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Enhancing surveillance of the northern Australian feral pig population for African swine fever and other high impact pests and diseases.

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Enhancing surveillance of the northern Australian feral pig population for African swine fever and other high impact pests and diseases.

Final Report

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Executive Summary

With the ongoing global spread of African Swine Fever (ASF), particularly in the Asia-Pacific region, ASF has become an emerging biosecurity concern for northern Australia. The goal of this project was to take a science-based approach to feral pig surveillance in the context of enhancing preparedness for and early detection of ASF in Northern Australian landscapes. We specifically trialled the application of two modern techniques, genomic sequencing (Component 1) and aerial thermal imagery (Component 2) to assess their efficiency and effectiveness for enhancing feral pig surveillance.

Component 1

This research aimed to determine if it was possible to assess meta-population connectivity and landscape resistance of feral pigs across northern Australia. To achieve this, we obtained DNA from over 2,000 feral pig tissue samples collected by our network of partners from across northern Australia. A subset of 796 of these samples was genotyped using single nucleotide polymorphism (SNP) genetic markers from across the pig genome. Patterns of genetic diversity among individuals were mapped to illustrate feral pig population connectivity across northern Australia. We also applied landscape resistance modelling based on the genetic data to determine what geographic features may aid or hinder pig movement and thus gene flow. The results showed that feral pig population connectivity was far greater in north Queensland than in the Northern Territory, and connectivity was greater in the Northern Territory than in northern Western Australia. Genetic clustering analyses identified major geographic features contributing to feral pig population connectivity across the landscape. There were regional differences in the effects of environmental variation on feral pig population connectivity, as estimated from genetic data. Across northern Australia and in the Northern Territory, topographic roughness was the primary limitation on gene flow among feral pig populations, whereas watercourses and dry season habitat suitability were better predictors of feral pig population connectivity in north Queensland. The SNP genetic marker panel and analytical pipeline provide an efficient and powerful approach to extend the mapping of population connectivity of feral pigs nationwide.

The key outcomes from this research were:

- A blueprint upon how to create and sustain a network of partners who will freely provide feral pig tissue samples.
- A network of partner organisations across northern Australia from which feral pig tissue can be collected into the future.
- A large panel of informative SNP genetic markers mapped to the pig reference genome, customised for the Australian feral pig population based on samples from northern, southern, eastern and western Australia.
- An extensive feral pig genetic database for northern Australia, illustrating the spatial extent of feral pig meta-populations and locations of population discontinuities.
- Regional maps of modelled patterns of feral pig movement across the landscape, featuring predicted areas of high and low feral pig movement.

Component 2

This research aimed to assess the utility of remotely piloted aircraft fitted with a thermal camera to detect both alive and dead feral pigs in the northern Australian savanna landscape and measure their body temperature remotely. Previous studies have shown that aerial thermal photography can detect free-ranging feral pigs in southern landscapes. However, no trials have been undertaken in northern landscapes, and as far as we are aware the proportion of feral pigs within a population that may not be detected using aerial thermal survey has never been assessed.

Here, we selected a study area representative of a large proportion of northern Australian landscapes where feral pigs are found. Remotely piloted fixed wing aircraft were used as the survey platform. The Remotely Piloted Aircraft Systems (RPAS) surveys were conducted at the peak of the wet season (February) and surveyed a known number of feral pigs within a large, fenced paddock comprised of mixed woodland savanna. Each pig was fitted with a GPS-based tracking collar and body surface temperature monitor. Aerial thermal and optical images were collected every 4 hours over four days using a fixed-wing remotely piloted aircraft fitted with dual-rigged thermal (radiometric) and optical (red-green-blue) cameras. The study found that detection probabilities for feral pigs during daylight hours were less than 20% (i.e., only 1 in every 5 present feral pigs was detected due to obscuration by canopy cover). However, this rose to around 60% during surveys undertaken in the late afternoon (after 6 pm). Termite mounds and wallabies produced a heat signature very similar to that of a feral pig, and therefore we recommend that aerial thermal photography be accompanied by optical

imagery. Aerial surveys during darkness are not recommended because it was not possible to obtain simultaneous optical imagery. The next stage is to provide the imagery from this study to software developers who could build upon our recommendations for northern landscapes. We also found that aerial thermal photography may prove challenging for identifying feral pigs with fever-induced elevated body temperatures, but further investigation is required.

The key outcomes from this research were:

- Documented methodology for using remotely piloted aircraft and aerial thermal imagery to complement existing feral pig surveillance activities in northern Australia.
- Guidelines to support image capture with the highest probability of autonomous feral pig detection within northern Australia woodland savanna during the wet season
- Extensive collections of simultaneous optical and thermal imagery from a known number of feral pigs with accurate geographic location data for the training and ground-truthing of Artificial Intelligence (AI) and Deep Learning routines.

Component 1 - The meta-population connectivity and landscape resistance of feral pigs across northern Australia.

Background

At present, Australian preparedness and response plans for ASF assume that feral pigs are a single homogenous population, and that disease will spread through the feral pig population in a random-like manner. A 2019 pilot study undertaken by Charles Darwin University (CDU) using samples collected by Northern Australia Quarantine Strategy (NAQS) staff and other partners demonstrated that Northern Territory feral pigs exist in strongly differentiated populations with defined boundaries, and that the extent of dispersal (and thus, the potential for disease spread) varies greatly across the landscape. The aim of this Biosecurity Innovation project funded by the Department of Agriculture, Water and the Environment (DAWE) was to provide richer information on the population dynamics of feral pigs across northern Australia (from Broome to Cairns), as well as identify where a disease such as ASF is likely to spread rapidly and identify natural breaks in the landscape that could enhance containment. This kind of information could be used to model potential patterns of disease spread if a new disease such as ASF were to enter the feral pig population at any point in northern Australia.

The aim of this study was to:

1. Obtain DNA from feral pig tissue samples across northern Australia, using a network of partners.
2. Genotype these samples using single nucleotide polymorphism (SNP) genetic markers.
3. Map patterns of population connectivity among sub-populations of feral pigs.
4. Identification of landscape features that may be promoting or inhibiting population connectivity and potential disease spread through the feral pig population.

Methods

Tissue sample collection and processing

The project collected over 2,000 blood samples across northern Australia from a range of partners. A subset of 796 was successfully genotyped after subsampling one individual per group for even spatial coverage of populations, and removal of a small fraction of poor-quality samples (Figure 1). The samples were collected during NAQS routine operations and through partnerships between CDU, State/Territory Governments, regional land management

organisations. Key external sample providers other than NAQS included the Australian Wildlife Conservancy and the Queensland Department of Agriculture and Fisheries (QDAF), and the Western Australia Department of Primary Industries and Regional Development (DPIRD). In addition to partnering with major organisations, we provided a collection kit to over 100 partners, including recreational hunters, across northern Australia. This included standardised sample collection and storage instructions and equipment, together with paid postage to the laboratory at CDU.

While NAQS sampling has been focussed on coastal areas, reflecting NAQS biosecurity survey regions, the new partnerships enabled greater sampling coverage inland. However, future additions to the database in inland areas would further improve the landscape connectivity analyses. This is important for a better understanding of population connectivity across the broader landscape in northern Australia. In addition to the sampling across northern Australia, which was the specific focus of the project, we included samples from southern New South Wales and southern Western Australia in the database (Fig. 1). By including these samples together with the core set of samples from across the north, we are in the position to develop a powerful set of SNPs to study feral pig population connectivity across the continent.

Sample processing

The protocol for collecting tissue for DNA extraction involved sample collection from individual pigs in the field and storage of blood or tissue samples in sterile vacuum tubes (blood) or ethanol-filled Eppendorf tubes. Samples were then couriered to CDU. The study found out that the DNA in pig blood and ear tissue was relatively robust. Samples could be stored for long periods (months) without freezing as long as the tubes were refrigerated. At the CDU lab a 5 µl sample of blood or 5 µg sample of tissue was placed into a 96 well PCR plate with 200 µl of ethanol. The plates were then sent to the Diversity Arrays P/L commercial laboratory in Canberra. The samples were genotyped at Diversity Arrays P/L (Canberra) using the "DartSeq" genotyping by sequencing method (Kilian et al., 2012). In brief, the method subsets a fraction of the genome of each pig (through restriction enzyme digestion and PCR), conducts high-throughput sequencing (Illumina NovaSeq) of the pooled, individually barcoded samples and uses bioinformatics approaches to identify variable sites in the genome (single nucleotide polymorphisms, or SNPs) that are sequenced to sufficient quality in a high proportion of sampled individuals.

Importantly, this process must be done in the way we have approached this project, i.e., by first characterising genomic diversity within and among the feral pig population across the country before selecting the SNPs to be used for analysis. If we had done this based on

samples from a small number of locations or used a commercial SNP array for domestic pigs, any extension of the project to new areas would result in the core analyses required to estimate genomic diversity in feral pigs and map population connectivity suffering from a problem known as "ascertainment bias" that confounds spatial analysis of genomic data.

