

Does genetic variation among invasive house mice in New Zealand affect eradication success?

Jamie W. B. MacKay^{1*}, Alana Alexander², Mark E. Hauber³, Elaine C. Murphy⁴ and Mick N. Clout¹

¹School of Biological Sciences, The University of Auckland, Private Bag 92019, Auckland Mail Centre, Auckland 1142, New Zealand

²Marine Mammal Institute and Department of Fisheries and Wildlife, Hatfield Marine Science Center, Oregon State University, Newport, OR 97365, USA

³Department of Psychology, Hunter College and the Graduate Center of the City University of New York, 695 Park Avenue, New York, NY 10065, USA

⁴Department of Conservation, PO Box 11 089, Christchurch 8443, New Zealand

*Author for correspondence (Email: j.mackay@auckland.ac.nz)

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Abstract: House mice (*Mus musculus*) were introduced to New Zealand accidentally in 1824 following the stranding of an Australian ship. Phylogeographic analyses have revealed many subsequent introductions from diverse sources. Mice have significant negative impacts on native ecosystems in New Zealand and elsewhere. This makes their eradication a desirable conservation outcome, yet a large proportion of mouse eradication attempts worldwide have failed for unknown reasons. We used a phylogeographic approach to identify mitochondrial DNA (mtDNA) D-loop haplotypes of mice obtained from 12 previously unsampled island and mainland sites to expand the previous sampling range for investigation of mouse genetics in New Zealand, and to test the hypothesis that eradication failure is linked to either mouse subspecies or source population as indicated by D-loop haplotype. We predicted that populations that had survived an eradication attempt would be of a different mouse subspecies or D-loop haplotype from those where eradication had succeeded. In addition, mouse populations at failed eradication sites may have a common D-loop haplotype, indicating a shared source population that may be more resistant to eradication attempts. Twenty-five complete mtDNA D-loop sequences were generated, describing six haplotypes including two D-loop haplotypes that had not previously been recorded in New Zealand linking New Zealand mice to populations in Portugal and Iran. A Portuguese haplotype was also recorded for the two geographic outgroup specimens sourced from Reunion Island, Indian Ocean; the first recorded mouse D-loop haplotype from that location. Mice sampled from six New Zealand populations where eradication outcome was known all possessed *domesticus* D-loop haplotypes. Mice in four of these six populations (three successful eradications and one failure) possessed the same D-loop haplotype (domNZ.04) making it difficult to infer a link between D-loop haplotype and mouse eradication success. Further sampling in New Zealand may uncover additional haplotypes linking New Zealand mice to other areas.

Keywords: eradication outcome; mtDNA D-loop; *Mus musculus domesticus*; New Zealand; phylogeography; Reunion Island

Introduction

Phylogeography uses genetic variation detected in populations to draw inferences about the origin of a population and its relationship with other populations of the same species (Avice 2000; Bloomquist et al. 2010). This is of particular interest in the study and management of invasive species, as identifying the potential source population of an invasion may influence which methods are used to control or remove the invasive species (Sakai et al. 2001). New Zealand was the last major land mass to be colonised by humans, around 730 years ago (Wilmshurst et al. 2008). Prior to the arrival of humans, the only extant terrestrial mammals present were three species of bats (King 2005); however, 31 species of land mammals have now become established in New Zealand following both deliberate and accidental transport (Parkes & Murphy 2003). The relatively recent arrival of invasive mammal species to New Zealand means it may be feasible to identify their original source populations through phylogeographic analysis, as insufficient time has passed since species introductions for mutations to have obscured phylogenetic links.

