Queensland University of Technology

FINAL REPORT

Project – Integrated Feral Pig Management for the Herbert cane area (Here Piggy Piggy!): Feral Pig Population Genetics Component

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Project Outline – The biggest impediment to successful feral pig control in Australia, including localised eradication, is reinvasion following *ad hoc* or poorly designed control programs based on operational ease rather than ecological knowledge of effective management units. This project, developed in consultation with Herbert Cane Productivity Services Ltd and partners, has investigated this problem. In this study, genetic methods were used to examine the population structure of feral pigs in the Herbert district of north Queensland to gain a better understanding of migration of feral pigs across the landscape. In total, 403 feral pigs have been screened for eight microsatellite loci and the population genetic structure, migration and ecological management units have been identified.

Background

Feral pigs pose a significant threat to World Heritage biodiversity values and agriculture including sugarcane and tropical fruit crops in north Queensland. Mitchell (2003) estimated that two to three million feral pigs inhabit north Queensland. The financial loss impact caused by feral pigs in 2009-10 in the Herbert cane district alone was in excess of $570,000; greater than any other pest (including cane grubs and rats) at present (Herbert Cane Productivity Services Ltd pers. com.).

To develop an efficient control program to reduce or eradicate feral pigs, management units (MUs) should be defined. Localised eradication can only be achieved if reinvasion following control does not occur (Choquenot et al. 1996, Hone et al. 1980), and therefore relies on the identification and management of demographically independent populations where dispersal is limited between populations. Currently, most feral pig control is undertaken in an *ad hoc* manner (individual farms or properties) or according to operational boundaries (council areas, catchments) rather than being based on ecological knowledge of the spatial distribution of populations. MUs that are designed using biological knowledge of natural population boundaries allow the identification of demographically independent units with clusters of populations that need to be eradicated simultaneously in order to maximize the long-term success of the operation (Robertson and Gemmell 2004).

When there is limited knowledge on dispersal pathways or when population boundaries are unknown, genetic data can be used. The population genetic structure of feral pigs in the Herbert cane region of north Queensland is currently unknown. To date managing feral pig populations in a cane landscape has had limited or varying degrees of success (Herbert Cane Productivity Services Ltd pers. com.). Anecdotal reports suggest that feral pigs routinely move between rainforest and agricultural crops, particularly during the dry season. While radio-tracking of feral pigs has been undertaken in the Wet Tropics World Heritage Area and has revealed that pigs are generally sedentary and move only short distances (approximately 1 km on average) from the centre of their home ranges (Mitchell et al. 2009), little is known about feral pig movements in the Herbert region.

Objectives

An examination of the population genetic structure of feral pigs in the Herbert region will provide valuable information on the population dynamics of this species and in particular, will identify:

1. if the region acts as a single demographic management unit (based on the extent of dispersal among locations) or whether it is composed of multiple management units;
2. the pattern or route of feral pig movement within the district and whether pigs are moving between cane, forest and non-forest areas;
3. source populations (if they exist) and provide recommendations for targeted implementation of control.

Methods

Sampling

403 feral pig tissue samples were obtained from 100 locations in the Herbert River region (see Appendix 1 for a list of samples). Feral pigs were trapped in all major habitat types in the region including, cane, crops, grazing land, forest and parks, by authorised and licenced contractors and feral pig tissue samples were provided to QUT by Herbert Cane Productivity Services Ltd (QUT Animal Ethics Tissue Notification # 1300000039).

Genetic Methods

Microsatellite DNA markers are extremely useful tools for examining population genetic questions of this nature and have been applied in a number of feral pig genetics studies (Hampton et al. 2004a, b, Spencer and Hampton 2005). Microsatellites are highly variable with rapid rates of mutation and are useful for revealing localised population structure. They consist of tandem repeats of short nucleotide sequences, are randomly distributed across the genome and occur at a high frequency in non-coding regions of eukaryotic DNA.

Total DNA from tail and ear tissue samples was extracted using the salting-out methodology of Miller *et al.* (1988). The tissue was digested overnight at 55° C in an incubation buffer containing Proteinase K. All PCR amplifications were performed on an Eppendorf Mastercycler. Reaction mixes contained 50-100 ng of template DNA, and final concentrations of 1.5mM $MgCl_2$, 1 x buffer, 0.2mM of each dNTP, 10 pmoles of each primer with fluorescent label (FAM, PET, NED, VIC) at the 5' end, and 0.5 units of *Taq* polymerase to a final reaction volume of 10µL. Eight loci that have previously been shown to be polymorphic and unlinked in *Sus scrofa* (Alexander et al. 1996) were analysed in this study (SW240, SW632, SW857, SW911, SW936, SW951, S0002, S0068), following conditions outlined in Hampton et al. (2004a), and were resolved using an ABI3500 genetic analyser.

Pig breed was determined by analysing mitochondrial DNA. A hyper-variable portion of the mitochondrial DNA (mtDNA) control region from 53 individuals was amplified using primers MT16498H (Meyer et al. 1990) and MT15996L (Campbell et al. 1995). The PCR reaction was performed in a 25 µl volume and contained 0.6 µM of each primer, 1 x MyTaq Red Buffer (Bioline, Australia), 0.5 unit of My Taq HS DNA polymerase (Bioline, Australia), 16.4 µl of ultra-distilled H2O

and 50-100 ng of template DNA. A Mastercycler ® ep (Eppendorf, Hamburg, Germany) was used and the PCR cycle protocol included an initial denaturation (94˚C) for 15 minutes, and then 30 cycles of 94˚C for 15 seconds, 50˚C for 15 seconds, 72˚C for 30 seconds, and a final extension at 72˚C for 5 minutes. PCR products were purified using an Isolate PCR and Gel Kit (Bioline, Australia) according to manufacturer's instructions. Purified PCR products were amplified in a sequence reaction containing 1.0 µl of PCR product, 1.0 µl of MT15996L (3.2 µM), 0.5 µl of version 3.1 ABI Prism ® Big Dye Terminators (Applied Biosystems, CA, USA), 3 µl of 5 x sequencing buffer and 14.5 µl of dH2O. The sequencing cycle protocol involved an initial denaturation at 94˚C for 5 minutes, followed by 29 cycles of 94˚C for 10 seconds, 50˚C for 5 seconds, 60˚C for 4 minutes, and then a final hold step of 4˚C for 10 minutes. Sequenced DNA was precipitated using a standard ethanol/EDTA protocol prior to analysis on an ABI3500 genetic analyser (Applied Biosystems, Australia). The mtDNA sequence data was aligned by eye using BioEdit v7.0.0 (Hall 1999). GenBank sequences from Asian domestic, European domestic, Asian wild boar and European wild boar were used to identify pig breed origins.

Data Analyses

From the original 403 samples, 385 samples produced adequate data (>4 loci successfully amplified and resolved) for further analysis. Unless otherwise stated, samples from locations less than 1 km apart were pooled into 50 resultant sites for data analysis. The final dataset consisted of 173 female, 191 male and 21 *S. scrofa* of unknown sex.

Population structure across the region

A Bayesian clustering approach implemented in STRUCTURE v2.3 (Pritchard et al. 2000; http://pritch.bsd.uchicago.edu) was used to estimate the number of populations or groups (*K*) in a sample and to assign individuals to one or more of these populations (*k*). Ten runs of *K* = 1 to 25 was performed at 100000 MCMC repetitions and 20000 burn-in period using no prior location information, independent allele frequencies and a model of admixture. The posterior probability was then calculated for each value of *K* using the estimated log-likelihood to choose the optimal number of populations. Individuals were assigned to each of the inferred populations based upon the highest percentage of membership (based on the percentage of ancestry that can be attributed to each inferred population). This approach was employed for the entire dataset (385 samples) to estimate the number of groups for *S. scrofa* across the Herbert River region.