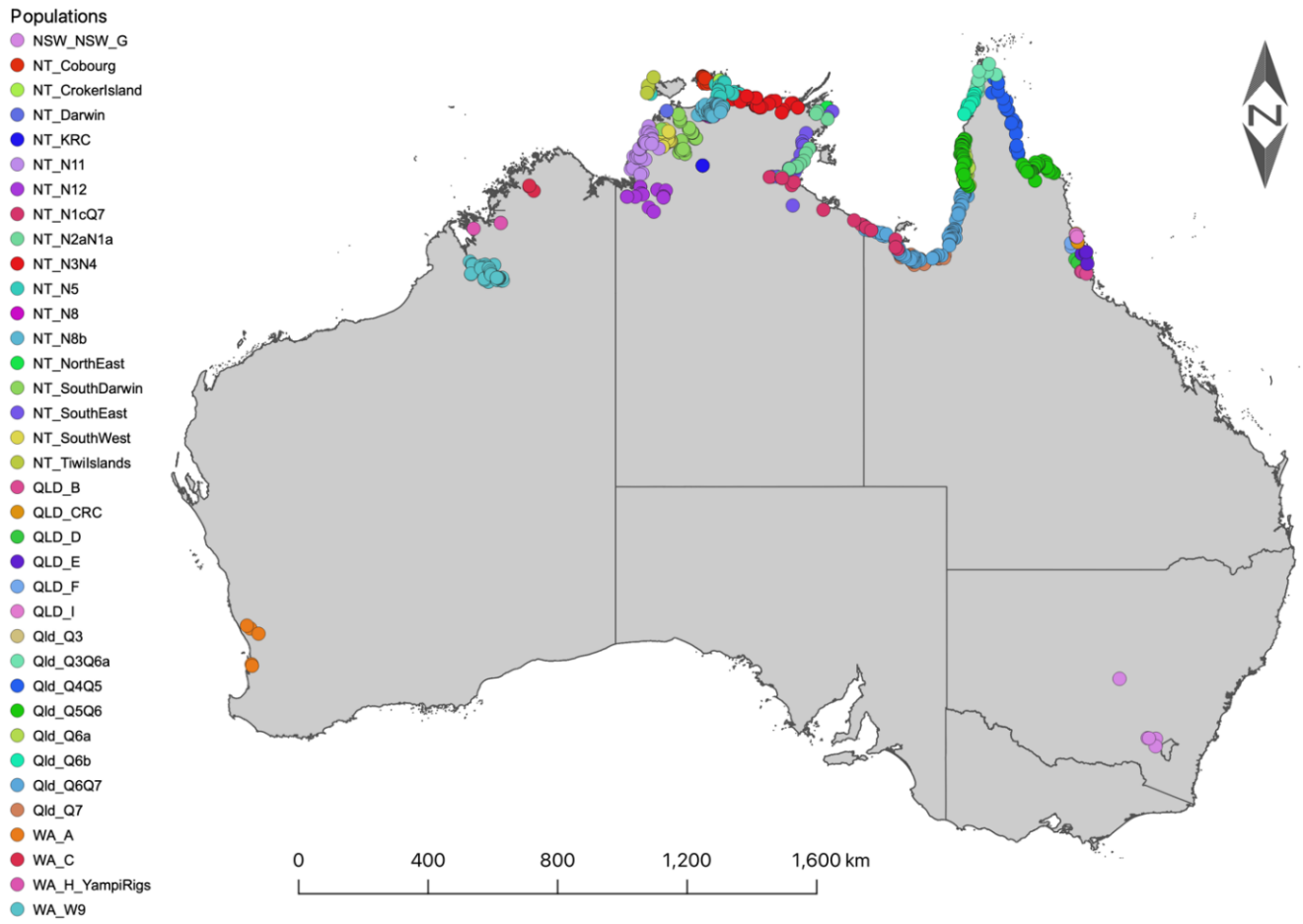

Figure 1: Distribution across Australia of feral pig genetic samples analysed for the project.

Results

Quality filtering of SNP data for statistical analysis

The 796 samples were genotyped at 48,830 single nucleotide polymorphism (SNP) genetic markers that were mapped to the most recent *Sus scrofa* reference genome assembly (Sscrofa10.2). The first component of the data analysis involves calculating basic SNP quality metrics to refine the set of markers used for each analysis according to specific criteria. The reduction in the number of SNPs and samples from before to after filtering is summarised in Figure 2.

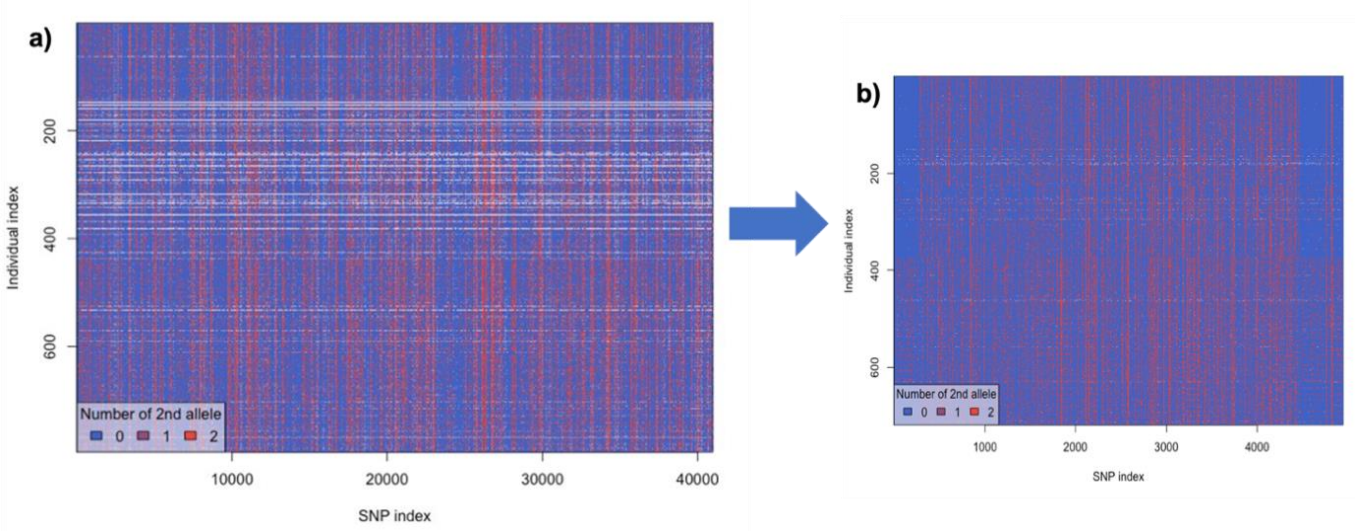

Figure 2: SNP rug plots showing the SNP x sample matrix before (a) and after (b) quality filtering, noting the reduction in the number of SNPs from 48,830 to 4,726 and the loss of a small number of samples.

The filtering steps involve dropping samples and SNPs with a high proportion of missing data, SNPs sequenced at low depth and other indicators of potential poor data quality (such as preferential detection of one of the SNP alleles over the other; Fig. 3).

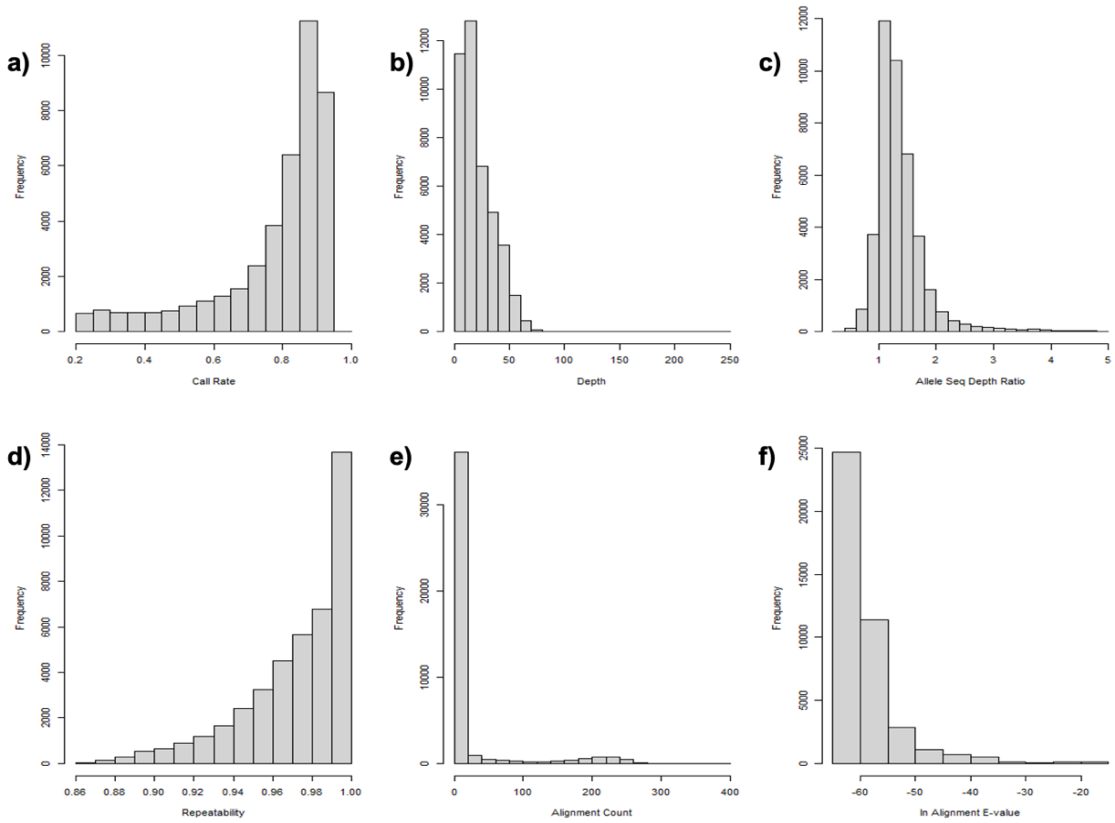

Figure 3. SNP quality metrics used for data filtering including call rate (proportion of samples called per SNP), depth (average number of sequence reads per SNP), depth ratio (the ratio of reads between alleles), repeatability of genotype calling based on replicated samples), alignment count and alignment e-value (representing quality of SNP mapping to the Sus scrofa reference genome).