The genus *Mus* probably originated in India (Boursot et al. 1993) and *Mus musculus* (hereafter: mice) radiated across Eurasia resulting in the three main subspecies known today: *Mus musculus musculus* (native to Eastern Europe), *M. m. domesticus* (Western Europe) and *M. m. castaneus* (South-East Asia) (Boursot et al. 1993; Din et al. 1996; Lundrigan et al. 2002). Mice were first recorded in New Zealand following a ship stranding in the far south of the archipelago in 1824 (Searle et al. 2009a). That ship originated from Australia (McNab 1907) and the mice that stowed away on the vessel would likely have been of British origin (Gabriel et al. 2011). Genetic diversity of New Zealand mice was recently described in a phylogeographic analysis of mouse mitochondrial DNA (mtDNA) haplotypes across the country (Searle et al. 2009a). That study uncovered high diversity, consistent with multiple colonisation events from different areas of the world. The approach used was sequencing of the D-loop; a region of the mitochondrial DNA (mtDNA) that has been extensively studied in mice (e.g. Prager et al. 1996, 1998; Gündüz et al. 2000). D-loop haplotypes can be used to determine subspecies

(Prager et al. 1996) as well as to make inferences about a population's source and its relationship with other mouse populations (Awise 2000; Bloomquist et al. 2010). The results revealed that the mouse population in New Zealand is made up of hybrids carrying genes derived from the three subspecies mentioned above, with mice possessing *domesticus* D-loop haplotypes the most prevalent in both abundance and range (Searle et al. 2009a).

Mice on islands are known to cause significant damage to native species and ecosystems (Newman 1994; Angel et al. 2009; St Clair 2011), and the eradication of invasive rodent populations from islands has become an important conservation tool (Howald et al. 2007). However, a large proportion of mouse eradication attempts worldwide have failed (MacKay et al. 2007). As part of a wider investigation into possible ecological and behavioural reasons for the failure of mouse eradication attempts (MacKay 2011), we obtained genetic samples from a number of island and mainland (North and South Island) areas in New Zealand that were the target of different mouse control regimes. These sites were chosen to expand upon the sampling range of the previous investigation of mouse genetics in New Zealand (Searle et al. 2009a), and to test the hypothesis that eradication failure is linked either to mouse subspecies or to source population, as indicated by

D-loop haplotype. Behavioural studies in laboratories have found variation in the way laboratory mice bred from different subspecies respond to identical situations (Le Roy et al. 1998; Koide et al. 2000; Fernandes et al. 2004). Also, in social interactions between wild mice, *M. m. musculus* individuals are more aggressive than *domesticus* individuals (Munclinger & Frynta 2000), indicating that subspecies differences are not restricted to the laboratory setting. These behavioural differences may result in one subspecies being better able to survive eradication attempts than the others.

To investigate this, we sequenced the entire D-loop for each mouse sampled at 12 island and mainland sites across New Zealand (Table 1) to identify subspecies of maternal origin and to infer the source population. Two additional mouse samples from Reunion Island were included as a geographical outgroup. Additional sequences from putative source populations and *M. m. gentilulus* (which acted as a phylogenetic outgroup) were acquired from NCBI GenBank (Table 2). We predicted that populations that had survived an eradication attempt would have a different mouse subspecies or D-loop haplotype to those where eradication had succeeded. In addition, mouse populations at failed eradication sites may have a common D-loop haplotype, indicating a shared source population that may be more resistant to eradication attempts.

Table 1. *Mus musculus* collection locations and population control management summaries. 'Site ref.' refers to the locations in Fig. 1, of which all but one (13) are in New Zealand.

