DNA profiling – a management tool for rat eradication

R. M. Fewster[1], S. D. Miller[2], and J. Ritchie[3]
[1]Department of Statistics, University of Auckland, Private Bag 92019, Auckland, NZ, [2]Department of Statistics, University of Waikato, Private Bag 3105, Hamilton, NZ, [3]Natural Logic Environmental Management, 21 Dormer Rd, RD2, Helensville 01875, NZ.
Corresponding author email: r.fewster@auckland.ac.nz

Abstract DNA profiling is a powerful tool for eradication planning and post-eradication management. We give an introduction to DNA methods for conservation, intended to be accessible to non-specialists with no previous knowledge of genetics. We illustrate the methods with a case study from Aotea/Great Barrier Island, New Zealand, where DNA methods have been used to manage eradications of ship rats (*Rattus rattus*). In initial management planning, DNA profiling gives evidence about reinvasion risk if an eradication is attempted. In the case of Great Barrier Island, we find that cliffs may be significant factors affecting reinvasion risk. After an eradication is attempted, DNA testing can determine whether new rats that appear are survivors of the eradication or reinvaders from another location, and can often determine precisely where the reinvaders have come from. This helps to focus management efforts for the prevention of future reinvasions.

Keywords: eradication, genetic boundary, island, microsatellite, *Rattus*, reinvasion

Introduction

The creation and maintenance of island sanctuaries free of rodents is a major conservation focus in New Zealand. The most problematic invasive rodents include Norway rats (*Rattus norvegicus*) and ship rats (*R. rattus*), both of which can swim hundreds of metres, or hitch-hike to islands on boats. As rat eradication attempts become more widespread and more ambitious, we need to advance our understanding of reinvasion processes, including the swimming capabilities and tendencies of rats, and the frequency of accidental boat transport.

DNA profiling of rat populations is a relatively new tool for eradication managers. Several studies attest to its usefulness for managing rat populations on islands (Robertson and Gemmell 2004; Abdelkrim *et al.* 2007; Russell *et al.* 2010) and the mainland (Abdelkrim *et al.* 2010). DNA profiling can inform island managers in two ways. Firstly, it can uncover patterns of swimming in existing rat populations, by assessing the level of gene flow between different islands. Some islands are genetically isolated from each other, suggesting that either there is little migration between them, or there are social factors that inhibit breeding after migration. Other islands are genetically linked, suggesting high migration and interbreeding. We can study features associated with isolated or linked populations, such as the size of the water crossing, presence of cliffs, and accessibility of landing points. An understanding of features associated with high or low gene flow can help to suggest candidate islands for eradication in the future.

Secondly, DNA profiling can determine whether rats found after an eradication attempt are survivors of the eradication, or reinvaders from another source. This is vital for targeting the management response, either for improving biosecurity in the case of reinvaders or for examining eradication protocols in the case of survivors. Both outcomes can enhance our understanding for the future as well as for a specific situation. Reinvaders help to calibrate

how genetic isolation translates to actual reinvasion rate. Survivors clarify our expectations about the short term effectiveness of a poison drop, especially among eradications that are eventually deemed 'successful' after the standard two-year follow-up period.

Our aim in this paper is to provide an accessible introduction to DNA profiling as a tool for eradication management, assuming no previous knowledge of genetics. Interpretation of DNA evidence is not always precise, and there is an immense and bewildering array of statistical analysis methods and software packages. Instead of aiming to be comprehensive, we will deliberately restrict our coverage to two genetic concepts, and attempt to explain these in enough detail for non-specialists to appreciate their power and limitations. The first concept is 'genetic distance' between populations, to measure genetic isolation of different islands, and we explore this using the distance measure F_{ST}. The second concept is of 'individual belongingness', which measures how well a single rat fits into each of several candidate populations, for example whether it is a survivor or a reinvader. For this we will describe the idea of genotype probabilities.