An Analysis of Molecular Variance (AMOVA) was conducted in ARLEQUIN v3.5 (Excoffier and Lischer 2010) to assess partitioning of variation within and among the two groups identified by Bayesian clustering (see Results). F_{ST} –like genetic distances were estimated for all populations with five or more individuals (24 populations). Isolation-by-distance (i.e., increasing genetic distance with geographical distance) was tested with 1000 iterations of the Mantel's test in ARLEQUIN v3.5 using population pairwise F_{ST} –like estimates and geographical distance (log transformed).

To estimate any influence of habitat on the genetic structure of *S. scrofa* in the Herbert River region, an individual's geographical distance (m) from nearest highland area (>200m above sea level) was recorded. Individuals from sites situated within forest were given a 'distance' of 1m to avoid values of 0 in the analysis and all individuals from the same population necessarily had identical geographical distance values. A correlation between individual geographical distance to highland area and the proportion of population membership to one of the groups identified by STRUCTURE analysis (see Results) was performed.

To determine whether female and male *S. scrofa* displayed gross differences in their genetic structure across the region, separate STRUCTURE analyses were run for each sex under the same conditions as above. To detect sex-biased dispersal of S*. scrofa* across the region GenAlEx v6.4 (Peakall and Smouse 2006) was employed to compare the distribution of assignment indices between the sexes using a U-test.

Spatial autocorrelation implemented in GenAlEx v6.4 was used to explore the patterns of individual genotypes in space across the entire region. An autocorrelation was generated that provides a measure of the genetic similarity between pairs of individuals whose geographical separation falls within a specified distance class. The analysis was run for 5, 10, 15 and 20km distance classes. The distance class size at which the autocorrelation co-efficient (r) is no longer significant provides an approximation of the extent of detectable positive spatial genetic structure.

Population structure within identified groups

STRUCTURE v2.3 was employed (using the same parameters as above) to estimate population genetic structure within the two geographically delineated groups (see Results) identified from initial analysis across the entire Herbert River region. Within group genetic structure can be used to explore finer scale patterns such as local dispersal between populations.

Isolation-by-distance was assessed for the two groups separately using an F_{ST}-like genetic distance, as described above. Spatial autocorrelation was also employed separately within the two groups. For the northwest (NW) group the analysis was run for 5, 10, 15 and 20 km distance classes (maximum distance between individuals was 60km). The south east (SE) group was analysed with 5, 10 and 15 km distance classes (maximum distance between individuals was 27km).

Dispersal among major groups in the Herbert River region

Three models of migration were compared using the program MIGRATE-N v3.6.4 (Beerli, 2009; Beerli and Palczewski, 2010) to assess which model of gene-flow possessed the highest likelihood, given the data. The dataset was split according to the two groups identified by STRUCTURE. One model described two-way dispersal (or gene-flow) between the groups and the remaining models allowed for dispersal from one group to the other (i.e., one-way) only. The first 10000 steps were discarded as burin-in, then 25 million steps were visited using parallel runs of 10 replicates. These conditions resulted in 50000 samples, recorded every 50 steps. To improve searching and also to calculate marginal likelihoods for model comparison, a heating scheme was applied using four chains (temperatures: 1.0, 1.5, 3.0 and 1000000). Start conditions involved a randomly generated genealogy and parameters estimated from an F_{ST} calculation.

Relatedness analysis

A relatedness co-efficient (R) was calculated between each pair of individuals using the GenAlEx v6.4 package. Pairs with R = 1.0 shared the same genotype (i.e., all alleles were identical across all loci) and were considered to be extremely highly related, while pairs with R values approaching -1.0 were considered to be unrelated. The relationship between R and geographical distance among highly related pairs (R > 0.75) was explored by correlation and the number of such pairs from the same population and those from different populations were recorded.

Proportion of inferred ancestry or group membership (from initial STRUCTURE analysis) was explored to identify individuals who possessed >75% ancestry from the group outside of that which they were sampled. Such individuals may represent potential migrants or recent translocations. The number of potential migrants was recorded, along with their location, sex and weight, and the location, sex and weight of their closest relative in the dataset, as determined by the pairwise R values.

Results & Discussion

Population structure across the region

The STRUCTURE analysis clearly indicated the presence of population structure, with two groups inferred, as represented by the two colours in Figure 1. These two groups were not associated with habitat type (forest, edge or non-forest crop or pasture). The majority of individuals in each group exhibited pure ancestry (>80% ancestry to one colour), representative of the group from which they were sampled, however, some individuals clearly exhibited ancestry from the group outside from which they were sampled potentially indicating recent dispersal or translocation.

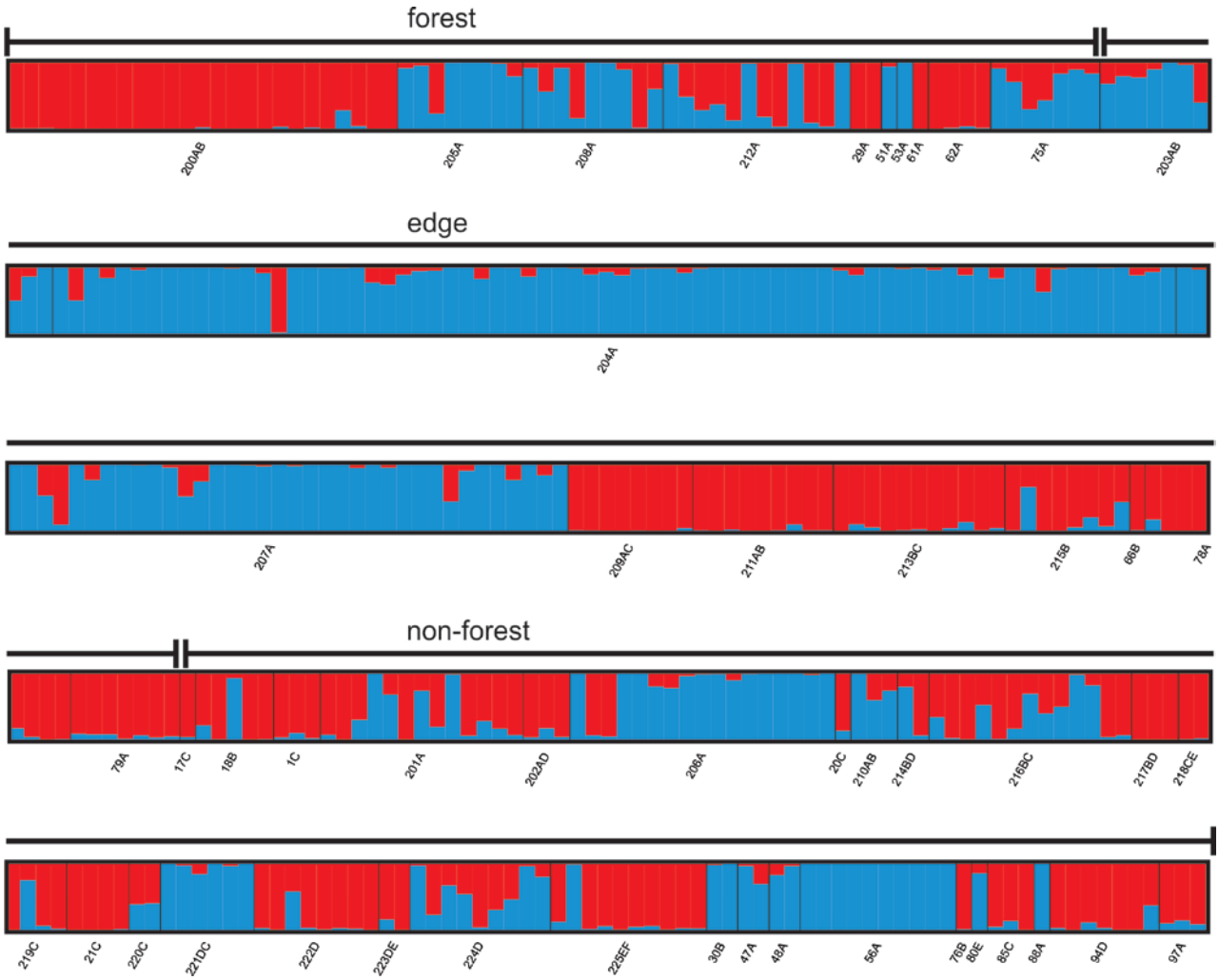


Figure 1: Structure bar plot with each bar showing the inferred ancestry of every sampled individual to the two inferred groups (red, blue) identified in the structure analysis.