| Site ref. | Location | Island or mainland | D-loop haplotype (number of individuals) | Control regime | Sample source |
|-----------|--------------------------------|---------------------|--|---|--|
| 1 | Bay of Islands Mainland | Mainland | domNZ.4 (2) | No control | Supplied by J. Russell, University of Auckland |
| 2 | Saddle Island, Mahurangi | Island | Mac.domNZ.1 (2) | Samples obtained prior to successful eradication | Collected for this research |
| 3 | Tawharanui, Rodney | Mainland | domNZ.9 (2) | Failed eradication at fenced site, ongoing ground-based poisoning | Collected for this research |
| 4 | Great Barrier Island, Auckland | Island | domNZ.4 (3) | No control | Supplied by J. Russell, University of Auckland |
| 5 | Motutapu Island, Auckland | Island | domNZ.4 (2) | Samples obtained prior to successful eradication | Supplied by R. Griffiths, Department of Conservation |
| 6 | Rangitoto Island, Auckland | Island | domNZ.4 (2) | Samples obtained prior to successful eradication | Supplied by R. Griffiths, Department of Conservation |
| 7 | Waiheke Island, Auckland | Island | domNZ.4 (2) | No control | Supplied by J. Russell, University of Auckland |
| 8 | Hauturu Island, Whangamata | Island | domNZ.4 (2) | Failed eradication | Supplied by J. Russell, University of Auckland |
| 9 | Mokoia Island, Lake Rotorua | Island | domNZ.4 (1) | Single invading mouse trapped 7 years after successful eradication | Supplied by T. Sachtleben, Department of Conservation |
| 10 | Cape Kidnappers, Hawke's Bay | Mainland | domNZ.3 (1), Mac.domNZ.2 (1) | Ongoing ground-based poisoning at fenced site designed to keep rats at low numbers; no targeted mouse control | Collected for this research by T. Ward-Smith, Cape Sanctuary |
| 11 | Adele Island, Abel Tasman | Island | domNZ.4(2) | Samples obtained prior to successful eradication | Collected for this research |
| 12 | Resolution Island, Fiordland | Island | casNZ.1 (1) | No control | Supplied by A. Veale, University of Auckland |
| 13 | Reunion Island, Indian Ocean | Geographic outgroup | Mac.domREU.1 (2) | No control. | Supplied by J. Russell, University of Auckland |

Table 2. National Center for Biotechnology Information (www.ncbi.nlm.nih.gov), GenBank accession number and collection locality for all sequences compared with those collected in this study.

| Accession numbers | Subspecies | Country of collection | Reference |
|-----------------------------|-------------------|-----------------------|----------------------------|
| AY172335 | <i>domesticus</i> | Inbred lab strain | Bayona-Bafaluy et al. 2003 |
| FM211596–FM211630 | <i>domesticus</i> | United Kingdom | Searle et al. 2009b |
| FM211632–FM211641 | <i>domesticus</i> | New Zealand | Searle et al. 2009a |
| GQ241989–GQ242005 | <i>domesticus</i> | Madeira | Förster et al. 2009 |
| GQ242006–GQ242020 | <i>domesticus</i> | Portugal | Förster et al. 2009 |
| U47431–U47497 | <i>domesticus</i> | Western Europe | Prager et al. 1996 |
| AJ286317–AJ286321 | <i>domesticus</i> | Iran and Turkey | Gündüz et al. 2000 |
| HQ241731, HQ241733–HQ241756 | <i>domesticus</i> | Scandinavia | Jones et al. 2010 |
| AJ286322 | <i>castaneus</i> | Iran and Turkey | Gündüz et al. 2000 |
| EF108342 | <i>castaneus</i> | Inbred lab strain | Goios et al. 2007 |
| FM211642–FM211644 | <i>castaneus</i> | New Zealand | Searle et al. 2009a |
| FM211645 | <i>musculus</i> | New Zealand | Searle et al. 2009a |
| U47504 | <i>musculus</i> | Eastern Europe | Prager et al. 1996 |
| AF074544, AF74545 | <i>gentilulus</i> | Yemen | Prager et al. 1998 |

Materials and methods

Sample collection

We obtained mouse tissue samples from 12 locations in New Zealand ranging from the Bay of Islands in the north of the North Island to Fiordland in the south of the South Island, as well as the geographic outgroup of Reunion Island, Indian Ocean (Table 1, Fig. 1). The sampling localities included six populations where eradication outcome was known; these comprised four successful eradications and two failures (Table 1). Mice from all locations except 2, 3, 5 and 6 (Fig. 1) were captured in snap-traps. Mice from locations 2, 3, 5 and 6 were trapped in Longworth live-traps (Chitty & Kempson 1949) and euthanased by cervical dislocation. All protocols were approved by the University of Auckland Animal Ethics Committee (approval R579). A small section of tail tip or other tissue was taken from each mouse and preserved in 70% ethanol before analysis. We sequenced up to three individuals from each population.