A component of the data filtering involves assessing the mapping of SNPs to the *Sus scrofa* reference genome, and the initial large SNP panel was reduced to only SNPs mapped confidently to autosomes, excluding X and Y chromosome markers (Fig. 4).

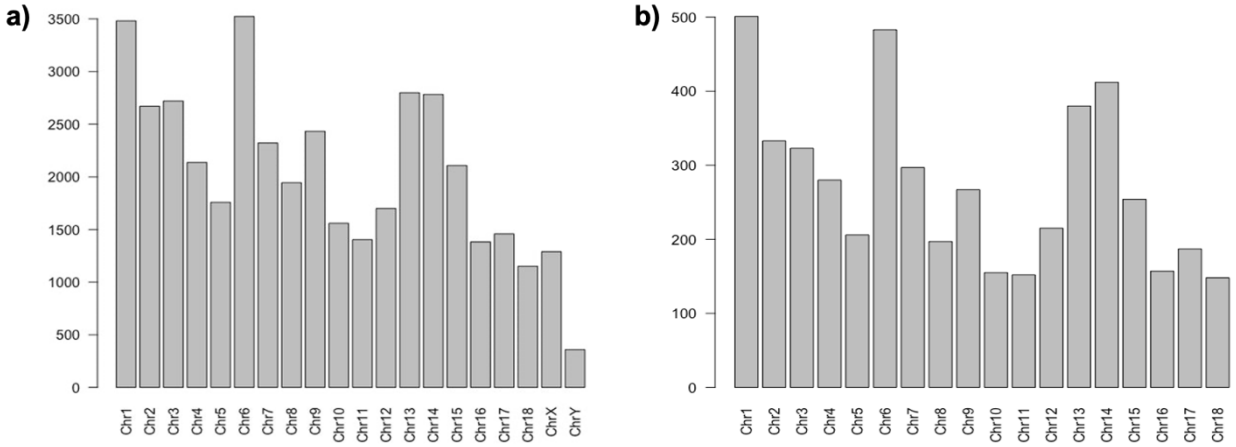

Figure 4: Histogram showing the number of SNPs mapped to the Sscrofa10.2 pig reference genome from before (a) to after (b) filtering, noting exclusion of sex chromosome (X, Y) markers.

Genetic diversity estimation across the samples analysed in the project

A critical first step of population genomics analysis is to quantify patterns of genetic diversity within and among populations. This provides important information that underpins the application of our genetic data to analyses for identifying population connectivity patterns. Overall genetic diversity levels at the SNP marker panel used were comparable to within-population genetic diversity in other studies, noting that such analyses filter datasets to a panel with sufficient diversity for statistical analyses (O'Leary et al. 2018), therefore, absolute values are not comparable between species. Of particular interest is that the inbreeding coefficient of 0.05 is low within 'populations' (defined by each collection of samples indicated in Fig. 1), and genetic differentiation between populations (F_{ST} = 0.3; Table 1) is relatively high, indicating strong power to identify patterns of genetic population structure and connectivity.

Table 1: Genetic diversity and SNP quality metrics from before to after filtering. Key genetic diversity metrics are average expected (HS) and observed (HO) heterozygosity within populations, average inbreeding coefficient (FIS) and average FST, a metric of genetic differentiation among populations that is affected by the degree of effective population connectivity (gene flow).

	n loci	**Call rate**	**Depth**	**Allele ratio**	**Repeat ability**	**H_S**	**H_O**	**H_T**	**F_{IS}**	**F_{ST}**
Post-filtering (mean)	4726	0.98	35.1	1.42	0.98	0.18	0.17	0.25	0.05	0.3
Post-filtering (SD)	4726	0.01	13.2	0.28	0.01	0.1	0.1	0.14	0.09	0.15
Pre-filtering (mean)	48830	0.79	21.29	1.37	0.97	0.16	0.11	0.25	0.32	0.3
Pre-filtering (SD)	48830	0.15	14.74	0.47	0.03	0.11	0.09	0.16	0.26	0.43

Levels of genetic diversity in the northern WA populations were substantially lower on average (expected heterozygosity within populations H_S = 0.073) than in the other jurisdictions (NT: 0.173, Qld: 0.192; NSW: 0.161), potentially reflecting smaller effective population size, greater isolation among local populations, or the founding of WA (Kimberley region) populations from a small initial number of individuals, leaving residual genetic effects of a population bottleneck. This pattern is reflected in further analyses of spatial variation in genetic diversity within populations. The sGD approach (Shirk and Cushman 2011) for estimating genetic variation across a landscape by calculating moving-window estimates of

genetic diversity (using a 100 km radius with a minimum of 10 samples used) revealed high genetic diversity by several metrics in Cape York feral pig populations, with lower values in the NT and lowest values in WA (Kimberley) populations (Fig. 5).

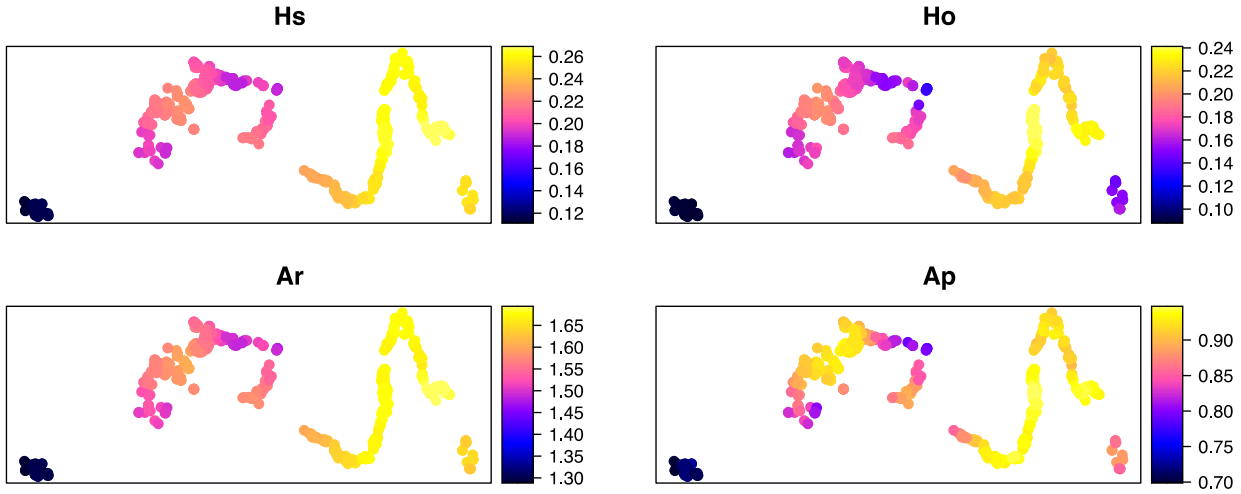

Figure 5: Interpolated spatial genetic diversity with a distance threshold of 100 km and minimum sample size of 10 individuals using the sGD method, with genetic diversity calculated as expected heterozygosity (HS), observed heterozygosity (HO), Allelic richness (Ar) and the proportion of total alleles in the sample represented in that 'window' (Ap).

Quantifying spatial genetic structure among individuals and populations

Analyses of genetic structure among individuals and populations are used to identify the spatial scale over which individual feral pigs are genetically similar due to recent shared ancestry. This scale is influenced by the distance moved by individuals from one generation to the next (dispersal distance) and may vary among regions due to different habitat types, feral pig population densities and other factors. Patterns of introduction/colonisation history and movement of pigs by humans can also influence these patterns.

The first set of analyses of genetic population structure partitioned the overall genetic differentiation metric (overall F_{ST} = 0.3) into F_{ST} values between the major regions, comprising NT, NSW, Qld, North WA and South WA. Pairwise F_{ST} (Table 2) between regions ranged from 0.14 (NT – NSW) to 0.39 (WA north to WA south). Figure 6 uses these pairwise F_{ST} values between sampling regions in a principal coordinates analysis to visualise patterns of genetic similarity among the sampling regions. Unusually, F_{ST} values among the major sampling regions were not clearly linked to geographic proximity, suggesting that continental-scale patterns of genetic differentiation reflect processes other than 'natural' pig movement (which is distance-limited) and most likely reflect the history of the introduction of the various stocks of pigs to different parts of the continent. Our analyses of finer-scale patterns of genetic

differentiation within northern Australia showed that genetic patterns were strongly linked to geographic distance, suggesting that individual movement shapes genetic patterns on sub-national scales.

Table 2: Genetic differentiation measured by FST between the sampling regions.