Broadly, the two groups were aligned with geographic location (Figure 2); sites close to highland forest in the northwest constitute one group (predominantly red) and sites in the southeast region constitute a second group (predominantly blue). These two groups are henceforth referred to as the northwest (NW) and southeast (SE) groups, respectively. A significant negative relationship was identified between an individual's proportion of 'red' group membership and geographical distance from the highlands (Pearson's R^2 = -0.5653, p = 0.000023). Hence, *S. scrofa* from locations close to highland areas are more likely to have 'red' ancestry, which is characteristic of the NW group. In contrast, there was no evidence of a correlation between an individual's proportion of 'red' (NW) group membership and highland area (p > 0.05).

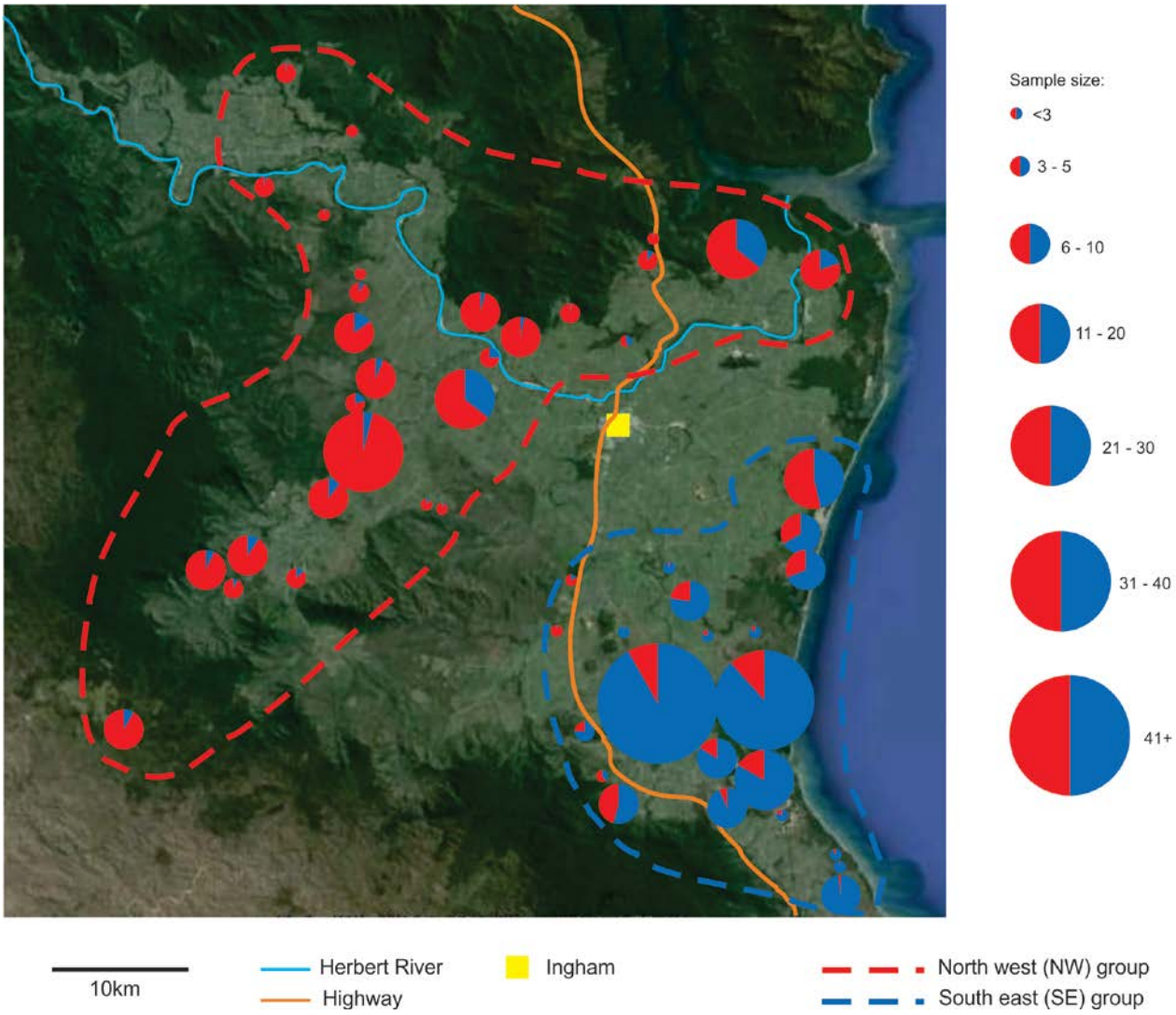


Figure 2: Proportion of individuals at each site with ancestry to each of the two inferred groups (red and blue) based on Bayesian structure analysis and positioned on a map of the study area. See Appendix 2 for a list by site of the proportion of ancestry to each of the two inferred groups.

No distinct natural (eg. Herbert River) or artificial (highway) boundaries appear to influence the population structure evident in Figure 2. A potential explanation for the difference between the highland and the coastal lowland pigs could be that the lowland pigs represent a separate introduction or release.

No evidence of sex biased population structure was identified for *S. scrofa* in the Herbert River region. STRUCTURE analysis for both sexes produced plots which were very similar to each other (results not presented) and to that of the initial STRUCTURE analysis of the entire dataset. Likewise, no difference was evident in the distribution of assignment indices between sexes in the Herbert River region.

Spatial autocorrelation analysis suggested that the extent of detectable positive spatial genetic structure for *S. scrofa* in the Herbert River region was between 26 and 34km (Table 1), but a plateau in distance was not reached (Appendix 3) indicating that the scale may in fact be larger than the sample area.

Table 1: Spatial Autocorrelation Results

Distance Class	Intercept
5km	26.29km
10km	28.33km
15km	31.65km
20km	33.83km

Assessing movement (gene flow) among sites

F_{st} estimates provided an indication of the amount of genetic difference among populations. F_{st} can range between 0 (no difference=high gene flow) and 1 (very different=no gene flow). Pairwise site comparisons of F_{st} estimates for the 24 sites compared ranged from -0.01 to 0.36 (Appendix 4). Over 94% of the pairwise F_{ST} estimates between the NW and SE groups were significant, while within the NW and SE groups there were over 80% and 76% of estimates were significant after appropriate Bonferroni correction. In total, these results reveal that most sites were significantly different from each other, suggesting low gene flow. AMOVA results supported this result, demonstrating that the majority of variation in the dataset occurred within sites (85.76% of

variation; F_{CT} = 0.0481), followed by among sites within the groups (9.43% of variation; F_{SC} = 0.0991) and among the NW and SE groups (4.81% of variation; F_{ST} = 0.14242). These results are consistent with a pattern of high variability within sites, and higher differentiation between the two groups (NW and SE) than among sites within a group.