Molecular methods

DNA extraction and processing was undertaken by EcoGene, Auckland, New Zealand. DNA was extracted from mouse samples using the automated standard tissue protocol on the Corbett X-tractor Gene equipment (Concorde, New South Wales, Australia), following the manufacturer's instructions. DNA was eluted in 70 µl of elution buffer.

Following extraction, we amplified a 947 base pair (bp) part of the control region using the primers MouseCRF (TCTTCTCAAGACATCAAGAAG)(RobynHowitt, EcoGene, pers. comm.) and H00072 (TATAAGCCAGGACCAAACCT) (Prager et al. 1993). Primer MouseCRF was designed to be internal to primers L15320 and H00072 (Prager et al. 1993) to overcome non-specific binding problems associated with primer L15320 (Robyn Howitt, EcoGene, pers. comm.). We performed PCR amplifications in 25 µl reactions containing 1 µl of DNA extract from tissue (a minimum of 5 ng of DNA), 1×PCR buffer with MgCl₂ (50 mM Tris/HCl, 10 mM KCl, 5 mM [NH₄]₂SO₄, 2 mM MgCl₂, pH 8.3), 0.4 µl BSA (10 mg ml⁻¹), 200 µM of each dNTP, 0.2 µM of each primer, and

1.25 U of FastStartTaq DNA Polymerase (Roche Diagnostics, Auckland, New Zealand). The thermocycler profile used was: initial denaturation at 95°C for 4 min; 37 cycles of 45 s at 94°C, 45 s at 58°C, 1 min at 72°C; and a final extension of 10 min at 72°C. We carried out these amplifications on a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Carlsbad, California, USA).

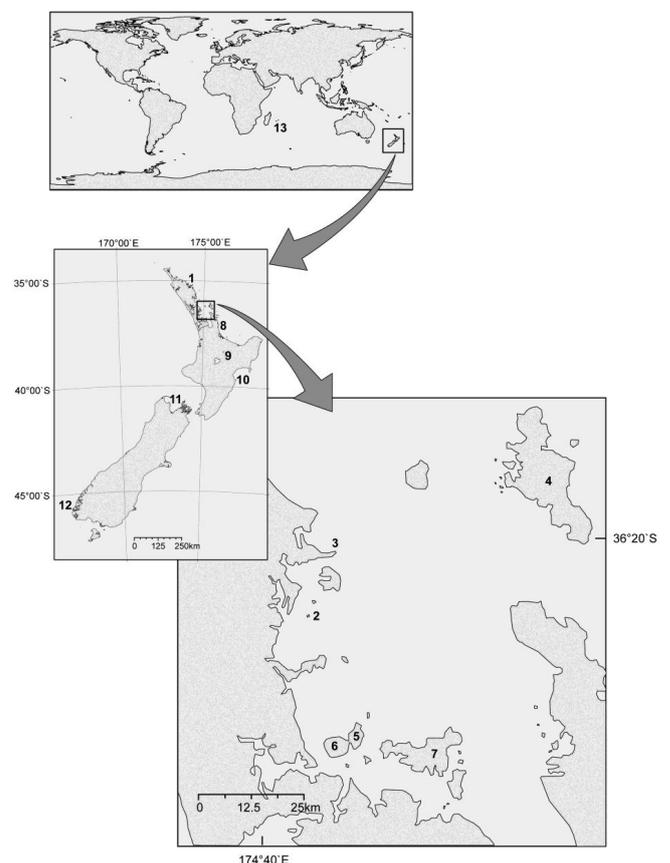


Figure 1. Mouse sampling locations. Numbers refer to site references in Table 1 and Fig. 1.

Following the manufacturers' recommended protocols, we directly sequenced purified products using BigDye™ Terminator Version 3.1 (Applied Biosystems). Products were run on an Applied Biosystems 3130xl genetic analyser using DNA Sequencing Analysis Software Version 5.3.1 (Applied Biosystems). Resulting DNA sequences were compared and edited manually using the programme Sequencher 4.6 (Gene Codes, Ann Arbor, Michigan, USA).