Our account is based on a study of ship rats in the archipelago surrounding Aotea/Great Barrier Island, New Zealand (28500 ha; Fig. 1). We report results from extensive DNA sampling from 2005 - 2008, and link genetic structure to features such as cliffs and water distances. In 2008, an ambitious eradication focused on Kaikoura Island (530 ha) in the west, and we report the contributions of the DNA work to sourcing post-eradication rats that appeared on Kaikoura from early 2009 onwards. In 2009, a further eradication took place on the Broken Islands 3 km south of Kaikoura. We consider how DNA evidence could contribute to ongoing management of this region.

Methods

Sampling

The Aotea/Great Barrier Island archipelago includes three island clusters: the Kaikoura chain comprising Kaikoura, Nelson, and Motuhaku; the Grey group of about 6 small islands; and the Broken Islands comprising Motutaiko, Rangiahua/Flat Island, Papakuri, Big Mahuki and Little Mahuki. From 2005 to 2008 we sampled a total of 270 rats from 12 locations (Fig. 1). We focused on the three island clusters, adjacent locations on the main island (Aotea), and two outgroups at Windy Hill and Awana. Rats were caught with snap-traps, and DNA samples corresponding to tail clips of about 4cm were preserved in 70% ethanol.

Eradications

In mid-2008, the Motu Kaikoura Trust began the eradication of rats from Kaikoura Island and nearby islands (Grey Group, Nelson and Motuhaku) using brodifacoum cereal baits spread by helicopter twice over two weeks in August and September. It was followed up with an intensive ground-based detection and response system on Kaikoura and the nearest parts of Aotea within swimming distance by rats. New rats were detected on Kaikoura by early 2009.

In June 2009, the Auckland Regional Council initiated rat eradication from the Broken Islands following a protocol similar to the protocol on Kaikoura. As of January 2010, no new rats had been reported from these islands.

Genetic loci and DNA profiling

A genetic *locus* (plural *loci*) is a position on a rat's DNA. For the type of loci that we use, every rat has two *alleles* at every locus, one inherited from each parent. The two alleles may

be the same as each other, or different. When the rat reproduces, one of its two alleles is selected at random to be passed on to its offspring.

Some genetic loci contain molecular code for a specific physical trait, such as hair colour, in which case the outcome of this trait for a given rat will be determined by which alleles it possesses. However, many loci contain 'junk' or non-coding DNA known as microsatellites. These loci surround the useful loci like packaging in a box. They follow the same rules of genetic inheritance, but do not correspond to any physical trait, so they are prone to harmless coding errors or mutations. Over millennia, mutations create numerous available alleles for these loci, none of which do anything. The resulting genetic variety means that different populations can have very different genetic profiles at junk loci, so these are the loci chosen for DNA profiling studies and forensics.

The key to DNA profiling is the different proportions of alleles in different populations. On one island, 80% of the alleles at a junk locus might be of type A and 20% of type B, whereas on a neighbouring island, there might be 70% of type B and 30% of type C. If an unknown rat has an allele of type A, it must be from the first island, while if it has type C it must be from the second island. Alleles of type B could be from either island but are more common on the second island, so we operate on the balance of probabilities. A conclusive decision requires not one but several loci, each of which sways the balance of probabilities one way or the other. The combined strength of about ten loci is often enough for a conclusive decision. This is the principle underlying *genetic assignment tests* – the process of assigning an unknown individual to a population.

Our study used ten microsatellite loci: D10Rat20, D11Mgh5, D15Rat77, D16Rat81, D18Rat96, D19Mit2, D20Rat46, D2Rat234, D5Rat83, D7Rat13 (Jacob *et al.* 1995). Details of the DNA extraction and amplification are given in Russell *et al.* (2010).

Can we tell the populations apart? Genetic distance

Some populations are more genetically distinguishable than others. For example, it is easier to distinguish between Asian and European populations of humans than between Scottish and English populations. The genetic differentiation depends upon the length of time since the populations split, their size, and the amount of ongoing migration between them. Similarly, the junk DNA in rats on an island can quickly develop allele profiles that differ from other islands, especially if the founding populations involved a small number of individuals. Substantial ongoing migration between island populations will keep them genetically similar.