A test of isolation by distance showed a significant correlation between log-transformed geographical distance and genetic distance (pairwise F_{ST}; Pearson's R^2 = 0.2156, p <0.0001). This suggests that genetic differentiation increases among *S. scrofa* as the geographical distance between individuals increases.

Population structure within identified groups

The STRUCTURE analysis suggested that the NW group contained three genetic units (K=3, 3 colours - green, pink, orange in Figure 3). Although most populations were highly admixed, slight geographical differentiation was evident that may be related to the Herbert River (less green was evident north of the river). A test of isolation by distance revealed a pattern of significant genetic distance with increased geographical distance (NW R^2 = 0.1795, p = 0.01).

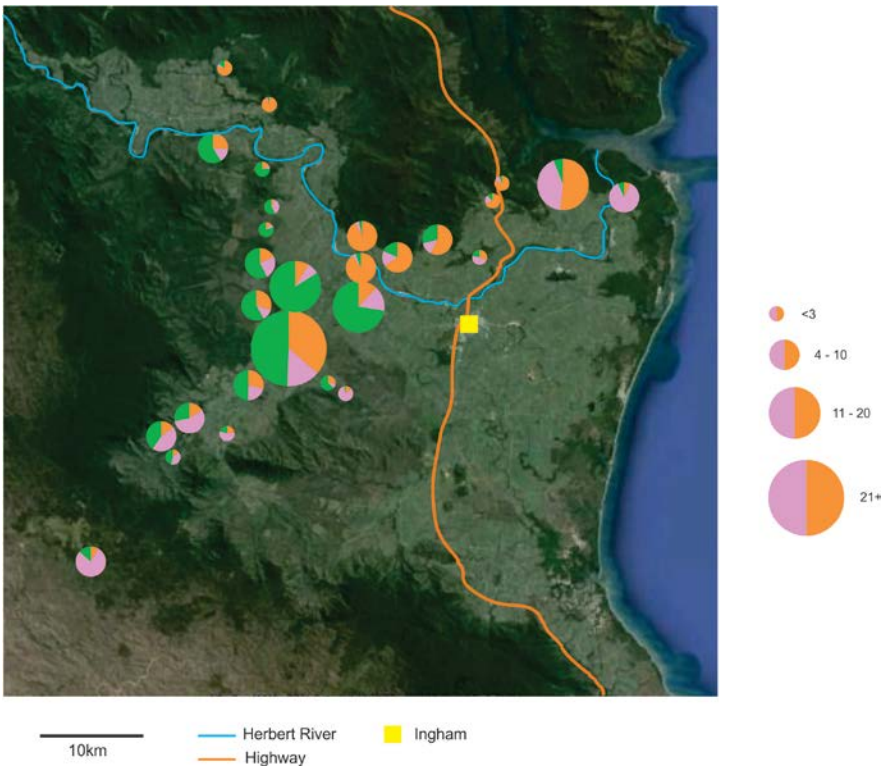


Figure 3: Proportion of individuals at each site in the NW group with ancestry to each of the three inferred groups (green, pink, orange) based on Bayesian structure analysis.

The SE group resolved into two genetic units but exhibited little structure that could be related to specific geographical features (K=2, 2 colours – pink, orange in Figure 4). Tests of isolation by distance revealed that the SE group exhibited a significant relationship between genetic distance and increasing geographical distance (SE R^2 = 0.2492, p = 0.007).

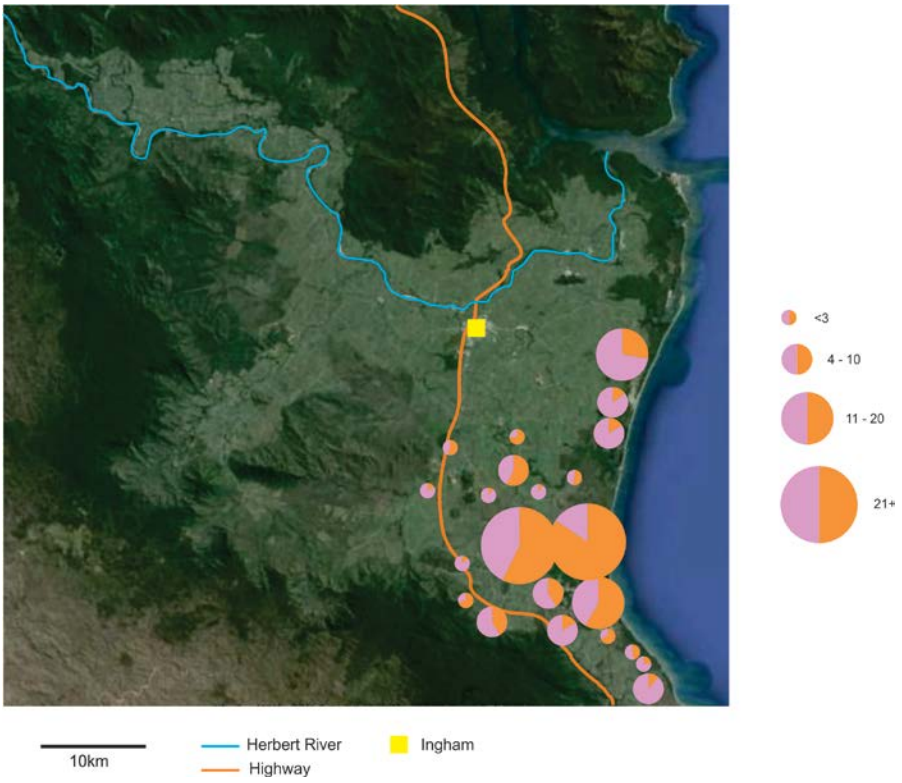


Figure 4: Proportion of individuals at each site in the SE group with ancestry to each of the two inferred groups (pink, orange) based on Bayesian structure analysis.

Spatial autocorrelation analysis suggested that the extent of detectable positive genetic structure was approximately 20 – 25km for the relatively more widespread NW group and 10 – 20 km for the SE group (Table 2).

Table 2: Spatial Autocorrelation Results – NW and SE groups

Distance Class	NW group Intercept	SE group Intercept
5km	21.7km	9.5km
10km	22.9km	17.4km
15km	24.1km	20.7km

Dispersal among major groups in the Herbert River region

Of the three models of gene-flow or dispersal compared by MIGRATE-N analyses, the model with the highest support was one which described directional gene-flow from populations in the SE to those in the NW of the Herbert River region (raw thermodynamic score = -70429.69, bezier approximation score = -12002.89). Both the model of two-way gene-flow between the groups and the model that described dispersal from the NW to the SE received less support (two-way model: raw thermodynamic score = -71941.13, bezier approximation score = -12235.63; NW to SE model: raw thermodynamic score = -70557.73, bezier approximation score = -12021.56).

Relatedness analysis

Values of relatedness (R) ranged from -0.809 – 1.0 for *S. scrofa* across the Herbert River region (mean R = 0.002). There were 231 highly related individual pairs (R>0.75) out of 73920 comparisons. Only 33.77% of highly related pairs were from the same sampled population, with the average geographical distance between highly related pairs estimated at 11.86km.

There were 20 *S. scrofa* identified as potential migrants or recent translocations (Appendix 5). These pigs possessed >75% ancestry from the group outside of that which they were sampled. No pattern influenced by sex, age or group membership was evident among the highly related pairs. For example, 11 of the possible migrants possessed ancestry characteristic of the SE group (blue) but were sampled from populations located in the NW, while the remaining nine possessed ancestry typical of the NW group (red) but were sampled from SE locations. Only three potential migrants had their identified closest relative in their own population, four were within 5km, six could be found within 5 – 15km and seven closest relatives were further than 15km away. Therefore most of the putative migrants were the result of pigs moving a distance of greater than 5km from the population from which they were sampled. These pigs were not from any particular demographic group (eg. all adult boars), but rather were a mix of males and females of different sizes (ie. ages).