We aligned the sequences in MEGA 4.0 (Tamura et al. 2007) using the Clustal-W algorithm (Larkin et al. 2007). For each sample, we obtained a 933-bp sequence between positions 15 367 and 16 299 relative to the mouse mtDNA reference (NCBI accession number AY172335) published by Bayona-Bafaluy et al. (2003). We truncated alignments to 894 bp between positions 15 406 and 16 299 to allow comparison with 141 sequences obtained from recent studies covering a wide geographic area (Table 2). *Mus m. musculus* (NCBI Accession numbers FM211645 and U47504) and *M. m. castaneus* (AJ286322, EF108342 and FM211642–FM211644) were included to allow us to identify these subspecies if they were present.

We used the Bayesian algorithm MrBayes plugin (Ronquist & Huelsenbeck 2003) for Geneious 5.3.4 (Biomatters, Auckland, New Zealand) to generate 50% majority-rule consensus trees with *M. m. gentilulus* sequences (AF074544 and AF074545) acting as phylogenetic outgroups (as in Rajabi-Maham et al. 2008). Following Searle et al. (2009a, b), we selected the GTR+I+Γ model of DNA sequence evolution as the sequences used in this study are similar or the same as those used by Searle et al. (2009a, b) so we assumed that the sample set in this study conforms to the same underlying model of evolution. Parameters were set following the methods described in Searle et al. (2009a, b): we ran two independent Markov

chain Monte Carlo analyses, each with one cold chain and four heated chains with the incremental heating parameter set at 0.2. We terminated the analyses after 5 million generations and the first 30% of trees were discarded as burn-in (Searle et al. 2009b).

We calculated nucleotide diversity for New Zealand *domesticus* samples using the Tamura and Nei model of nucleotide substitution (Tamura & Nei 1993) in ARLEQUIN 3.5 (Excoffier & Lischer 2010). We also used ARLEQUIN to calculate haplotype diversity for samples from this study combined with the samples from Searle et al. (2009a) for comparative purposes.

In order to infer the population of origin of the mice sampled in this study, we used trees to describe phylogenetic relationships between the individual sequences generated and those from other publications. We considered haplotypes from previous studies identical to those found in this study suggestive of potential source populations from within the native ranges of house mice. We compared the haplotypes present in the populations where eradication outcome was known to attempt to establish a link between D-loop haplotype and eradication outcome.

Results

We generated complete D-loop sequences from 23 New Zealand mice and two mice from Reunion Island (Table 1). New Zealand mice represented six haplotypes and the Reunion Island mice possessed a haplotype that was distinct from the New Zealand samples and is the first reported mouse D-loop haplotype from that island (Fig. 2, Table 3). Twenty-four mice, including both Reunion Island samples, had *domesticus* D-loop sequences and