The degree to which different populations are genetically distinguishable can be measured by a *genetic distance*. A widely accepted distance measure is F_{ST} (Wright 1978), where *F* denotes 'Fixation index', and 'ST' denotes 'Subpopulation within the Total population'. The 'subpopulations' can be seen as different islands and the 'total population' as the combined subpopulations.

To illustrate F_{ST}, we can think of two islands with the same numbers of rats, and a single locus with two possible alleles, A and B. If all A and B alleles from rats on both islands were put together and one allele drawn at random, the selected allele would vary between A or B. F_{ST} is the proportion of this variance that is explained by the differences in allele frequencies between islands. For example, suppose the two islands are identical, each with 50% allele A. Knowing which island a selected allele comes from gives no information about which allele it is, so the genetic distance between islands is $F_{ST} = 0$. However, suppose allele A is possessed

by no rats on island 1 but all rats on island 2. The combined proportion of A from both islands is still 50%, but in this case, knowing which island the selected allele is from specifies exactly which allele it is. The island differences therefore explain 100% of the variance in allele selection, so their genetic distance is $F_{ST} = 1$. The same idea of partitioning variance can be extended to calculate F_{ST} when there are different population sizes, multiple alleles, and multiple loci.

In summary, F_{ST} measures genetic distance on a scale from 0 to 1. At 0 the populations are genetically indistinguishable and at 1 they are completely distinguishable, i.e. they are fixed for different alleles. A useful rule of thumb is that F_{ST} values from 0 to 0.05 denote little genetic distance; 0.05 to 0.15 denote moderate distance; and 0.15 and above signal large genetic distance and easily distinguishable populations (Wright 1978).

In this study, we calculated F_{ST} for pairs of adjacent populations using Genepop on the Web (Rousset 2008), available free from *http://genepop.curtin.edu.au/*. We further used the software FreeNA (Chapuis and Estoup 2007, *http://www.ensam.inra.fr/URLB/*) to correct our F_{ST} estimates for the possible presence of null alleles, which are alleles that do not show up on the DNA profile of an individual due to a mutation just outside the microsatellite region. Although we use the corrected estimates here, there is negligible difference between these and the estimates gained from Genepop. FreeNA uses the method of Weir (1996) to calculate F_{ST} after possible null alleles have been excluded.

Where does a rat come from? Genetic 'belongingness'

F_{ST} is used for measuring the genetic distance between two populations, such as two islands. We also need a method for measuring how well an individual rat fits into a given population, which is useful for two reasons. Firstly, if a rat has unknown origin – for example it is a reinvader to an island that has previously been eradicated – we can examine its fit to all possible source populations to estimate where it came from. This process is *genetic assignment*. Secondly, we can routinely examine the fit of all rats to all populations, which can reveal individual anomalies such as rats caught on one island that have the genetic characteristics of a different island. Such anomalies provide direct evidence of migration by swimming or by boat, and they cannot be detected by population-level distances such as F_{ST}.

To understand how an individual measure of 'genetic belongingness' works, we will use another example from human populations. If a blond man is seen walking down the street in Zanzibar, we might want to know where he comes from. Blondness is common in Sweden – perhaps 80% of Swedes are blond – but people are also blond in many other countries. If we give the man an 80% belongingness probability for Sweden, it means that 80% of Swedes are blond, not that the man is 80% likely to be Swedish. Unfortunately, there is no way of calculating the man's probability of being Swedish, much as we would like to. We can only say how common his blond characteristic is in Sweden, and compare with how common it is in other countries.

This idea is a common source of confusion in genetic reporting. If we replace the human analogy with rats on islands, we can change our 'blond man' to a 'rat with its observed set of alleles', and replace Sweden by a possible island source for the rat. All we can say about the rat is that it is more or less typical of different islands, just as blond men are common in Sweden but less common in Italy. We cannot say that the rat is 80% likely to come from any island, just as it is absurd to suggest that 80% of blond men in Zanzibar are automatically forced to be Swedish.

