

Supplementary Material

Appendix S1. Supplementary methods and molecular analysis. Mice were thawed and a 2 mm³ thigh muscle sample was taken for DNA extraction using a high salt method (Sunnucks & Hales 1996). DNA was diluted to 10 ng μL^{-1} for polymerase chain reaction (PCR). The complete mitochondrial D-loop and flanking regions were amplified with PCR primers L15774 (AACGACGGCCAGTACATGAATGGGAGGACAACCAG), H16498 (CCTGAAGTAGGAACCAGATG) (Gunduz et al. 2000), L15735 (CCAATGCCCTCTTCTCGCT) and H00072 (TATAAGGCCAGGACCAAACCT) (Prager et al. 1993) in two overlapping fragments. We used PCR condition as specified in Gunduz et al. (2000) and Prager et al. (1993). PCR products did not require quantifying or cleaning before sequencing. Two mtDNA PCR products per mouse were sequenced (Macrogen, Korea) using L15774 or L15735 respectively to provide one continuous 1187 bp sequence when the overlapping region was aligned. Our mitochondrial DNA sequence alignment comprised 106 bp at the 5' end of Cytochrome *b*, the tRNAs Threonine (67 bp) and Proline (67 bp), the entire D-loop (877–879 bp) and tRNA Phenylalanine (68 bp). Each haplotype was compared to sequences on the NCBI database using BLASTn (Acland et al. 2013). Published D-loop sequences from New Zealand collected mice are shorter than our mtDNA sequences, so we used a trimmed dataset to identify haplotypes previously reported. All the mouse mtDNA sequences observed in our study belong to the *Mus musculus domesticus* haplogroup (Fig. S1). Five haplotypes have already been reported in New Zealand mice (Genbank accessions: KP411761; KP411763; FM211632; FM211634; FM211635), but five D-loop sequences were new (Table 1). These five trimmed new haplotypes were aligned with 23 *M. musculus domesticus* haplotypes reported from New Zealand (King et al. 2016) and a network inferred using the minimum spanning approach (Bandelt et al. 1999) in PopART (Leigh & Bryant 2015; Fig. S1).

To visualise the relationship between the DNA sequences from Hawkes Bay, haplotype networks were inferred using the full sequences and the minimum spanning approach (Bandelt et al. 1999) in PopART (Leigh & Bryant 2015). The aligned

1187 bp of mtDNA from 61 mice had 1096 identical sites and consisted of ten haplotypes. INDELs occurred at two positions; one was parsimoniously inferred at an AT tandem repeat and these positions were recoded as AA in those sequences missing the repeat, which effectively treated the INDEL as a single mutational event for network inference. A mononucleotide repeat region varied in length from 6C to 8C, and in this case, each inferred gap position was recoded as G for network inference. Four haplotypes were detected in the Waikawa Island sample ($n = 14$). The common haplotype (domNZ.12; King et al. 2016), was also present in the Mahia and Gisborne samples, but two haplotypes were recorded on Waikawa Island only. Three haplotypes (domNZ.12; domNZ.14; domNZ.3) were widespread in Hawkes Bay (Fig. 1). Six of the seven rare haplotypes differed by one or two point mutations from one of the common haplotypes; a pattern typical of expanding populations (Slatkin & Hudson 1991). Waikawa Island haplotypes differed by a maximum of 11 substitutions from one another, two were common and two were observed only in the island sample ($\pi = 0.0015$; Fig. 1).

Our full D-loop sequences plus adjacent tRNAs were used for population genetic analyses. To test for evidence of population differentiation, we estimated pairwise Φ_{ST} between each population using the software Arlequin 3.5 (Excoffier et al. 2005). If our mouse samples were taken from a single population Φ_{ST} would not differ from zero (Excoffier et al. 1992) so we tested to see if pairwise Φ_{ST} estimates were significantly >0 using 1000 permutations and a 0.0025 significance level because of multiple tests. Data are available from http://evolves.massey.ac.nz/DNA_Toolkit.htm.