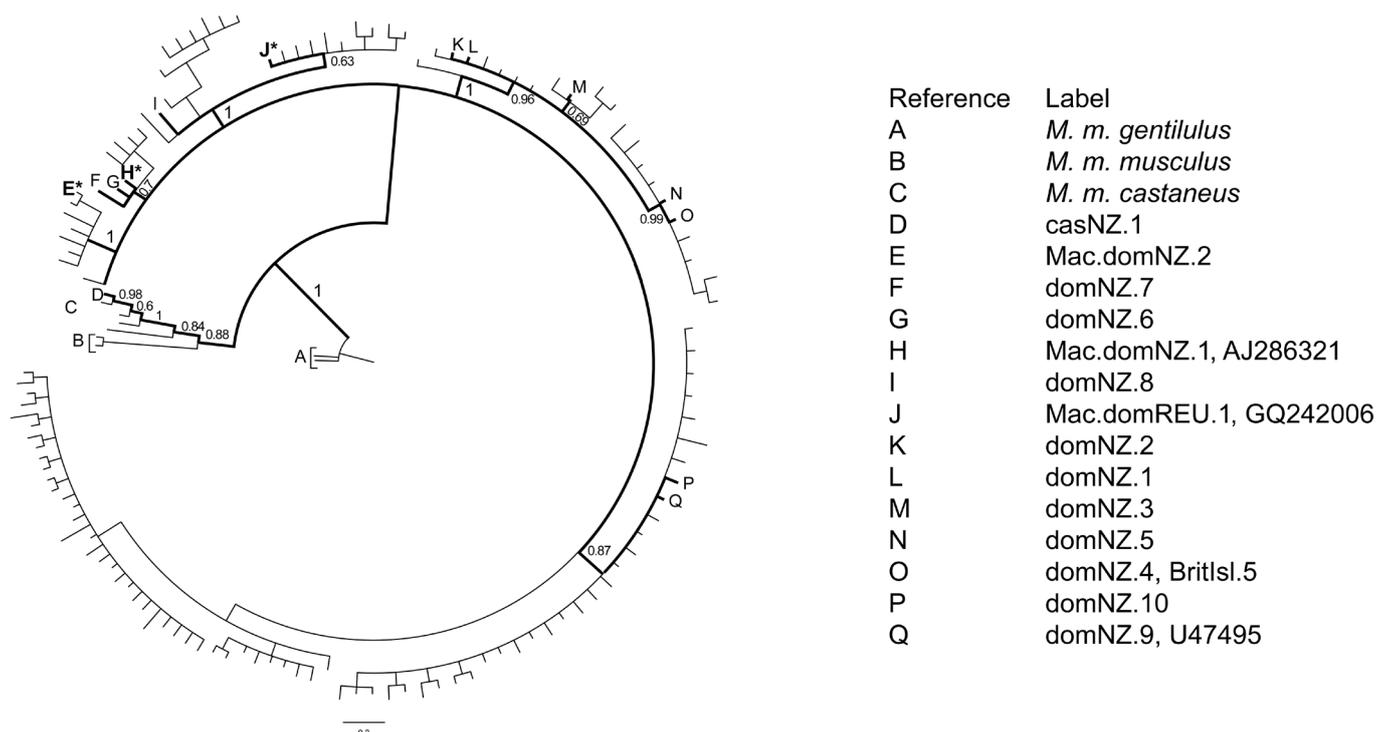


Figure 2. Phylogenetic tree for *Mus musculus domesticus*, derived from Bayesian analysis. Posterior probabilities are displayed for branches leading to haplotypes found in this study. An asterisk indicates a haplotype first recorded in this study for either New Zealand or Reunion Island. Table 2 lists the sequences used to construct the phylogeny that were not derived from this study.

Table 3. Variable sites that define the *domesticus* D-loop haplotypes found in this study. Sample AY172335 is the reference sample from Bayona-Bafauly et al. (2003) and sites are numbered with reference to this sample. A dot indicates that the sequence is identical to the reference sequence.

| | 1 | 5 | 4 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 6 | 6 | 7 | 9 | 9 | 9 | 9 | 0 | 2 | 2 | 2 |
|--------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AY172335 | T | T | T | C | A | C | A | C | C | T | C | T | T | A | C | C | A | A | G | A | T | | |
| Mac.domNZ.1 | A | . | . | . | . | . | . | . | . | C | T | C | . | . | T | . | . | . | . | . | . | . | C |
| Mac.domNZ.2 | A | C | . | . | . | . | . | C | T | . | T | . | . | T | T | T | T | . | A | G | C | | |
| domNZ.3 | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| domNZ.4 | . | . | . | A | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| domNZ.9 | A | . | C | T | G | . | . | . | T | . | T | . | . | . | . | T | . | . | . | A | . | . | C |
| Mac.domREU.1 | A | . | . | . | . | T | T | . | . | . | T | . | C | . | T | . | . | C | . | . | . | . | C |

one mouse from Resolution Island in Fiordland had *castaneus* mtDNA (casNZ.1). We detected no *musculus* individuals within these 25 samples. New Zealand *domesticus* haplotypes were positioned in five regions of the phylogenetic tree (Fig. 2) and haplotype domNZ.4 was the most abundant haplotype, with 16 of 25 mice from eight out of the 12 New Zealand locations possessing this haplotype (Table 1).