To help to keep the distinction clear, we will refer to the probabilities as measures of *genetic 'belongingness'* or '*fit'.* A blond man looks as if he *belongs* or *fits in* to Sweden, but this does not exclude him from fitting equally well or better to Denmark or elsewhere. Similarly, given a specific rat, we calculate the probability of this rat's alleles in each of our potential islands. Because every rat is unique, these probabilities will usually be very small, so we take logs to convert tiny numbers back to a manageable scale.

The measure of 'belongingness' or genetic fit that we use is called the *log genotype probability.* The *genotype* is the particular set of alleles that the rat possesses at the junk loci in our study: for example it might have alleles A and B at the first locus, G and G at the second locus, and so on. Every island has its own allele frequencies, so the rat with genotype AB/GG will have different belongingness probabilities for every different island – just as a blond man might have a belongingness probability of 0.8 to Sweden and 0.3 to England. If it is known that an island has 60% alleles of type A and 40% of type B at the first locus, and 30% alleles of type G at the second locus, our rat's log genotype probability for this island would be $\log\{(2\times0.6\times0.4) \times (0.3\times0.3)\}$. The contribution for the first locus is multiplied by two because the AB alleles could have arisen two ways, either getting A from the mother and B from the father, or the reverse. These calculations rely on two assumptions: firstly that each locus is in *Hardy-Weinberg Equilibrium (HWE)*, so that the genotype probabilities for the locus can be obtained by multiplying the allele probabilities as above; and secondly that the loci used are independent (described as *linkage equilibrium*), so that the probabilities for different loci can be multiplied together. Some loci may have to be discarded if tests indicate that there are substantial deviations from Hardy-Weinberg or linkage equilibrium.

In practice, we will not know that the island has exactly 60% allele A, 40% allele B, and so on. These numbers have to be estimated from the animals caught on the island. The sampling error in estimating these frequencies is accommodated in the log genotype probabilities, so the approach is not quite as simple as inserting the sample frequencies 0.6 and 0.4. In particular, if an allele C is not sampled on island 1, it doesn't mean it is absent there. The log genotype probabilities account for the possibility that allele C might be present at low frequency, and will not completely exclude the island as a possible source for a rat with allele C. This is accomplished through a Bayesian method, so the belongingness probabilities are sometimes called *log posterior genotype probabilities*, with *posterior* indicating that they are the probabilities obtained after the allele frequencies have been estimated. The methods we use for belongingness computation are identical to those found in the free program GENECLASS2 (Piry *et al.* 2004), using the Bayesian criterion of Baudouin and Lebrun (2001).

Given a rat's observed alleles, we calculate the genotype probabilities, or belongingness probabilities, for each of the possible source islands in our study. A powerful way of conveying this information is to plot it on a graph. If there are two possible source islands, we plot the belongingness probabilities for the two different islands on a two-dimensional scatter-plot, where each point gives the two belongingness probabilities for a single rat. We have found this visual method to be an effective way of communicating genetic structure quickly and easily. It requires an imputation method for dealing with missing genetic data, described in Russell *et al.* (2010). We omit from the plot any rats with missing data at more than three loci. If there are more than two populations, a multivariate plotting method is required, which we do not show here.

If the rat's origin is unknown, for example it has been detected on an island following an eradication attempt, we can *assign* it to a possible source population by selecting the population for which it has the highest belongingness probability. This is akin to estimating that all blond men seen in Zanzibar come from Sweden, on the basis that Sweden has the highest proportion of blonds in the world, so the interpretation should be treated with caution. This is why we recommend the visual approach, which might reveal that the blond man has an excellent fit to Sweden but also a perfectly reasonable fit to England. Nonetheless, it is useful at times to collapse the findings to a single selected population source. The population to which the rat has the highest 'belongingness' is given by the percentage scores output by GENECLASS2.