		F_{ST} between regions				
	F_{ST} within region	NT	Qld	WA_N	WA_S	NSW
NT	0.18		0.20	0.32	0.20	0.14
Qld	0.10	0.20		0.36	0.20	0.17
WA_N	0.25	0.32	0.36		0.39	0.28
WA_S		0.20	0.20	0.39		0.143
NSW		0.14	0.17	0.28	0.14	

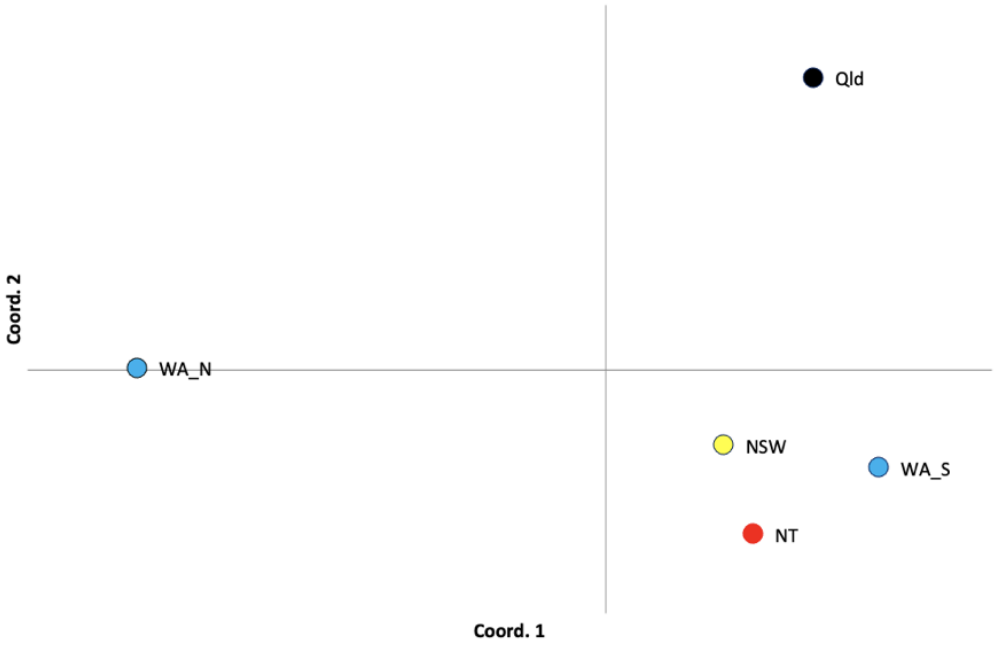

Figure 6: Principal coordinates plot showing clustering among broad sampling regions based on pairwise genetic differentiation (FST) among samples.

Repeating the principal coordinates analysis at the individual level (using pairwise genetic distances among individuals calculated from allele-sharing at each SNP), the sample cluster broadly into populations and sampling regions (Fig. 7). We used this result as a rationale to conduct our detailed analyses of landscape-scale population connectivity separately for QLD and NT samples, as these appear to fall into discrete genetic units, potentially due to independent population histories (separate introduction events).

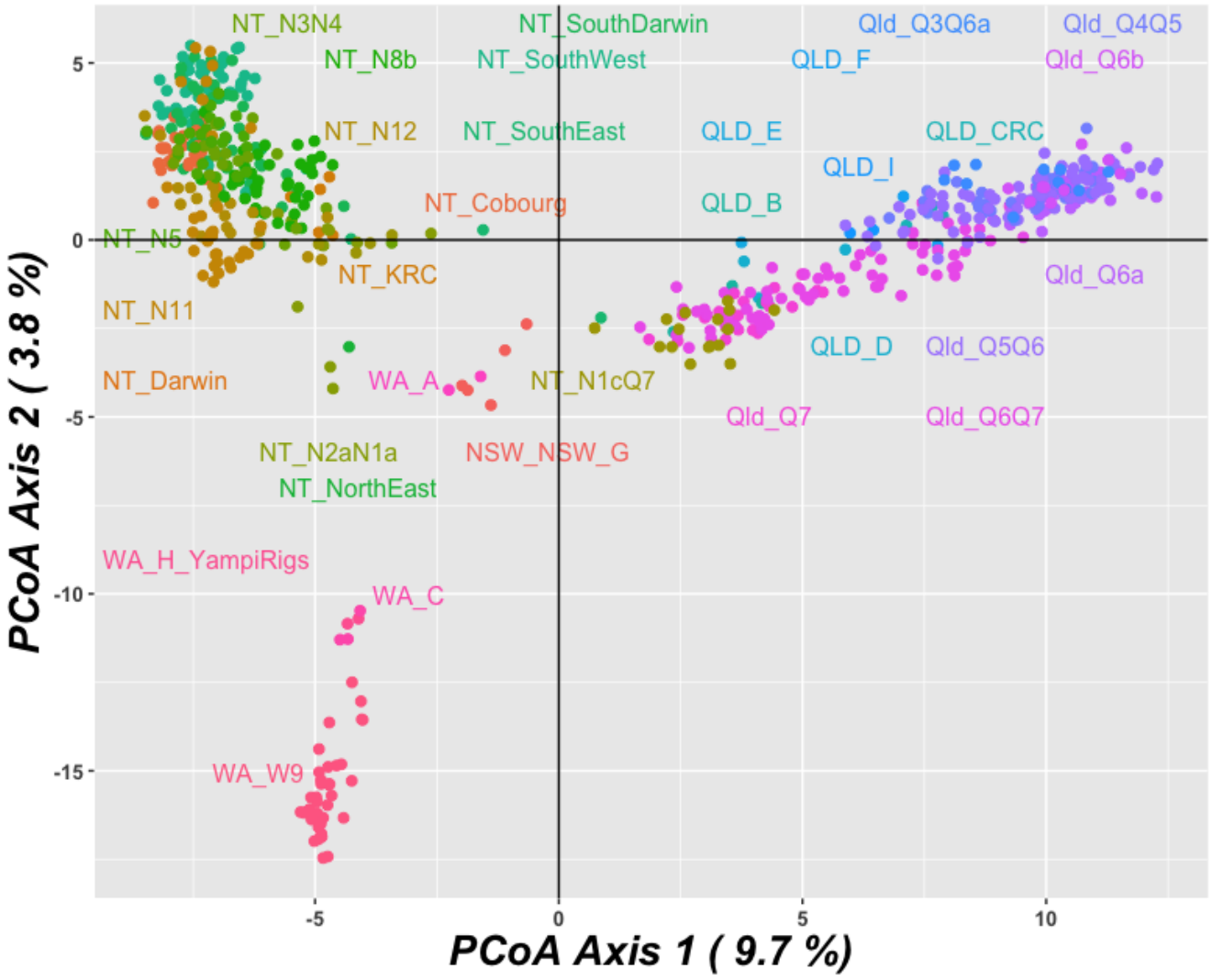

Figure 7: Principal coordinates analysis using genetic distances among individual pigs, colour-coded by "population".

We used a spatial autocorrelation analysis to identify the magnitude and scale of clustering of genetically similar individuals. The autocorrelation coefficient, r, represents the correlation between genotypes separated by incremental distance intervals. High *r* values at low distances with a rapid drop in *r* with increasing distance indicate low rates of dispersal across the landscape, leading to clustering of genetically related individuals. Higher levels of dispersal lead to weaker *r* values at low distances but the extension of patterns of genetic similarity (moderate *r* values) over greater distances (Banks and Peakall, 2012). Figure 8 shows these patterns by state/territory in northern Australia. The key result from this analysis is that the spatial autocorrelation signal is consistent with gene flow and population connectivity over a much larger scape in north Queensland relative to WA. The sample size from northern WA is not comparable to Qld and NT, but the analyses are consistent with the greater spatial genetic structure on fine scales (reduced dispersal) relative to Qld and NT.

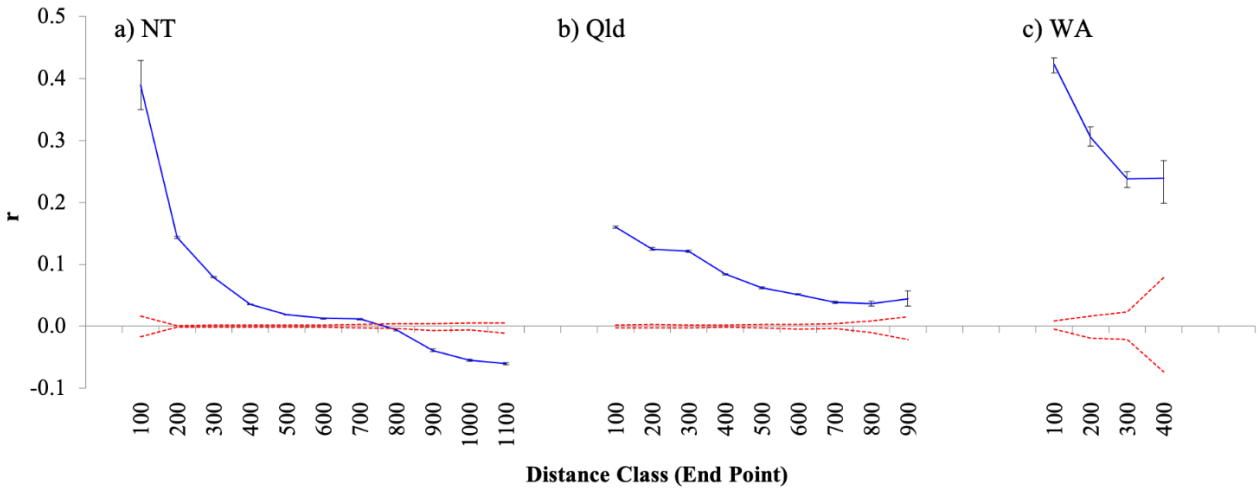

Figure 8: Spatial correlograms from genetic distances among individual pigs sampled in a) NT, b) Qld, and c) WA. Blue lines connect the autocorrelation coefficient (r), representing the degree to which individuals in a particular distance class are genetically similar (correlated) relative to the entire population. The red lines represent the bounds of a randomisation-based significance test. Values outside these bounds are significantly different to a random sample of individuals from the population.