The nine mice without haplotype domNZ.4 (Table 1) comprised: the two mice from Tawharanui (Fig. 1, site 3 – haplotype domNZ.9); the two mice from Saddle Island (site 2), which both possessed a haplotype not previously described in New Zealand (Mac.domNZ.1); one Cape Kidnappers (site 10) mouse, which had haplotype domNZ.3, and the second sample from Cape Kidnappers, which possessed an additional haplotype (Mac.domNZ.2) not previously found in New Zealand – this latter haplotype is positioned in a separate clade in comparison with other New Zealand samples (Fig. 2); the two Reunion Island samples (Mac.domREU.1); and a *castaneus* haplotype (casNZ.1) from Resolution Island, South Island, New Zealand. We have deposited the two haplotypes previously undiscovered in New Zealand (Mac.domNZ.1 and Mac.domNZ.2), and the haplotype discovered in the two Reunion Island samples (Mac.domREU.1) in GenBank (JN091566–JN091568).

The variable sites of the *domesticus* D-loop haplotypes recorded in this study are given in Table 3. Haplotype diversity of the New Zealand *domesticus* samples ($n = 22$) combined with the haplotypes from Searle et al. (2009a) was 0.63 ± 0.05 and nucleotide diversity of the same samples was 0.005 ± 0.003 . Searle et al.'s (2009a) samples alone had haplotype and nucleotide diversities of 0.64 ± 0.05 and 0.004 ± 0.003 , respectively.

All sampled mice from the six populations where eradication outcome was known were *domesticus*, and mice at four of these sites had haplotype domNZ.04. D-loop haplotype domNZ.04 mice have been successfully eradicated from three sites (Rangitoto, Motutapu and Adele) and the fourth site (Hauturu) represents a failed eradication. The remaining two sites comprise a failed eradication (Tawharanui, haplotype domNZ.09) and a successful one (Saddle Island, Mac.domNZ.1).

Discussion

This new geographic survey of genetic diversity provides further evidence of the diverse origins of invasive house mice in New Zealand. Our data confirm the patterns previously described by Searle et al. (2009a), reinforcing the strong linkage between New Zealand and Europe. We found nucleotide and haplotype diversity values that were similar to those obtained by Searle et al. (2009a), confirming that the earlier study was large and geographically diverse enough to be representative of the majority of underlying *domesticus* diversity present in New Zealand.

As previously reported by Searle et al. (2009a), domNZ.4 was the most abundant haplotype, with 16 of 25 mice from 8 out of the 12 New Zealand locations possessing this haplotype (Table 1). This haplotype had been previously recorded throughout New Zealand, Australia, the UK, Ireland, and continental Europe (Gabriel et al. 2011; Searle et al. 2009a, b).

Two *domesticus* haplotypes not previously known in New Zealand were identified in this study. The first (Mac.domNZ.1) came from Saddle Island, north of Auckland (North Island), and had been previously recorded in Iran (AJ286321; Gündüz et al. 2000) and Turkey (AJ843824; Gündüz et al. 2005). This haplotype represents the first Middle-Eastern haplotype discovered in New Zealand, although it may now be extinct given that the mouse population on Saddle Island was eradicated in 2008 (MacKay et al. 2011). The second new haplotype found in this study (Mac.domNZ.2) came from Cape Kidnappers in Hawke's Bay, North Island. The two mice from this site were caught within a few metres of each other (Tamsin Ward-Smith, Cape Kidnappers Sanctuary, pers. comm.) but possessed divergent D-loop haplotypes (Mac.domNZ.2 and domNZ.3; Fig. 2). Haplotype Mac.domNZ.2 is positioned in a separate clade to other New Zealand samples (Fig. 2) and the closest, but not identical, haplotype to it comes from Lisbon, Portugal (GQ242020; Förster et al. 2009). Haplotype domNZ.3 (Table 2) was previously recorded in nearby Napier (Hawke's Bay) and also in Ireland, the UK, and Germany (Searle et al. 2009a). Cape Kidnappers was the only site sampled in this study that had multiple haplotypes present. Samples from Great Barrier ($n = 3$) and Waiheke ($n = 2$) islands, off

the North Island, were collected at sites up to 10 km apart yet all samples from both islands were domNZ.4. However, sampling for this research was limited, so it may be that other haplotypes were present at nearby sites or intermixed within the same population.