We test for deviations from Hardy-Weinberg and linkage equilibrium using Genepop on the Web (Rousset 2008). For Hardy-Weinberg proportions, we use the option for an exact test when there are fewer than five alleles at a locus, and for the remaining loci we use Guo and Thompson's (1992) unbiased estimate of the exact *p*-value.

Keeping out the neighbours: genetic boundaries, cliffs and water crossings

Using the tools of genetic distance (F_{ST}) and belongingness, we can investigate associated geographical features. We construct a genetic relatedness diagram for F_{ST} and search for genetic boundaries using the Monmonier algorithm (Monmonier 1973) from the package ADEGENET (Jombart 2008) in R (R Development Core Team 2009). The Monmonier algorithm finds the pair of islands with the highest F_{ST} between them, and grows boundaries until it can no longer find island pairs with an F_{ST} above a pre-set threshold, which we set at 0.13.

Using the genetic relatedness diagram, we determined a *separation type* between each pair of islands on the diagram, on the basis of maps, aerial photographs, and fieldworker reports. If islands are separated by a water gap of 1km or more, their separation type is recorded as 'long water'. For gaps of less than 1km, the type is recorded as 'cliff' if the separation is severely cliffy or otherwise inaccessible on one or both sides of the crossing, and 'beach' otherwise. The other separation types are 'land' if the locations are connected by land, even if the distance is considerable; and 'none' when assessing belongingness for a rat into its own population.

We can investigate the impact of separation type on both F_{ST} and belongingness. For F_{ST} we plot the pairwise F_{ST} estimates according to separation type. For belongingness, we conduct a simple linear regression with response of log genotype probability for every rat into every population in the network, and predictors given by two categorical variables, the first being separation type between the rat's sampling population and the target population, and the second with a different level for each target population. The results of interest are the estimated levels for the different separation types: beach, cliff, long water, and land, which show the impact of separation type on belongingness probability.

Results

Genetic boundaries

Genetic boundaries plotted onto the map (Fig. 2) visually appear to correlate with long water crossings and cliffs. In particular, there are strong genetic boundaries between the tiny Grey Group Islands and all other locations, corresponding to long water crossings. There are also clear boundaries along the cliffy areas from the main island (Aotea) to the Broken Islands, from Kaikoura to Nelson, and from Nelson to Motuhaku. The beach crossings between

Kaikoura and Fitzroy / Red Cliffs areas, and between Motutaiko and Flat Islands, and Flat and Mahuki Islands, are of similar sizes to the cliffy crossings but do not present genetic boundaries.

We also calculated genetic distances, and belongingness coefficients from the regression, categorized by separation type (Fig. 3). Both methods reflect the same picture: long water crossings create the largest genetic boundaries, followed closely by cliff crossings, then land and beach separations represent substantially less genetic difference.

Survivors or reinvaders?

The exact test for Hardy-Weinberg equilibrium indicated significant departures from equilibrium at three loci: D10Rat20, D20Rat46, and D5Rat83. For a conservative approach, we present results with these three loci excluded from assignment analyses; however, there are no substantive changes in our conclusions when these loci are included (see also Table 1).

The linkage disequilibrium tests revealed only minor evidence of linkage disequilibrium among the Broken Island rats.

Rats were eradicated on Kaikoura, Nelson, Motuhaku, and Grey Group in August 2008. New rats were caught in traps on Kaikoura from March 2009 onwards, and a total of 11 rats and two mice (*Mus musculus*) were caught up to November 2009 and submitted for DNA testing. One of the rats was discovered by DNA analysis to be Pacific rat (*Rattus exulans*). Neither mice nor Pacific rats had been detected on Kaikoura before the eradication, despite 61 ship rats being trapped from 2005 - 2008. If they were present before the eradication, these species might have been undetected due to competition for bait from the more dominant ship rats. Pacific rats and mice are considered unlikely to be swimmers, so their post-eradication presence suggests either survivors of the eradication, or transport by boat. Of the remaining ten ship rats, eight were fresh enough when preserved to provide good DNA.