Following the principal coordinates analyses, we used the *snmf* function of the LEA R package (Frichot and François, 2015) to conduct a spatial genetic clustering analysis to map patterns of shared ancestry among individuals across the landscape. This model takes a user-defined number of genetic groups and clusters individuals into those groups in a manner that matches theoretical expectations about population genetic diversity patterns. For the analysis, a model of between one and 14 genetic groups were used. This approach is somewhat artificial as it imposes a scenario of discrete genetic groups on a continuous population across the landscape, but the spatial patterns can be informative. Insights can be drawn from models with different numbers of genetic groups. Here, we present results from a model featuring 12 genetic populations, for which there was good support indicated by a low cross-entropy score (Fig. 9). This partitioned individuals predominantly into discrete groups (Fig. 9).

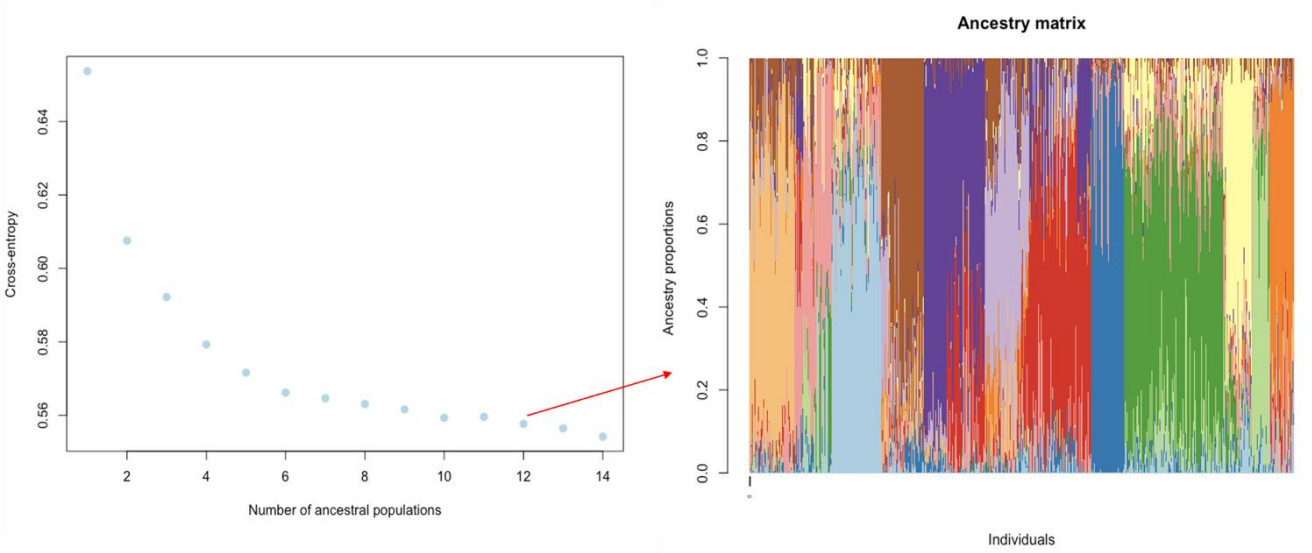

Figure 9: Evaluation of support for LEA *snmf* models of 1-14 ancestral genetic populations. The figure panel on the left shows the cross-entropy statistic, lower values indicating greater model support. The figure on the right shows individual genetic group membership for the 12-group mode. 'Assignment' of individuals to each population is indicated by colour coding on the bar plot.

Mapping of the inferred ancestry of each pig among the 12 groups provides insights into the types of geographic features that contribute to population structure. This can be seen in Figure 10, which reveals some of the major rivers (Mitchell, Roper, East Alligator) associated with different genetic groupings of individuals, as well as other patterns such as the known shared ancestry among Daly River and Bathurst Island feral pig populations, as well as gene flow from the first site of pig introduction to the NT (Cobourg Peninsula) across the northern Arnhem Land coast.

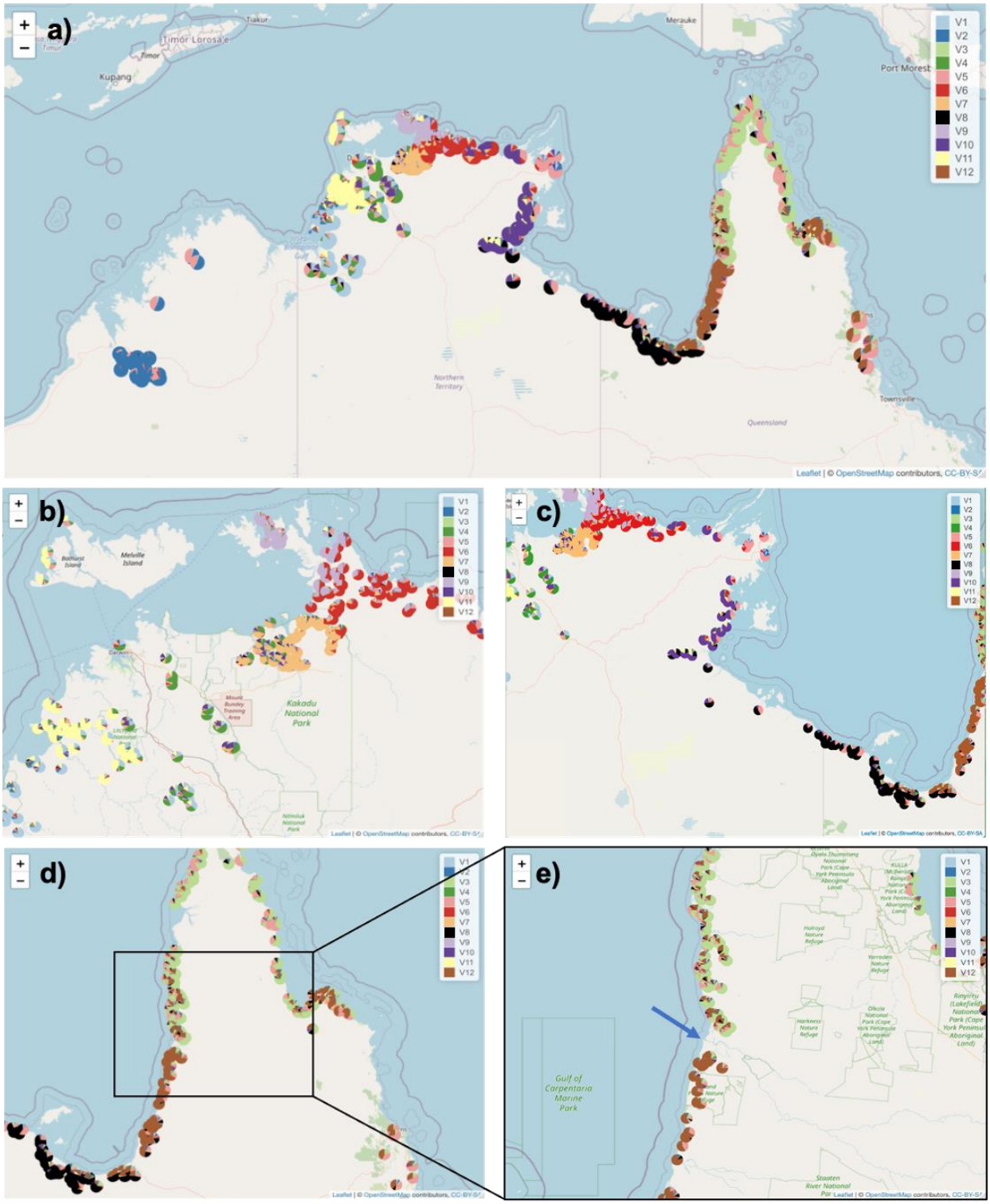

Figure 10: Pie charts represent estimated ancestry of individual pigs to the genetic groups in the 12-group snmf model for northern Australia (a). These can provide a qualitative assessment of the locations of major population boundaries. For instance, the Darwin region figure (b) reveals known recent ancestry of Bathurst Island pigs in the mainland Daly River region (group 11, coded yellow) and population boundaries near major rivers such as the East Alligator River (groups 6/7 boundary, coded red and tan) and c) Roper River (groups 8 and 10, coded black and purple). The Cobourg Peninsula (group 9, coloured mauve) was the first known introduction of pigs to the NT. The major geographic boundary between groups 3 and 12 on western Cape York is the Mitchell River (d and e, indicated with a blue arrow in e).