There were two ‘pigs of note’ from site 204A who were identified as the closest relative to more than one pig from another site (Table 3).

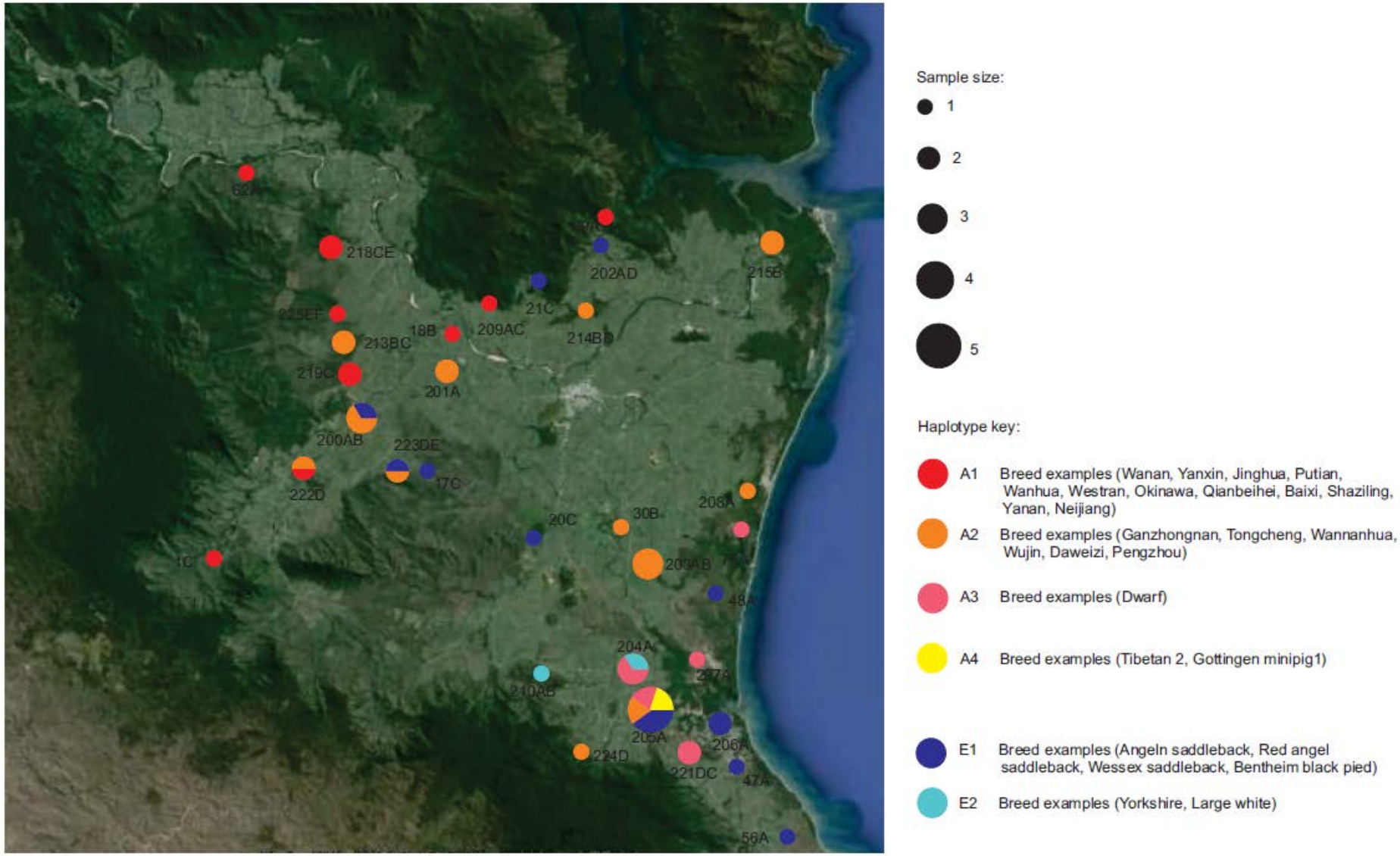
Table 3: Potential migrants

Pig	Closest relative
pig 194 (male, 10-40kg, site 204A)	pig 423 (R = 0.881; male, 10-40kg, site 201A) pig 18 (R = 0.881; female, 60-80kg, site 205A) pig 229 (R = 0.881; female, 10-40kg, site 207A) pig 460 (R = 0.881; female, 10-40kg, site 212A)
pig 263 (female, 5-10kg, site 204A)	pig 118 (R = 1; male, 10-40kg, site 18B) pig 461(R = 0.689; male, 10-40kg, site 212A) pig 15 (R = 1; female, 10-40kg, site 214BD)

Analysis of pig breed

Analysis of mtDNA to determine pig breed revealed a geographic pattern associated with pig breed (Figure 5); Asian A1 breeds (colour coded red) were only found in the NW region, while Asian A3 (pink) and A4 (yellow) and European E2 (light blue) breeds were only found in the SE region. Asian A2 (orange) and European E1 (dark blue) found throughout study area.

Figure 5. Pie chart showing mitochondrial DNA haplotype frequencies at each sample site and colour coded according to pig breed.



Conclusions & Recommendations:

The overall objective of this study was to examine the population genetic structure of feral pigs in the Herbert River district of north Queensland. Our results indicate that the study area constitutes two demographically independent management units; one comprising sites located in the north-west (NW) of the study region and associated with the highlands, and the second located in the south-east (SE) lowland area of the study. These two groups were not associated with habitat type (forest, edge or non-forest crop or pasture) and therefore, pig populations were not confined to a particular habitat such as cane, but were mixed across the landscape.

Our results indicate that within each MU, feral pigs are moving over distances up to between 10 and 25km within one to two generations. This result is consistent with studies undertaken in Western Australia that described feral pig management units at a scale of 25km and limited migration between populations (Hampton et al. 2004). Our results also support the findings of traditional radio-tracking studies that have found feral pigs to be relatively sedentary in tropical habitats and have defined home ranges (Caley 1997; Mitchell et al. 2009), a result that is consistent with the theory that an animal's home range size will be small in resource abundant habitats. Mitchell et al. (2009) reported that feral pigs in far north Queensland have an average home range size of 8 km^2 and move an average distance of 1 km. In the current study, the NW population was larger in scale relative to the SE population and it is likely that the NW population is in fact larger than the study area.

No distinct natural (eg. Herbert River) or artificial (highway) boundaries appear to influence the population structure evident in the study area. It is probable that the difference between the highland NW and the lowland SE pigs is due to the lowland pigs resulting from a separate introduction or release. The mtDNA analysis supports this hypothesis with Asian dwarf, Gottingen minipig and Tibetan breeds, together with European large white and Yorkshire breeds, only found in the SE region. Furthermore, the dispersal analysis provided highest support for a scenario of directional gene flow from populations in the SE to the NW of the Herbert River region (relative to a model of SE to NW direction or two-way migration). In total, these results suggest that pigs from the SE are gradually dispersing from the lowland area out and up towards the north and west.

The Fst pairwise site analysis indicated that most sites were genetically different from each other, indicating limited gene flow that was associated with geographic distance (ie. isolation by

distance). The population structure analysis showed that the majority of individuals in each management unit exhibited pure ancestry (>80% ancestry to one colour), representative of the management unit from which they were sampled, however, a limited number of individuals clearly exhibited ancestry from the management unit outside from which they were sampled indicating recent dispersal or translocation. These putative migrants were subjected to a relatedness analysis to determine the location of their closest relative. The results indicate that in most cases, the closest relative was located more than 5km away. Furthermore, the twenty putative migrants identified were not from any particular demographic group (eg. all adult boars), but rather were a mix of males and females of different sizes (ie. ages).