We amplified a nuclear gene, vitamin K 2,3-epoxide reductase subcomponent 1 (*vkorc1*) using a combination of primers (Song et al. 2011) to provide 1205 bp of sequence from the beginning of exon 1 to the end of exon 2. We amplified and sequenced exon 1 of *vkorc1* (260 bp fragment of which 171 bp are coding) from 30 mice from six locations (Table 1). DNA sequences were aligned and edited as appropriate using Geneious, version 8.1.4 (Kearse et al. 2012).

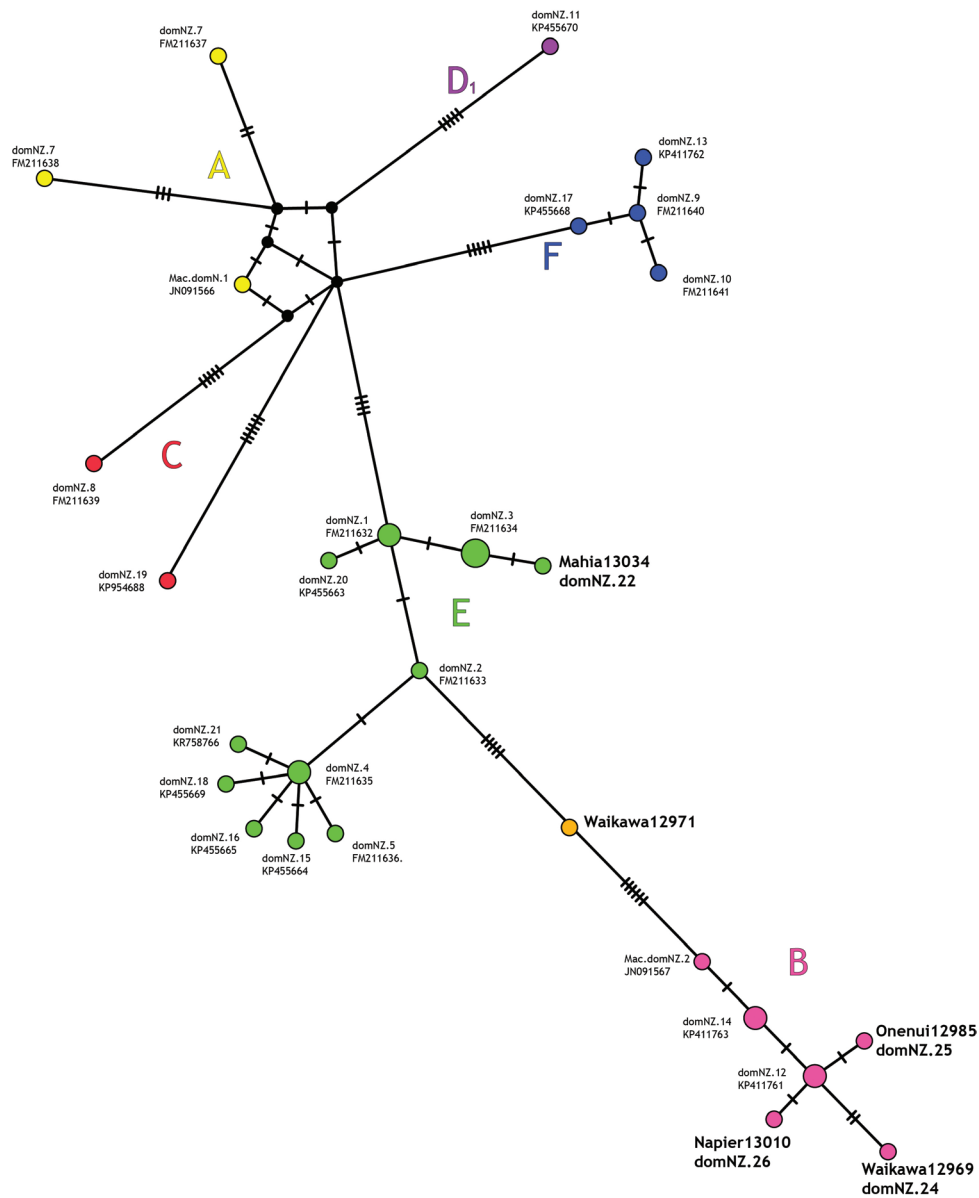


Figure S1. Network of *Mus musculus domesticus* mtDNA haplotypes sampled in New Zealand. Minimum spanning network inferred from mitochondrial DNA sequences of *M. musculus* D-loop haplotypes (879 bp; sampled from New Zealand populations). Sequences representative of the *M. musculus domesticus* haplogroup are shown only, and coloured according to previously reported clusters (King et al. 2016). Clusters A, B, E, and F have been detected in the North Island, C and D in the South Island only, B was common in much of New Zealand, and E was detected only in Hawkes Bay. Few informative sites separate haplotypes and therefore, a phylogenetic approach is not appropriate for these data. Haplotypes labelled in bold are new to this study and have codes domNZ.22 – domNZ.26. Genbank accession numbers provided for published sequences and mouse codes provided for new haplotypes.