Modelling and mapping landscape effects on genetically estimated *population connectivity*

These analyses use pairwise genetic distances among locations or individuals to estimate patterns of gene flow, or effective population connectivity, across the landscape, associating these patterns with landscape features to enable predictions of connectivity patterns across the region. Through these analyses, we will identify how the landscape influences connectivity among feral pig populations across northern Australia. We can estimate these effects directly from our data in conjunction with a set of land cover layers predicted to influence feral pig movement. We can also evaluate support for previously developed feral pig population connectivity models based on habitat suitability.

The "response" variable used for these models is a pairwise matrix of genetic distances among individuals and populations. We used a Euclidean distance calculated from the first ten axes of a principal coordinates analysis of the genetic dataset, as simulation research has shown this distance metric to be more informative than others (Winiarski et al., 2020). The relationship between this distance metric and geographic distance among individuals is shown in Figure 11.

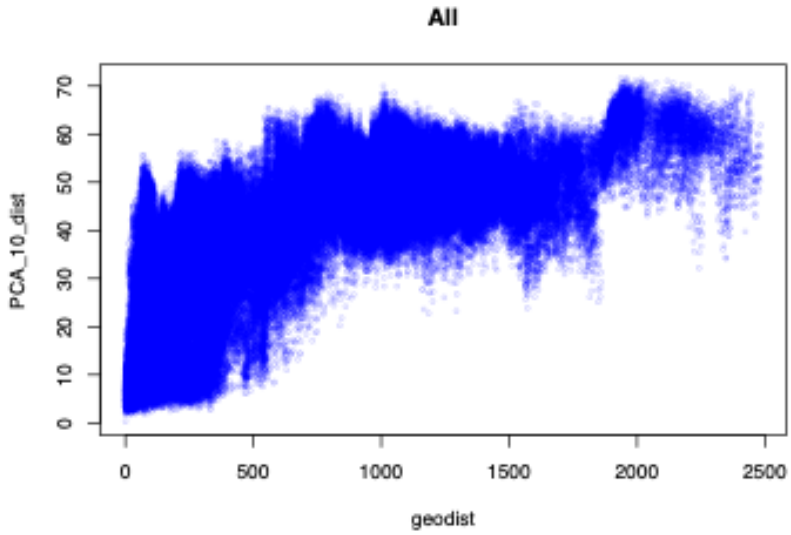

Figure 11: Genetic distance among individual feral pigs (10-axis PCA distance) plotted against geographic distance among samples.

Following exploratory analysis, we used pairwise distances among local populations rather than among individuals to remove some of the local 'noise' in genetic data at an individual level. To do this, we split the samples across northern Australia into 90 fine-scale groupings of at least five individuals and calculated the 10-axis PCA distance among those units.

We identified a set of landscape features as candidate resistance surfaces influencing feral pig population connectivity. These included a layer of topographic roughness (TRI) calculated from the Geoscience Australia 1 second SRTM Digital Elevation Model (Geoscience Australia, 2011), the ANUCLIM Annual Mean Rainfall raster layer (NSW Department of Planning, Industry and the Environment, 2020), national surface water hydrology lines spatial layer (Crossman & Li, 2015), the Geoscience Australia GEODATA COAST 100K 2004 (Geoscience Australia, 2004) layer representing a uniform landscape with sea boundaries, and dry season and wet season feral pig breeding habitat models (Froese et al., 2017). Feral pig habitat model data was accessed from: https://data.gov.au/data/dataset/5c49cb00-0b76-44e3-a211-7cc2b38a7c7b.

These layers were transformed where required to conform to resistance surface requirements. The uniform landscape (LandSea) layer comprised cell values of '1' for land and 'NA' for sea and was used as a mask layer for all other surfaces. The "RiverSea" surface classified dry land with a resistance value of 10, non-perennial rivers with a resistance of 5 and perennial rivers with a resistance of 50, based on preliminary analysis. The mean precipitation (AvPrec) and wet and dry-season predicted feral pig breeding habitat surfaces were inverse-transformed with an inverse monomolecular transformation in ResistanceGA (Peterman, 2018). The candidate surfaces are shown in Figure 12.

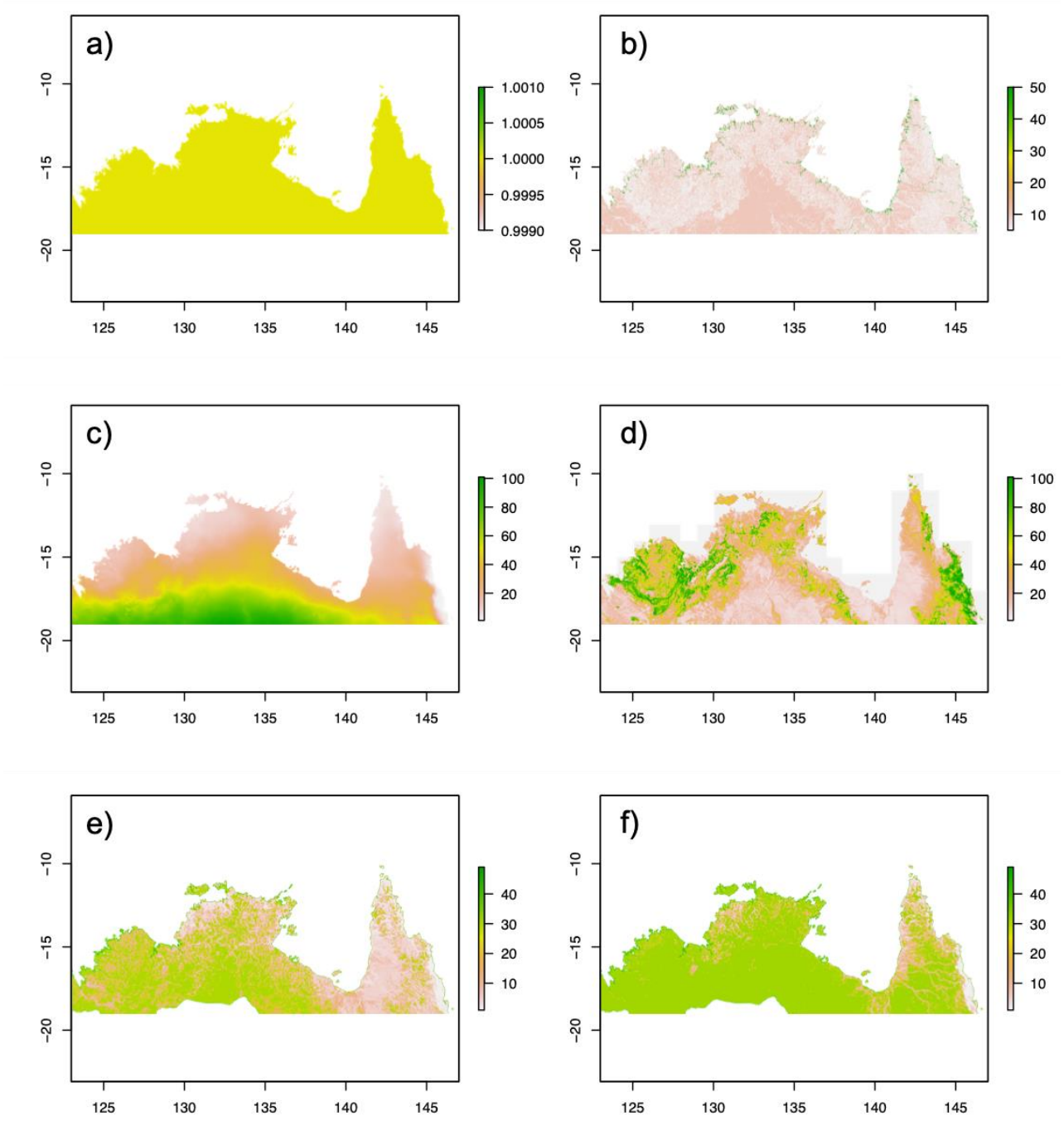

Figure 12: Candidate resistance layers including a) 'LandSea', b) 'RiverSea', c) 'AvPrec', d) 'TRI', e) 'Wet season feral pig habitat', and f) 'Dry season feral pig habitat', after transformation.

We used the program 'Circuitscape' (McRae, 2006) to estimate the resistance distance between all local populations for each resistance surface. This method uses electrical circuit theory to estimate the resistance to movement across the landscape, given a particular model of landscape resistance. We then used the 'ResistanceGA' R package to fit generalised least squares regression models of genetic distance using each of the six resistance datasets (one from each candidate resistance surface) with the maximum-likelihood population effects

correlation structure that accounts for pairwise data (Clark 2002). We fitted these models for the overall north Australian data and separately for NT and Qld datasets (there were not sufficient WA samples to do this at this stage).

For the overall data and the NT dataset, we found the strongest support (via Akaike's Information Criterion) for the topographic roughness model (TRI), whereby topographically complex areas act as barriers to feral pig movement (Table 3). The patterns in Queensland were somewhat different, with the best resistance model featuring the 'RiverSea' surface, with large rivers as barriers and small, non-perennial rivers as corridors. The second-best model was well-supported and featured the inverse of dry season breeding habitat quality as a resistance surface.