Management Implications

- The two management units identified in this study should be considered as operational units for feral pig control. However, it is likely that the NW population exists beyond the limits of the current study area and will be more difficult to control due to the terrain (proximity to highland areas) and the broad geographic extent of this management unit. The SE population represents a separate introduction of feral pigs into the Herbert River district in the lowlands area. Management of the SE population may be more achievable due to the ease of access in the lowland terrain and the relatively small extent of the management unit at the current time (compared with the NW). It is possible, given the different breeds found in the SE, that the potential source (pig farm?) may be easily identifiable and action taken to prevent further escapes.

- Feral pig populations in the Herbert River district are not constrained according to habitat type (forest, edge or non-forest crop or pasture), but rather are mixed across cane, pasture and forest areas. Localised control of feral pigs at the property level (eg. control of pigs in one cane farm, but not in surrounding banana crops or rainforest) is not likely to be effective in the long term because recolonisation of controlled areas will occur. Coordinated feral pig control of all properties within a management unit at the same time is required.

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Appendix 1: Samples, GPS coordinates, site code and locations

Forest = 1, Edge (<15m from forest edge) = 2, Non-forest (>15m from forest) = 3, N/A = information unavailable

Sample (pig) Number	Latitude	Longitude	Site Code	Forest (1), Edge (2) or Non-forest (3)
20	-18.6611	145.9929	200AB	1
161	-18.6611	145.9929	200AB	1
162	-18.6611	145.9929	200AB	1
163	-18.6611	145.9929	200AB	1
164	-18.6611	145.9929	200AB	1
165	-18.6611	145.9929	200AB	1
166	-18.6611	145.9929	200AB	1
167	-18.6611	145.9929	200AB	1
168	-18.6611	145.9929	200AB	1
169	-18.6611	145.9929	200AB	1
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307	-18.6611	145.9929	200AB	1
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106	-18.6611	145.9929	200AB	1
125	-18.6611	145.9929	200AB	1
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303	-18.6611	145.9929	200AB	1
19	-18.8152	146.2251	205A	1
185	-18.8152	146.2251	205A	1
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14	-18.8152	146.2251	205A	1
12	-18.8152	146.2251	205A	1
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462	-18.6949	146.2894	212A	1
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205	-18.5300	146.1870	29A	1
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127	-18.4655	145.9845	61A	1
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202	-18.8060	146.2148	204A	2
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212	-18.8060	146.2148	204A	2
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173	-18.6244	146.0011	213BC	2
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216	-18.5498	146.2994	215B	2
217	-18.5498	146.2994	215B	2
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219	-18.5498	146.2994	215B	2
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283	-18.5498	146.2994	215B	2
284	-18.5498	146.2994	215B	2
215	-18.5498	146.2994	215B	2

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358	-18.7335	145.9022	78A	2
356	-18.7335	145.9022	78A	2
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360	-18.7335	145.9022	78A	2
357	-18.7335	145.9022	78A	2
361	-18.7335	145.9022	78A	2
359	-18.7335	145.9022	78A	2
371	-18.8354	145.8243	79A	2
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369	-18.8354	145.8243	79A	2
366	-18.8354	145.8243	79A	2
373	-18.8354	145.8243	79A	2
372	-18.8354	145.8243	79A	2
9	-18.7013	146.0508	17C	3
113	-18.5981	146.0662	18B	3
121	-18.5981	146.0662	18B	3
188	-18.5981	146.0662	18B	3
189	-18.5981	146.0662	18B	3
190	-18.5981	146.0662	18B	3
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305	-18.6096	146.0500	201A	3
322	-18.6096	146.0500	201A	3
422	-18.6096	146.0500	201A	3
420	-18.6096	146.0500	201A	3
423	-18.6096	146.0500	201A	3
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418	-18.6096	146.0500	201A	3
419	-18.6096	146.0500	201A	3
174	-18.5446	146.1837	202AD	3
364	-18.5446	146.1837	202AD	3
365	-18.5446	146.1837	202AD	3
8	-18.8229	146.2406	206A	3
204	-18.8229	146.2406	206A	3
258	-18.8229	146.2406	206A	3
269	-18.8229	146.2406	206A	3
285	-18.8229	146.2406	206A	3

286	-18.8229	146.2406	206A	3
287	-18.8229	146.2406	206A	3
288	-18.8229	146.2406	206A	3
289	-18.8229	146.2406	206A	3
290	-18.8229	146.2406	206A	3
291	-18.8229	146.2406	206A	3
292	-18.8229	146.2406	206A	3
300	-18.8229	146.2406	206A	3
272	-18.8229	146.2406	206A	3
273	-18.8229	146.2406	206A	3
274	-18.8229	146.2406	206A	3
275	-18.8229	146.2406	206A	3
7	-18.7483	146.1252	20C	3
309	-18.8442	146.1714	210AB	3
310	-18.8442	146.1714	210AB	3
415	-18.8442	146.1714	210AB	3
15	-18.5986	146.1772	214BD	3
196	-18.5986	146.1772	214BD	3
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399	-18.5416	146.2319	216BC	3
429	-18.4361	145.9505	217BD	3
428	-18.4361	145.9505	217BD	3
110	-18.4361	145.9505	217BD	3
10	-18.5596	145.9948	218CE	3
199	-18.5596	145.9948	218CE	3
49	-18.6409	145.9956	219C	3
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304	-18.6409	145.9956	219C	3
200	-18.5761	146.1310	21C	3
203	-18.5761	146.1310	21C	3
998	-18.5761	146.1310	21C	3
999	-18.5761	146.1310	21C	3
23	-18.8677	146.1604	220C	3
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1095	-18.8545	146.2239	221DC	3
82	-18.8545	146.2239	221DC	3

83	-18.8545	146.2239	221DC	3
85	-18.8545	146.2239	221DC	3
90	-18.8545	146.2239	221DC	3
87	-18.8545	146.2239	221DC	3
68	-18.6859	145.9696	222D	3
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117	-18.6859	145.9696	222D	3
62	-18.6859	145.9696	222D	3
66	-18.6859	145.9696	222D	3
69	-18.6859	145.9696	222D	3
77	-18.6859	145.9696	222D	3
108	-18.6859	145.9696	222D	3
184	-18.6933	146.0384	223DE	3
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22	-18.8878	146.1685	224D	3
24	-18.8878	146.1685	224D	3
25	-18.8878	146.1685	224D	3
26	-18.8878	146.1685	224D	3
27	-18.8878	146.1685	224D	3
28	-18.8878	146.1685	224D	3
29	-18.8878	146.1685	224D	3
30	-18.8878	146.1685	224D	3
31	-18.8878	146.1685	224D	3
124	-18.5941	146.0065	225EF	3
156	-18.5941	146.0065	225EF	3
112	-18.5941	146.0065	225EF	3
101	-18.5941	146.0065	225EF	3
123	-18.5941	146.0065	225EF	3
126	-18.5941	146.0065	225EF	3
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131	-18.5941	146.0065	225EF	3
427	-18.5941	146.0065	225EF	3
170	-18.7406	146.1899	30B	3
214	-18.7406	146.1899	30B	3
44	-18.8358	146.2456	47A	3
45	-18.8358	146.2456	47A	3
197	-18.7862	146.2469	48A	3
198	-18.7880	146.2473	48A	3
81	-18.9314	146.3075	56A	3
84	-18.9314	146.3075	56A	3
86	-18.9314	146.3075	56A	3
93	-18.9314	146.3075	56A	3
94	-18.9314	146.3075	56A	3
95	-18.9314	146.3075	56A	3
97	-18.9314	146.3075	56A	3
98	-18.9314	146.3075	56A	3
99	-18.9314	146.3075	56A	3