Of the eight post-eradication Kaikoura rats, one had a strong assignment to the Broken Islands. Its belongingness score for the Broken Islands was in the centre of those from genuine Broken Islands rats. Only four of the 211 rats sampled from outside of the Broken Islands in 2005-2008 equalled or surpassed this score (none if all ten loci were used). This presents very strong evidence that this rat came from the Broken Islands. The distance is too far for swimming, and implies boat transport.

Each of the remaining seven rats were given two belongingness probabilities (log-genotype probabilities) identified in Fig. 4a: one for the hypothesis that it is a survivor from Kaikoura Island, the other for the hypothesis that it came from the main island (Aotea), grouping together the locations Fitzroy, Red Cliffs, and Mainland from Fig. 1. Circles on the plot denote rats sampled on Kaikoura before the eradication from 2005-2008. Triangles denote rats sampled in the aforementioned three mainland sites in 2005-2008. Squares denote the post-eradication rats whose source we wish to determine. A high value on either axis represents a good fit to the corresponding population, and the diagonal line represents an equally good fit to both.

We found a large overlap between the two populations, in keeping with the low F_{ST} values and accessible landings on Kaikoura. This makes it very difficult to distinguish between the survivor and reinvader hypotheses for these rats. However, six of the seven rats fell below the diagonal line (Fig. 4a), favouring the hypothesis that they are survivors of the eradication

from Kaikoura. Although the hypothesis of swimmers from Aotea cannot be excluded for any of these rats individually, it is extremely unlikely (p=0.001) that a group of seven swimmers would yield six or more with a better belongingness to Kaikoura than to their native Aotea. Thus we have very strong evidence that these seven rats include some survivors of the eradication.

The leftmost of the post-eradication rats in Fig. 4(a) has a poor fit to all our sampled populations on Great Barrier Island, having the worst all-round fit out of all 270 rats we have sampled in the archipelago. This raises the possibility that it might have arrived by boat from outside the region.

The Broken Islands are separated from Aotea by rugged terrain on the Aotea side. By contrast with Kaikoura, the plot for the Broken Islands (Fig. 4b) clearly distinguishes between rats from the Broken Islands and those from Aotea, even though the water gap is less than 300m. We thus have much greater power to discriminate between survivors and reinvaders for the Broken Islands case, should new rats be detected. The plot, and the statistics in Table 1, indicate that Broken Islands genetics form a subset of Aotea genetics, in the sense that Broken Islands rats largely have a good fit to the Aotea population (i.e. circles have a high score on the vertical axis in Fig. 4(b)), but Aotea rats do not have a good fit to the Broken Islands population, shown by the low scores of triangles on the horizontal axis of Fig. 4(b).

Discussion

Our results from Great Barrier Island suggest that cliffs may be a significant factor in limiting gene flow for ship rats between two islands over short water crossings. Ship rats are capable climbers, so this is perhaps a surprising result. There are many possible behavioural reasons for cliffs to act as boundaries. However, it is also possible that the cliffs on Great Barrier Island are not the cause of the separation, but are simply associated with some other factor, such as water currents.

The genetic results from post-eradication Kaikoura Island, together with an unexpected Pacific rat and two mice, provide strong evidence that in early 2009 there were survivors of the August 2008 eradication. While disappointing, we do not know how unusual this result is, because there is often no post-eradication monitoring until two years after the eradication has taken place. No evidence of breeding was found among the post-eradication rats in early 2009. At least one other rat was almost certainly transported by boat from the Broken Islands. The genetic diagrams show that it will be a challenge to keep Kaikoura rat-free, and that we cannot be conclusive in discriminating between survivors and swimmers. Some threats have been removed by the additional eradications that took place in 2009 on the Broken Islands and the main island. Future risk can be reduced by publicity among boat users in the area, and further control on the mainland fringe.