The haplotype present on Reunion Island, Indian Ocean, also has Portuguese origins, having previously been found in Lisbon (Förster et al. 2009). Historical accounts state that Reunion Island was discovered by Portuguese sailors in the early 16th century (Allen 1999, p. 9,) and these sailors may have introduced mice to the island. However, the island is now part of France and over the intervening centuries since the island was discovered mice from many different regions may have been introduced making it serendipitous that the two mice we sampled were linked to some of the earlier settlers.

Mice are the only rodent species resident within the predator-fenced sanctuary at Tawharanui where they continue to be present at very high densities (Goldwater et al. 2012). The two mice sampled from Tawharanui possessed haplotype domNZ.9, which is part of the Orkney lineage, UK (Searle et al. 2009b) and is also identical to a sample from Croatia (U47495; Prager et al. 1996). This haplotype had also been previously recorded in Ruatangata (North Island) and Ashburton (South Island), New Zealand, by Searle et al. (2009a). Previous laboratory trials showed that mice from the Orkney lineage are more aggressive than those from other lineages (Ganem & Searle 1996). Tawharanui therefore provides an excellent arena for further comparative behavioural research into the lineage-specific behaviour of house mice in relation to successful and unsuccessful eradication histories. Similarly, future comparative work should address the genetic make-up and diversity of populations at sites in New Zealand where anticoagulant toxins are used for sustained control of mouse populations rather than eradication. These sites may be at increased risk of developing anticoagulant resistance (Greaves 1994; Billing 2000) and should be tested for mutations known to confer resistance (Pelz et al. 2005; Rost et al. 2009). If anticoagulant resistance is detected at these sites, limiting the transport of mice from these lineages will be essential for successful control of mice in other areas.

The single *castaneus* mouse found in this study came from Resolution Island in Fiordland, South Island. *Castaneus* is the dominant subspecies found throughout the south of the South Island (Searle et al. 2009a) and the presence of this subspecies on Resolution Island suggests the island was colonised by animals from Fiordland or Southland rather than from elsewhere in New Zealand or beyond. The wider phylogeny of *castaneus* is not well defined and at present the original source population of this subspecies is unknown (Searle et al. 2009a).

Our sampling did not reveal any *musculus* individuals. In the previous survey only one *musculus* individual was found, near Wellington (Searle et al. 2009a), an area we did not sample. It would appear that *musculus* mice have a localised range within New Zealand. In cage trials, *domesticus* mice are more aggressive than *musculus* (Munclinger & Frynta 2000) and this could be an explanation for the limited distribution of *musculus* individuals in New Zealand.

Previous studies into mouse behaviour have demonstrated that there are behavioural differences between the subspecies in laboratory situations (Le Roy et al. 1998; Koide et al. 2000; Munclinger & Frynta 2000; Fernandes et al. 2004). However, limited sample size and the dominance of *domesticus* mice affected our ability to make firm conclusions regarding the influence of D-loop haplotype on eradication outcome.

Haplotype domNZ.04 was associated with both successful and failed eradications and it would appear that there is no link between D-loop haplotype (indicating both subspecies and source population) and eradication outcome. Subspecies differences would be better investigated in controlled laboratory situations using wild-caught mice from a range of New Zealand locations.

Overall, two new D-loop haplotypes have been found in New Zealand to add to the 10 published by Searle et al. (2009a). These haplotypes suggest new linkages between New Zealand and Portugal and the Middle East. Future sampling in additional regions may uncover interesting links to other populations, even if overall nucleotide and haplotype diversity values do not change. Areas such as East Cape in the North Island and the West Coast and interior of the South Island have not yet been sampled, so investigating these areas may reveal novel links.

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Editorial Board member: Joanne Hoare

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