100	-18.9314	146.3075	56A	3
321	-18.7831	146.1073	76B	3
377	-18.7781	146.2125	80E	3
325	-18.5729	145.9942	85C	3
327	-18.5729	145.9942	85C	3
431	-18.5729	145.9942	85C	3
323	-18.5729	145.9942	85C	3
396	-18.7818	146.1655	88A	3
449	-18.7232	145.9128	94D	3
447	-18.7232	145.9128	94D	3
445	-18.7232	145.9128	94D	3
446	-18.7232	145.9128	94D	3
451	-18.7232	145.9128	94D	3
448	-18.7232	145.9128	94D	3
450	-18.7232	145.9128	94D	3
425	-18.7424	145.9494	97A	3
452	-18.7424	145.9494	97A	3
424	-18.7424	145.9494	97A	3
5	N/A	N/A	N/A	N/A
324	N/A	N/A	N/A	N/A
398	N/A	N/A	N/A	N/A
432	N/A	N/A	N/A	N/A
433	N/A	N/A	N/A	N/A
453	N/A	N/A	N/A	N/A

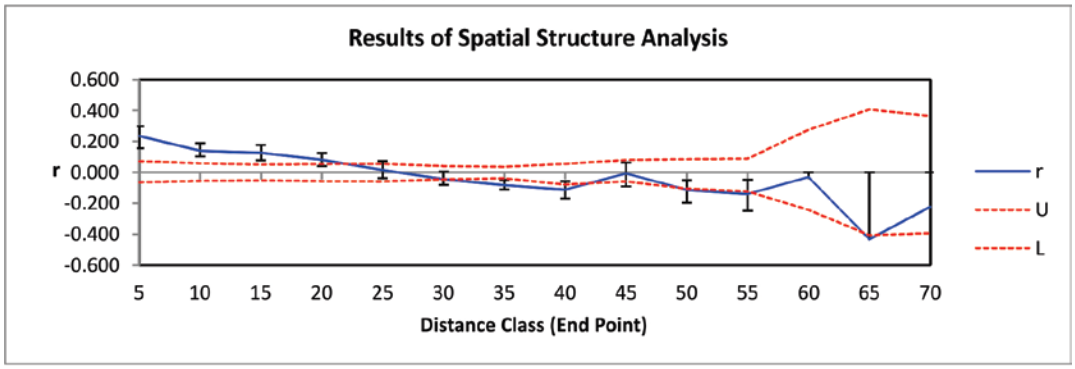
Appendix 2: Whole dataset proportion of ancestry to blue SE group and red NW group from Structure analysis. Bold values indicate dominant ancestry >0.75.

Site code	blue	red	Sample size
200AB	0.0371	**0.9629**	25
201A	0.3561	0.6439	13
202AD	0.102	**0.898**	3
29A	0.0223	**0.9777**	2
203AB	0.772	0.228	10
204A	**0.9177**	0.0823	72
205A	**0.839**	0.161	8
206A	**0.8337**	0.1663	17
47A	**0.8028**	0.1972	2
48A	**0.8704**	0.1296	2
207A	**0.884**	0.116	38
208A	0.6638	0.3362	9
51A	**0.9117**	0.0883	1
53A	**0.9804**	0.0196	1
56A	**0.9804**	0.0196	10
209AC	0.0315	**0.9685**	8
61A	0.0192	**0.9808**	1
62A	0.0404	**0.9596**	4
210AB	**0.7614**	0.2386	3
75A	0.6886	0.3114	7
79A	0.0818	**0.9182**	7
211AB	0.042	**0.958**	9
88A	**0.9843**	0.0157	1
212A	0.4633	0.5367	12

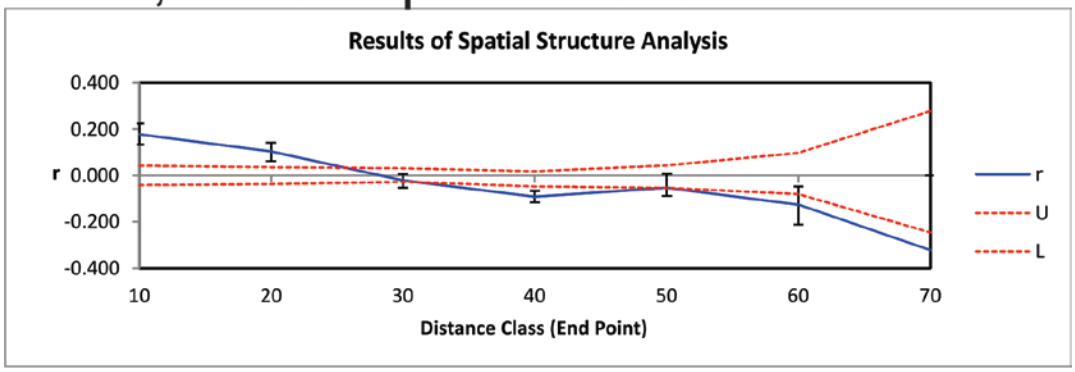
Site code	blue	red	Sample size
97A	0.1228	**0.8772**	3
213BC	0.0606	**0.9394**	11
18B	0.2469	**0.7531**	5
214BD	0.4288	0.5712	2
30B	**0.9535**	0.0465	2
215B	0.1948	**0.8052**	8
66B	0.0399	**0.9601**	1
76B	0.0334	**0.9666**	1
216BC	0.3577	0.6423	13
217BD	0.021	**0.979**	3
1C	0.0761	**0.9239**	3
78A	0.0691	**0.9309**	8
218CE	0.0392	**0.9608**	2
219C	0.2165	**0.7835**	4
17C	0.0597	**0.9403**	1
20C	0.1542	**0.8458**	1
21C	0.019	**0.981**	4
220C	0.3944	0.6056	2
85C	0.0785	**0.9215**	3
222D	0.1035	**0.8965**	8
223DE	0.094	**0.906**	2
224D	0.543	0.457	9
221DC	**0.9345**	0.0655	6
94D	0.0921	**0.9079**	7
225EF	0.1476	**0.8524**	10
80E	**0.8302**	0.1698	1

Appendix 3: Spatial Autocorrelation plots of r versus distance class.