Table S1. Summary statistics for three body variables from six population samples of mice collected from Hawkes Bay, New Zealand.

Location	Sample size	Weight (g) Mean (SD)	Body length (mm) Mean (SD)	Tail length (mm) Mean (SD)
Gisborne	5	13.36 (2.86)	76.50 (6.64)	72.17 (2.74)
Mahia	18	12.74 (2.92)	78.03 (6.23)	71.80 (5.78)
Napier	9	13.26 (6.08)	74.73 (11.07)	72.57 (8.34)
Onenui	5	13.33 (1.73)	79.20 (17.29)	73.33 (4.06)
Waikawa Island	10	20.27 (7.51)	81.50 (10.21)	81.70 (7.66)
Wairoa	9	12.57 (2.38)	74.56 (5.09)	72.06 (4.20)

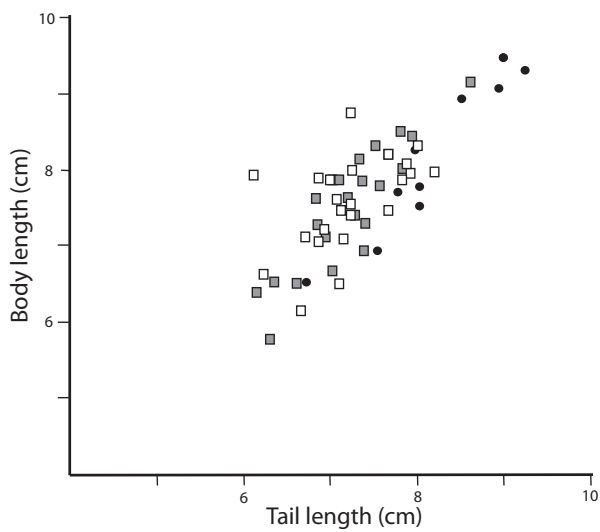


Figure S2. Variation in the ratio of tail-length to body-length in *M. musculus* from Waikawa Island and adjacent mainland New Zealand locations. Waikawa Island mice are filled circles, Mahia Peninsula mice are open squares, and Hawkes Bay mice are grey squares.

Table S2. A linear model to explain weight variation (response variable) of mice collected from six locations in Hawkes Bay, New Zealand.

	S.E.	d.f.	<i>t</i>	<i>P</i>
(Intercept)	-24.3661	4.3111	-5.652	6.79E-07
Mahia	0.1252	1.3059	0.096	0.924
Napier	0.5731	1.4615	0.392	0.6965
Onenui	-1.2466	1.6537	-0.754	0.4544
Waikawa Is.	4.066	1.5584	2.609	0.0118
Wairoa	0.0711	1.5392	0.046	0.9633
Tail length	0.788	0.8913	0.884	0.3807
Body Length	4.1876	0.6964	6.013	1.83E-07

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