australia (Carmelet-Rescan et al. 2022), implying potential for high diversity in the invasive population. We found representation of a subset of Australian lineages, among the New Zealand possum population consistent with the limited historical information about translocations from southern Australia and Tasmania (Pracy 1974; Campbell et al. 2021) (Figure 1). Possum mitochondrial lineages from northern and western Australia are not represented in our sampling from New Zealand, but we nevertheless found high haplotype diversity (Hd = 0.879; Table 3). This clearly reflects the high diversity in southeast Australia, several sources among the New Zealand founders (Figure 1), and high retention of haplotypes in a situation with rapid population increase. Most of the haplotypes sampled in New Zealand are very similar to those from Tasmanian possums. More localised within New Zealand were haplotypes more similar to those sampled only in the state of Victoria, Australia.

For example, a common haplotype in possums from Nelson and Wellington were just four nucleotide steps different from haplotypes in Bendigo possums and Northland haplotypes differed by five nucleotide from those at Sutton Grange (Figure 4). Among the population samples from Australia, haplotype diversity is high (Hd = 0.89) with some containing representatives of more than one main haplotype lineage (e.g, Brisbane and Bendigo Figure 4). This might be the result of natural connectivity and/or human-mediated translocation within Australia (Baker and Gemmell 1999; Cooper et al. 2018). Nevertheless, we observed only one haplotype shared among the native Australian and invasive New Zealand population samples, which in part reflects our relatively small-scale sampling in Australia. A coalescent outcome of population expansion in terms of mtDNA lineages is the star-like network associated with an excess of rare mutations (Slatkin and Hudson 1991; Hillis et al. 1994). This effect is apparent in the data with several rare DNA sequence variants associated with the most common haplotypes in New Zealand (Figure 4).

Although our sampling of wild possums in Tasmania (*T. v. fuliginosus*) did not resolve a unique mitochondrial lineage for the population, the New Zealand haplotypes suggests a high Tasmanian component. This is consistent with historical records that black possums from Tasmania were highly valued and early liberations focused on this subspecies, especially releases in West Coast, South Island (Pracy 1974) (Figure 1). Indeed the only shared haplotype in our data set is recorded in Tasmania and West Coast, South Island. The intensive efforts to establish and actively disperse possums from a mixture of sources across New Zealand might have resulted in homogenisation of the invasive population, but haplotype data show spatial structure and our increased number of sampling locations compared to previous work (25 cf 13; Taylor et al. 2004) reveals genetic structure across small geographic distances not defined by main islands (cf Figure 2). Our genetic structure analysis of possums in New Zealand using relatively few polymorphic loci identified five genetic clusters with little association to geography, including samples as little as 60 km apart with distinct genetic composition. Importantly, our data suggest that a high Tasmanian component of the genome is not restricted to South Island populations (see Table 1).

An absence of Tasmanian genotypes in North Island inferred from initial sampling (Taylor et al. 2004; Figure 2) can be now seen as an incomplete picture. The inclusion of additional microsatellite loci (4–7) compared to Taylor et al. (2004) in our analysis did not increase the number of clusters among New Zealand samples, however the locations sampled in the two studies were not the same. Despite population expansion of this invasive species we do not detect widespread homogenisation. In both datasets, we see adjacent population samples frequently share alleles but differentiation is not maintained by geographic distance or physical barriers. For example, the possum sample from Purua in Northland was genetically distinct from both Tapora and Pukewharaiki samples despite being geographically close with no known barrier to gene flow. By considering mtDNA haplotype evidence with nuclear genetic structure we see a signature of Tasmanian origins in East Cape North Island population samples, but a signal of admixture in southern North Island. This new finer level of genetic resolution and inference of origins is relevant to the evidence of regional variation in toxicity of 1080 within the invasive population (Table 1).

Management

Documenting the genetic structure on a country wide scale for brushtail possums in New Zealand provides resources for planning eradication units and will allow us to document the effectiveness of future culling operations and where dispersing individuals come from during reinvasion. Predator-free 2050 will need this type of base-line data for all mammalian species that are targeted. In the case of grey squirrels in UK culling had little influence on their genetic structure (Simpson et al. 2013). Other studies have detected reduced genetic diversity resulting from management operations but not loss of genetic structure (Zalewski et al. 2016). Local culling operations provide the opportunity to incorporate population history and reinvasion pathways into future strategies if we continue to collect samples. Our data here provides an essential base for incorporating new samples following population management. Importantly, the markers used here are appropriate for temporal monitoring as they are inexpensive and samples can be processed piece-meal and easily incorporated into new analyses with published data.

Changes in density and rates of dispersal resulting from culling have implications for management strategies that aim to suppress and eradicate pest species (Synnott et al. 2023), and the challenges are not merely numerical. Increased dispersal of possums means increased movement of TB infected individuals with direct implications for agriculture. The reliance on 1080 toxin for large scale possum culling means strong selection for any naturally occurring tolerance for this toxin. It is well known that brushtail possums in Western Australia show elevated tolerance of 1080 due to natural exposure in the plants they eat (King et al. 1978 Carmelet-Rescan et al. 2022;; Carmelet-Rescan et al. 2024). Toxicity experiments also indicate variance in tolerance to 1080 among possums from southeast Australia and New Zealand, although on a narrower scale (Table 1). Variation among studies may have a methodological component (McIlroy 1981, 1983), but if the available 1080 LD_{50} data reflect genetic variation (c.f. behavioural conditioning: Morgan 1982) then intense, sporadic culling with 1080 will result in selection for resistance alleles in New Zealand. Specifically, the elevated LD_{50} recorded in some New Zealand possums might be associated with individuals from populations with a high Tasmanian component of their genome (i.e. Rangiora, Greymouth) compared to populations with high South Australian genotypic assignment (i.e. Whanganui, Table 1). Thus for management it may be important to consider the potential for elevated 1080 tolerance and genetic origins at a finer spatial scale within the New Zealand invasive possum populations (McIlroy 1983 Taylor et al. 2004;).

Author contributions

NP, MMR, and SAT conceived the idea and designed the study. NP and SAT did analyses. NP wrote the draft ms. MMR and SAT developed the final version. SAT obtained samples and funding.

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Data availability statement

The data used in this paper can be accessed by contacting the corresponding author.

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