Table 3: Akaike's Information Criterion values (delta AIC) for regression models of genetic and resistance distance. Models with delta AIC = 0 (models with lowest AIC) are best supported, and we have also used models with delta AIC <2.

Resistance model	**All**	**NT**	**Qld**
LandSea (Null landscape)	284.79	2.088	7.788
AvPrec	788.76	14.476	26.255
Wet Season Habitat	470.27	491.895	26.63
Dry Season Habitat	258.62	142.69	1.507
RiverSea	339.29	188.994	**0**
TRI	**0**	**0**	210.86

Table 4: Coefficients in highest-ranked resistance models for feral pigs across northern Australia and in the NT and Queensland.

		Value	**Std. error**	**t-value**	**p-value**
All (best model)	(Intercept)	-2.488	1.017	-2.445	0.015
	TRI	0.287	0.003	97.035	0
NT (best model)	(Intercept)	-4.771	1.528	-3.123	0.002
	TRI	0.379	0.011	35.377	0
Qld (best model)	(Intercept)	-5.948	1.098	-5.419	0
	RiverSea	0.746	0.031	23.897	0
Qld (2nd best model)	(Intercept)	1.43	1.809	0.791	0.432
	Dry season habitat	0.174	0.044	3.981	0

Building on the resistance modelling, we used Omniscape (Landau et al., 2021) to map likely patterns of pig connectivity across the landscape. We used each of the well-supported models in Table 4 and, for each of these, generated two current maps, one corresponding to wet season habitat (with the wet season habitat layer as a current source strength surface) and one corresponding to dry season habitat (with the dry season habitat layer as a current source strength surface). Maps are shown for northern Australia (Fig. 13), the Top End of the Northern Territory (Fig. 14) and the Cape York and Gulf regions of Queensland (Fig. 15). These maps show predicted current flow (overall predicted volume of feral pig movement) but not directionality. Maps of gene flow directionality can be produced. However, they would be most informative if matched to specific scenarios, such as mapping sources of recolonisation following planned culling in a target area or most likely paths of host-mediated pathogen spread following a disease incursion in a particular locality.

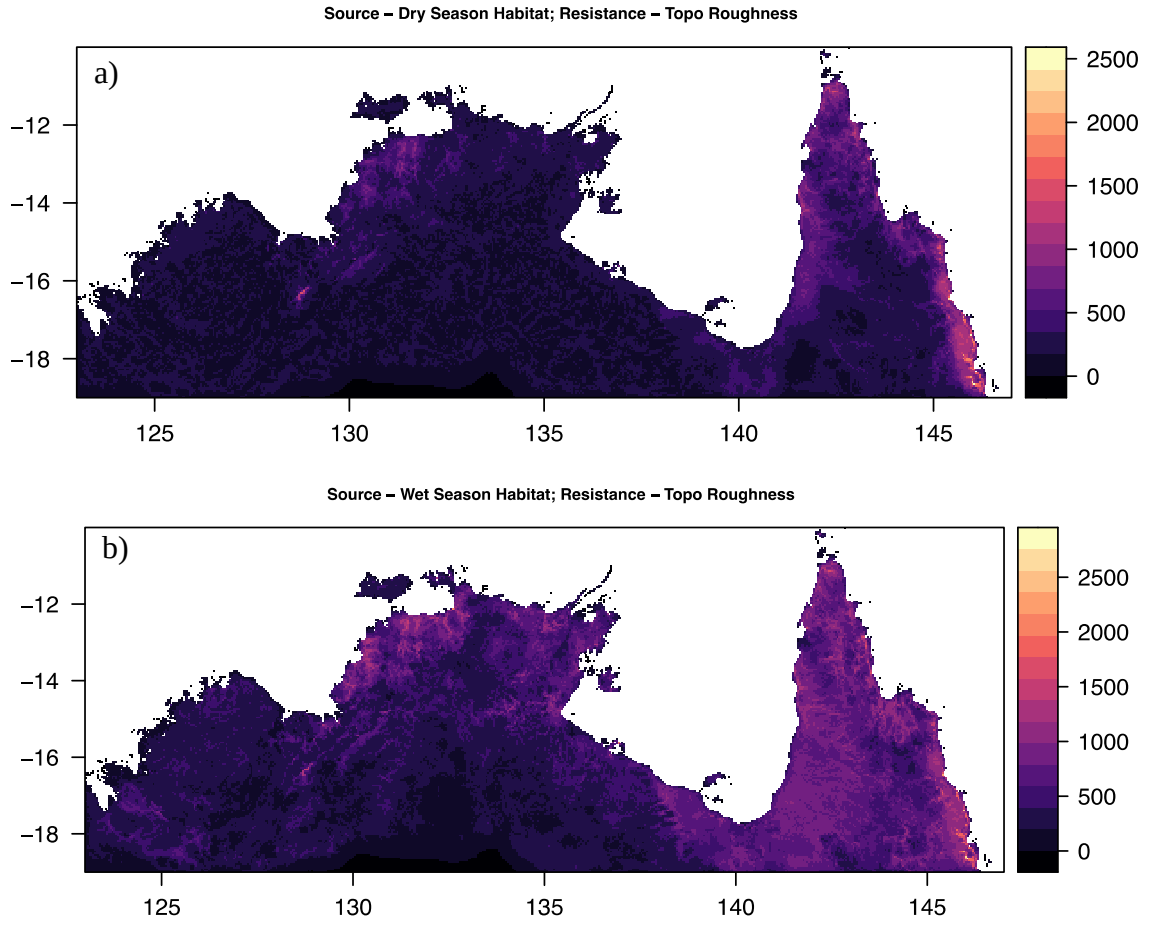

Figure 13: Current maps of potential feral pig gene flow across northern Australia from the best-supported landscape resistance model and current sources corresponding to dry (a) and wet (b) season feral pig breeding habitat quality. High current values show predicted 'flow' of feral pigs through each cell based on the best-supported resistance surface, from current sources (coded as dry and wet season breeding habitat quality) to any cell on the landscape (all coded with equal current 'sink' values).

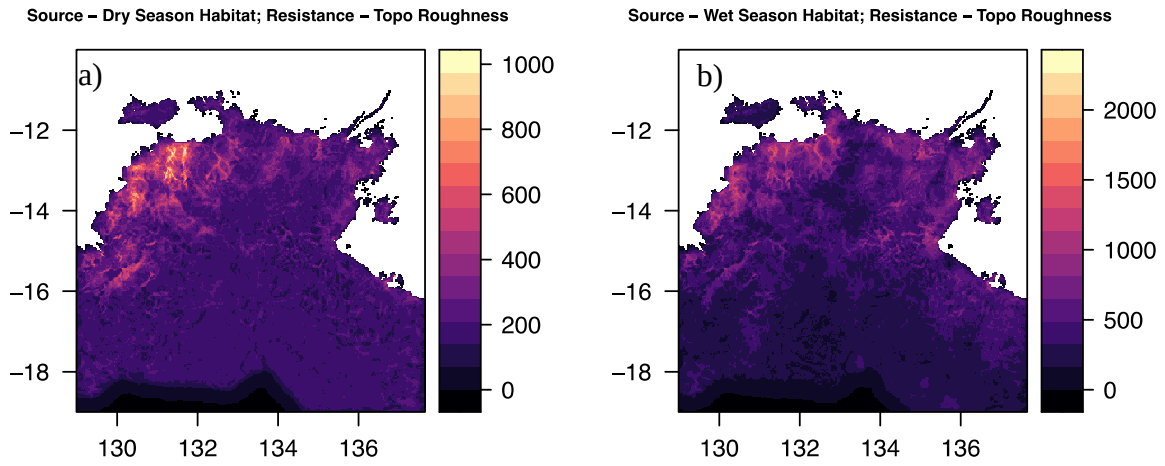

Figure 14: Current maps of potential feral pig gene flow across the Top End of the Northern Territory from the best-supported landscape resistance model and current sources corresponding to dry (a) and wet (b) season feral pig breeding habitat quality.

