Trichosurus vulpecula

Two lineages of brushtail possums (*T* - *c . ec a*) were historically introduced to Aotearoa New Zealand, and these two subspecies have different phenotypic forms. Despite over 100 years of potential interbreeding, they appear to retain morphological differences, which may indicate reproductive isolation. We examined this using population samples from 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. We also sought

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invertebrates (Cowan 2005; Tompkins 2018). Initially introduced to establish a fur industry (Pracy 1974), this species is now recognised as a crucial challenge to conservation of native biosystems and as a vector of bovine tuberculosis- a major issue for the agriculture

effects on genetic structure within our sample, and then

phosphates (dNTP), 0.25 l M each primer, and 0.05U DreamTaq DNA polymerase. Thermocycling used 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-min extension step of 72 °C. Amplification products were sequenced using Big-Dye® 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 (p) diversity, were estimated 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 and Bryant 2015).

within these data. However, if possums had mated randomly with respect to subspecies, we expected to see a mixing of genotypes towards genetic homogeneity. Some spatial differences in genetic composition might remain that reflect initial timing and pattern of genotype introduction. We applied a naïve modelling approach that infers genotypic clusters and assigns individual genotypes using Structure 2.3.2 (Pritchard et al. 2000). Assigned clusters (K) are based on the assumptions that the populations (not the samples) 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.

Bayesian Markov-Chain Monte Carlo (MCMC) clustering of the individual genotypes was run with a the Burnin period set to 5000 and the MCMC repeats thereafter set to 50,000 (Porras-Hurtado et al. 2013). An admixture model was used specifying that the predefined populations based on collecting location 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 determined using the Evanno method (Online Resource 3) 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 (Fig. 1).

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 3.5.2.2 and a Bonferroni correction for multiple tests (0.01667). Pairwise F_{ST} was estimated for microsatellite genotypes of 132 individuals combined and 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 (0.01667). Assortative mating with respect to fur colour would lead to deviations from Hardy–Weinberg proportions so we examined the full dataset and subdivided by fur colour phenotype. We also looked for evidence of genotypic partitioning that would be expected if fur phenotypes were reproductively segregated using the naïve modelling analysis Structure 2.3.2 (Pritchard et al. 2000) with K values of 1 to 3.

Overall haplotype diversity (h) was 0.56 and nucleo-

Additional markers will provide resolution for a national wide study of this species' invasion.

Notably the brushtail possums in Aotearoa New Zealand exhibit fur colour variation that distinguishes the two subspecies of *T c   ec a* introduced to the country (Fig. 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 patterns of gene flow in this invaded landscape remain largely concealed. Analysis of neutral genetic loci and phenotypic information found evidence that distinct brushtail possum lineages established in the North Island of New Zealand from separate introductions from Australia have remained partitioned (Sarre et al. 2014; Campbell et al. 2021). These analyses imply ongoing assortative mating of genotypes characterised by different fur colour (and origin), highlighting the potential for cryptic lineages (Bastos et al. 2011; Jarić et al. 2019; Perry et al. 2018).

We conducted a fine-scale analysis of genetic structure of brushtail possums collected from Kenepuru Peninsula (Fig. 2), which is a near closed system with a barrier to dispersal from the mainland in the form of a narrow isthmus. Within this landscape we sought evidence of genetic partitioning using seven nuclear microsatellite markers and a mitochondrial marker to resolve reproductive exchange among possums of mixed ancestry. Phenotypic variation based on morphological data suggested 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 (Fig. 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

Peninsula was assisted by Steve Podjursky (Bowron, Jackson and Harvey properties 2016), Emily Osbourne and Mitch Bartlett (Skiddaw property, Koutuwai 2019), Alistair Jenner (Millig and Waitaria Bay properties 2019), Kristen and Mike Gerard (Hopai and Port Gore properties 2019), and Landcare Research (Kenepuru, 2003). We thank Andrea Taylor and team for making raw data from the 2004 possum study available for reanalysis.

Declarations

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