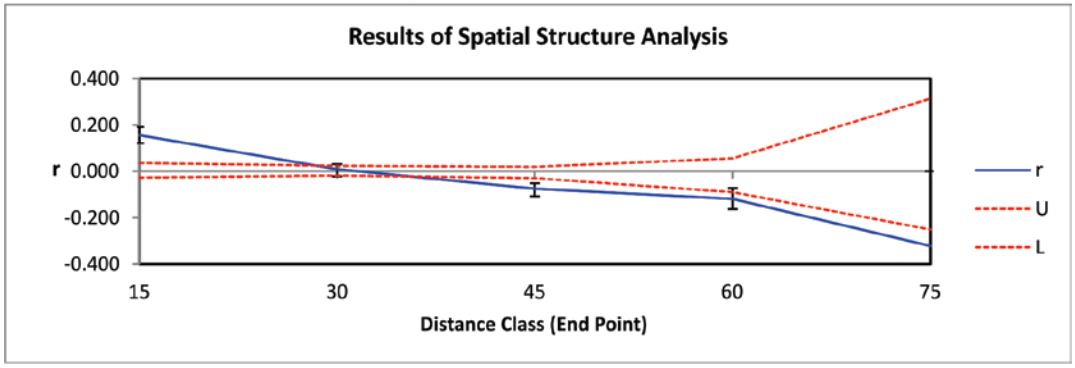
5km, r intercept = 26.286km



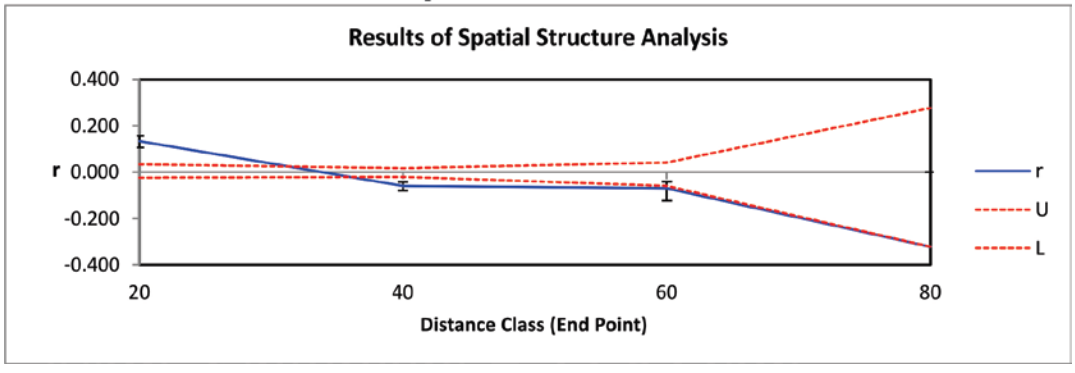
10km, r intercept = 28.329km



15km, r intercept = 31.648km



20km, r intercept = 33.833km



Appendix 4: Pairwise Fst estimates between sites

			NW													SE										
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
NW	200AB	1	0																							
	201A	2	0.102	0																						
	209AC	3	0.033	0.095	0																					
	79A	4	0.190	0.187	0.099	0																				
	211AB	5	0.161	0.222	0.069	0.226	0																			
	213BC	6	0.132	0.104	0.081	0.127	0.211	0																		
	18B	7	0.165	0.152	0.040	0.180	0.087	0.139	0																	
	215B	8	0.155	0.186	0.101	0.174	0.271	0.239	0.254	0																
	216BC	9	0.165	0.119	0.064	0.122	0.112	0.157	0.079	0.142	0															
	78A	10	0.093	0.133	0.065	0.124	0.205	0.149	0.213	0.174	0.144	0														
	222D	11	0.019	0.075	0.019	0.117	0.143	0.077	0.172	0.139	0.084	0.064	0													
	94D	12	0.098	0.156	0.050	0.099	0.200	0.137	0.167	0.158	0.145	0.010	0.071	0												
	225EF	13	0.047	0.050	0.045	0.138	0.152	0.082	0.113	0.132	0.092	0.088	-0.01	0.092	0											
SE	203AB	14	0.167	0.113	0.087	0.133	0.153	0.149	0.154	0.188	0.048	0.143	0.107	0.148	0.091	0										
	204A	15	0.151	0.094	0.117	0.149	0.198	0.152	0.159	0.159	0.074	0.167	0.104	0.180	0.075	0.045	0									
	205A	16	0.160	0.097	0.088	0.105	0.182	0.130	0.167	0.157	0.065	0.154	0.090	0.144	0.060	0.045	0.019	0								
	206A	17	0.135	0.091	0.103	0.137	0.166	0.129	0.140	0.202	0.089	0.147	0.084	0.158	0.069	0.059	0.015	0.005	0							
	207A	18	0.183	0.116	0.140	0.174	0.216	0.134	0.167	0.203	0.103	0.226	0.150	0.228	0.119	0.064	0.028	0.051	0.038	0						
	208A	19	0.175	0.123	0.100	0.153	0.176	0.140	0.082	0.189	0.058	0.160	0.106	0.159	0.080	0.050	0.066	0.077	0.085	0.111	0					
	56A	20	0.334	0.240	0.259	0.252	0.361	0.288	0.343	0.320	0.211	0.240	0.277	0.266	0.276	0.182	0.197	0.190	0.199	0.256	0.158	0				
	75A	21	0.194	0.140	0.101	0.149	0.247	0.162	0.166	0.173	0.132	0.183	0.136	0.114	0.099	0.113	0.118	0.089	0.139	0.160	0.085	0.279	0			
	212A	22	0.158	0.130	0.083	0.128	0.110	0.123	0.067	0.208	0.040	0.140	0.096	0.145	0.097	0.059	0.067	0.060	0.063	0.088	0.049	0.208	0.117	0		
	224D	23	0.127	0.085	0.055	0.126	0.154	0.110	0.175	0.158	0.052	0.118	0.080	0.123	0.058	0.038	0.027	-0.01	0.020	0.048	0.082	0.216	0.108	0.055	0	
	221DC	24	0.245	0.200	0.158	0.109	0.293	0.174	0.274	0.221	0.119	0.132	0.140	0.156	0.143	0.133	0.117	0.072	0.128	0.178	0.127	0.203	0.194	0.144	0.082	0

p<0.0001 Bonferroni corrected

Appendix 5: Relatedness analysis

possible migrant?	pop	pop dominant colour?	sex	weight	most closely related to?	from what pop?	sex	weight	first R	if same pop, next indiv out of pop?	next pop?	sex	weight	next R	1st or next pop dominant colour?	same colour?	distance between (km)?	potential dispersal distance (first)? (same=same, <5km=short, <15km=med, <35km=long)
201	201A	red	f	10-40kg	238	207A	f	10-40kg	1.000						**blue**	diff	30.8	Long
423	201A	red	m	10-40kg	194	204A	m	10-40kg	0.881						**blue**	diff	27.9	Long
233	204A	**blue**	m	40-60kg	228	207A	m	10-40kg	0.784						**blue**	same	3.8	Short
18	205A	**blue**	f	60-80kg	194	204A	m	10-40kg	0.881						**blue**	same	1.5	Short
204	206A	**blue**	m	80-100kg	167	200AB	f	0-5kg	0.568						**red**	diff	31.7	Long
258	206A	**blue**	m	60-80kg	351	200AB	f	0-5kg	0.748						**red**	diff	31.7	Long
229	207A	**blue**	f	10-40kg	194	204A	m	10-40kg	0.881						**blue**	same	3.8	Short
188	18B	**red**	m	10-40kg	263	204A	f	5-10kg	1.000						**blue**	diff	27.9	Long
444	208A	blue	f	10-40kg	17	203AB	m	10-40kg	0.534						**blue**	same	10.9	Med
152	208A	blue	m	10-40kg	443	208A	f	10-40kg	0.424	23	220C	m	80-100kg	0.370	red	diff	22.7	Same
426	212A	red	m	60-80kg	209	204A	f	10-40kg	0.580						**blue**	diff	14.6	Med
460	212A	red	f	10-40kg	194	204A	m	10-40kg	0.881						**blue**	diff	14.6	Med
461	212A	red	m	10-40kg	263	204A	f	5-10kg	0.689						**blue**	diff	14.6	Med
416	212A	red	m	60-80kg	202	204A	m	40-60kg	0.655						**blue**	diff	14.6	Med
15	214BD	red	f	10-40kg	263	204A	f	5-10kg	1.000						**blue**	diff	23.4	Long
458	216BC	red	f	0-5kg	457	216BC	f	0-5kg	0.821	86	56A	m	10-40kg	0.669	**blue**	diff	44.1	Same
459	216BC	red	f	0-5kg	455	216BC	m	0-5kg	0.734	263	204A	f	5-10kg	0.689	**blue**	diff	29.5	Same
27	224D	blue	f	40-60kg	23	220C	m	80-100kg	0.605						red	diff	2.4	Short
24	224D	blue	f	60-80kg	54	203AB	f	10-40kg	0.720						**blue**	same	14.7	Med
156	225EF	**red**	m	80-100kg	115	1C	m	40-60kg	0.577						**red**	same	19.8	Long