DNA profiling can be a powerful tool in conservation management, both for understanding underlying behaviour and for sourcing individual rat invaders. To best exploit the opportunities offered, coordination is needed among different management and research groups. Genetic results from different labs are only comparable if they use the same genetic loci and share control samples for calibration. The ideal would be to collate genetic results from around the country into a national database, accessible to any management groups with reinvaders to source. Crucially, we encourage managers to take DNA samples before any eradication is attempted. Studies should aim for samples of at least 30 rats from each source

population, including the island for eradication, although some islands will provide a strong genetic signature with fewer samples.

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Table 1: Summary statistics for genetic assignment analyses shown in Fig. 4. H_e , H_o and Hardy-Weinberg tests were calculated using Genepop on the Web (Rousset 2008). An individual is heterozygous at a locus if its two alleles are different. H_e gives the mean (across loci) of the proportion of individuals that would be heterozygous at that locus under Hardy-Weinberg equilibrium, and H_o gives the equivalent mean proportion observed in the sample. The HW exact test has null hypothesis that the genotype proportions are in HWE, and alternative hypothesis that they are not.

	Kaikoura	Mainland	Broken Islands
Number of rats included	60	54	60
Mean number of distinct alleles per locus	7.7	8.4	5
Expected heterozygosity, H_e	0.71	0.71	0.57
Observed heterozygosity, H_o	0.65	0.67	0.53
p-value for HW exact test	0.07	0.34	0.48

Fig. 1: Sampling locations on Great Barrier Island and surrounding islands. Numbers in brackets give the number of rats from each location for which DNA samples were submitted for genotyping. The Broken Islands are the group of Motutaiko, Flat, and Mahuki.

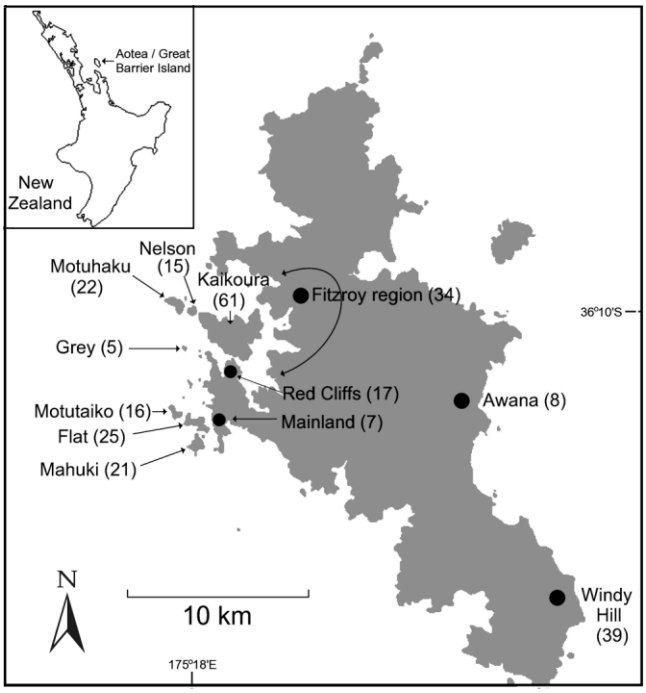

Fig. 2: Genetic relatedness network. Numbers on the lines are F_{ST} values multiplied by 100 and rounded to the nearest integer. Values of 13 and above constitute a genetic 'boundary', marked by thick lines. The map on the right shows the physical locations of the genetic boundaries (four dashed lines). Cliff regions are marked on the map with bold black lines.

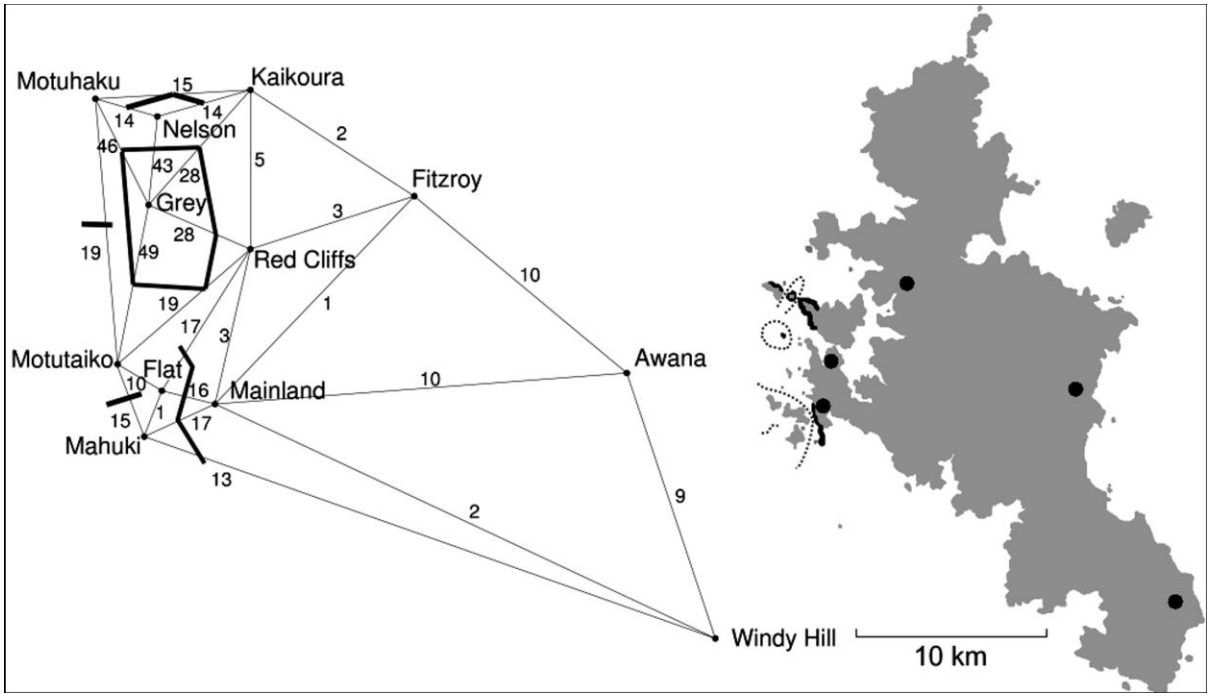

Fig. 3: Impact of separation type on belongingness coefficients (top) and F_{ST} (bottom). For belongingness coefficients, the most negative effects suggest the greatest barriers to genetic relatedness. For the genetic distance F_{ST} , the most positive values give the greatest barriers. Both measures give the same ordering of separation types.

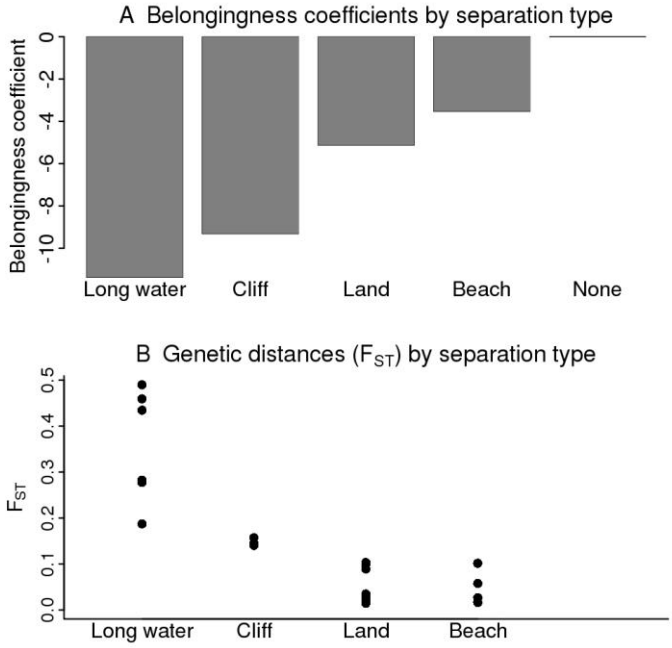

Fig. 4: Belongingness diagram for Main Island rats, sampled from Fitzroy, Red Cliffs, and Mainland areas on Fig. 1, against (A) Kaikoura rats, and (B) Broken Island rats.