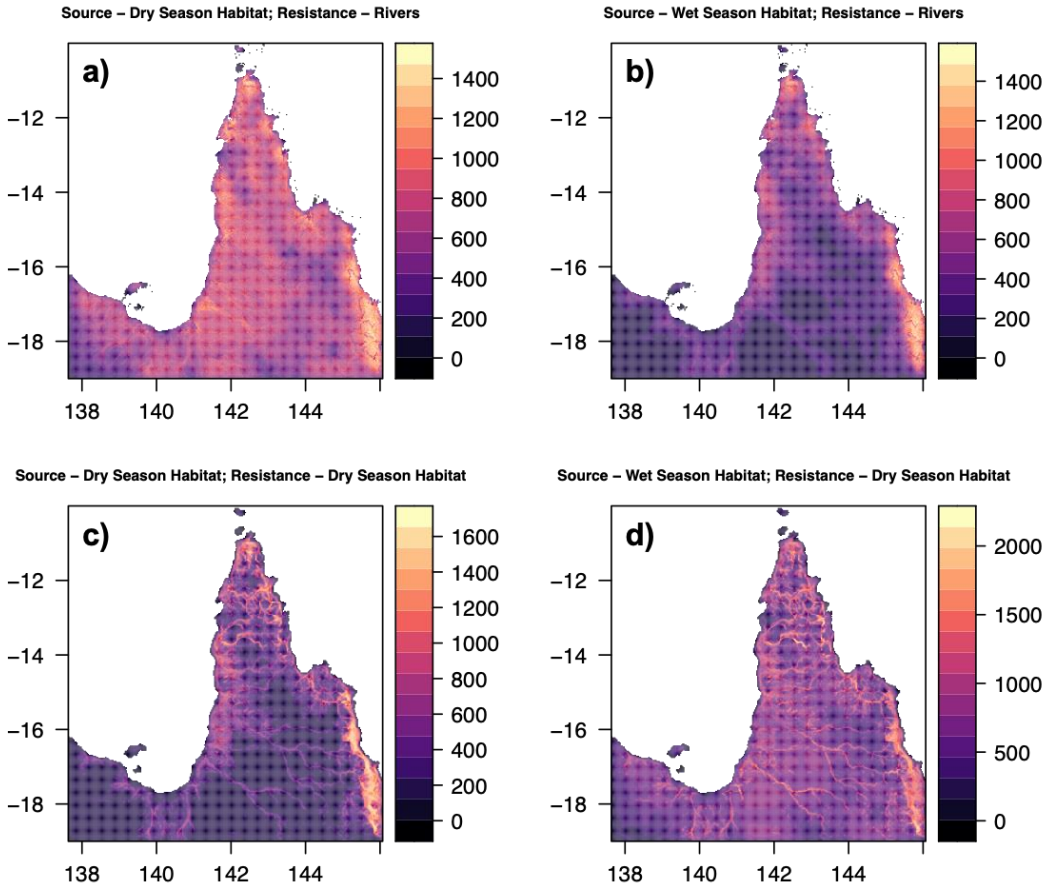

Figure 15: Current maps of potential feral pig gene flow across the Cape York and Gulf regions of Queensland from the best-supported landscape resistance models and current sources corresponding to wet and dry season feral pig breeding habitat quality.

Combined insights into feral pig population connectivity in northern Australia

The results enable inference about feral pig population connectivity from a set of different analyses.

Key findings

- On a national scale, genetic structure most likely reflects processes other than natural dispersal, reflected in nationwide genetic patterns that do not reflect a simple 'isolation by distance' scenario, which would correspond to progressive expansion of feral pigs across the country from a single point. The national-scale genetic patterns most likely correspond to feral pig introduction/escape history.
- Across northern Australia, genetic patterns are consistent with that expected under predominantly natural dispersal (isolation-by-distance) with some evidence of local translocations (e.g., Tiwi Islands) and colonisation history (e.g., Cobourg Peninsula).
- Spatial genetic analyses suggest population connectivity is far greater in north Queensland than the Northern Territory and greater in the Northern Territory than the Kimberley region of Western Australia.
- Genetic clustering analyses identify major geographic features like large rivers in Queensland and the Northern Territory (Mitchell, East Alligator, Roper) contributing to genetic structure across the landscape. These features act as localised barriers to movement.
- Resistance modelling of genetic distances identifies differences in the most prominent environmental correlates of population connectivity in Queensland and the Northern Territory. We were not able to run standalone analyses for Western Australia at this stage but will do this as more samples become available.
- Resistance surfaces can be linked to source habitat for feral pig dispersal to map likely 'current flow' representing the net movement of pigs across the landscape. We used published habitat models based on expert opinion in this report, but habitat suitability models based on modelling of occurrence records and survey-based density estimates will refine the spatial predictions. Connectivity maps can be generated for specific scenarios, such as mapping predicted recolonization sources following local culling operations or predicting host-mediated pathogen spread from an incursion point.

Future development of a national feral pig connectivity mapping platform

The datasets and resources developed in this project provide a solid platform upon which to develop a national-scale feral pig genotyping and connectivity mapping service. Here, we have developed a powerful dataset and genotyping approach feeding into spatial analyses

to map patterns of population connectivity in feral pigs across Northern Australia. This information can be used for several management and research activities including improving effectiveness of feral pig control efforts (by mapping culling areas against natural barriers to recolonization) and mapping corridors and barriers for likely host-mediated disease spread in the event of a novel pathogen incursion. There is strong potential to scale up from this project to a national-scale program enabling sample submission, tracking, online data access and interactive visualisation for government and non-government organisational and individual stakeholders.

Key benefits of the approach we have developed include:

1. *SNP marker panel with unbiased sampling of the genome for feral pig population analysis*

   Having developed the SNP marker panel from first principles, including hundreds of samples from northern, southern, eastern, and western Australia, we have the only suitable SNP panel for national-scale feral pig population connectivity analyses without the risk of ascertainment bias during data analysis. The genotyping protocol provides approximately 50,000 SNP loci mapped to the pig genome that can be filtered as appropriate for diverse analyses.

2. *Commercial genotyping provider with public accessibility to the genotyping service*

   Our partnership with Diversity Arrays P/L means that the protocol we have developed is available to any stakeholder to generate a comparable dataset and to add to a communal dataset on feral pig genomics across Australia.

3. *Cost-effectiveness*

   Having developed a foundational database from feral pigs across the country, we can subset the most informative markers from our panel to generate a targeted SNP genotyping array for a cheap ($15/sample) genotyping service, providing genotypes with very high quality for 5,000 – 10,000 SNPs per individual. Enabling data sharing and partnerships for genetic analysis of feral pig populations across the continent.

4. *Sample tracking and data sharing*

   Our genotyping provider (Diversity Arrays P/L) can co-develop a sample labelling and tracking app to enable samples to be tracked from the point of collection to final data visualisation. The KDDart and EcoKDDart platforms managed by Diversity Arrays provide opportunities for data storage, public access and visualisation.

5. *Existing partnerships*

The project has formed strong partnerships with Commonwealth, State and Territory governments in Queensland, the Northern Territory, Western Australia and New South Wales, as well as with major NGOs (Australian Wildlife Conservancy, Territory NRM), recreational hunter groups and regional councils. This includes strong collaboration with scientists and pest animal managers within these organisations who contribute directly to the research (e.g., pig habitat mapping), sample provision, management applications and facilitating collaboration with other initiatives such as EcoCommons and the Atlas of Living Australia for data storage and spatial analysis. Through these partnerships, we already have a set of over 2,000 samples for the project and have genotyped nearly half of those within this project.

Progress towards a national-scale program, the next steps include:

- Improved sampling density and coverage away from coastal regions in northern Australia, and across southern Australia. While the analyses were informative, the information quality will further improve if we can add sampling density in Western Australia and inland sites within the broad sampled regions. We are also working on implementing methods for fitting multivariate resistance surfaces to draw simultaneously on different sources of information to parameterise feral pig landscape resistance surfaces.
- Inclusion of alternative habitat suitability and population density maps in the connectivity modelling process.
- Subsetting the SNP marker panel for very cost-effective genotyping (< $15 sample), only by achieving this low-cost rate can we expect to have buy in from stakeholders across Australia.
- Development of sample tracking apps for mobiles or tablets that could be used in the field and ensure tissue samples are accurately tracked through to sequencing, analysis and mapping.
- Building shared data storage and open-access online options to visualise and interrogate the database for specific management applications. Providing this incentive would encourage a wide range of stakeholders from across Australia to genetically sequence feral pig samples upon their property, whilst ensuring data are stored collectively within a national database.

Component 2 – Assessing the utility of aerial thermal photography and remotely piloted aircraft for assessing the abundance and measuring body temperature in free-ranging feral pigs in northern Australia

Background

Traditional surveillance, using direct observation by the human eye, is a core component of NAQS operations. A significant proportion of NAQS resources are required to enable NAQS to undertake surveillance safely and effectively, including direct operational costs and the costs associated with maintaining training and safety standards. The objective of this component of the project was to trial remote aircraft and thermal photography as a means of undertaking feral pig surveys. This component of the project was originally to be delivered by the Six Seasons Investments Pty Ltd. Six Seasons were to undertake the flights and collaborate with Microsoft to incorporate the study findings within the Healthy Country AI dashboard. However, these partners pulled out of the project due to staffing issues. Thus, Charles Darwin University undertook the aerial surveys and collected thermal photography. Therefore, some of the deliverables were changed, and these were reported upon at each of the project milestones.

The application of thermal photography as a surveillance tool to detect feral pigs has been demonstrated in southern Australian landscapes, and there is currently a DAWE funded project that is developing AI routines to automate the interrogation of thermal imagery surveyed using crewed aircraft. Here we wished to assess how effective thermal photography was at detecting feral pigs in northern savanna landscapes. This is in terms of determining the probability of false negatives (not detecting pigs when they are present) and false positives (identifying items as feral pigs when they are not). This robust ground-truthing of aerial thermal imagery has not previously been attempted in any landscape, so whilst the results are applicable to northern landscapes, the methodologies will be suitable for aerial feral pig surveys in any landscape.

Crewed aircraft was cost prohibitive for the project, and therefore, we used remotely piloted aircraft systems (RPAS, more commonly known as drones) as the survey platform.

Methods

Feral pig abundance assessment in the aerial survey area

This research was undertaken at the CDU Katherine Rural Campus (Fig. 16). The property is around 10,000 acres and is located approximately 20 km north of Katherine in the Northern Territory. The vegetation on the property is made up of open grassland and mixed woodland.

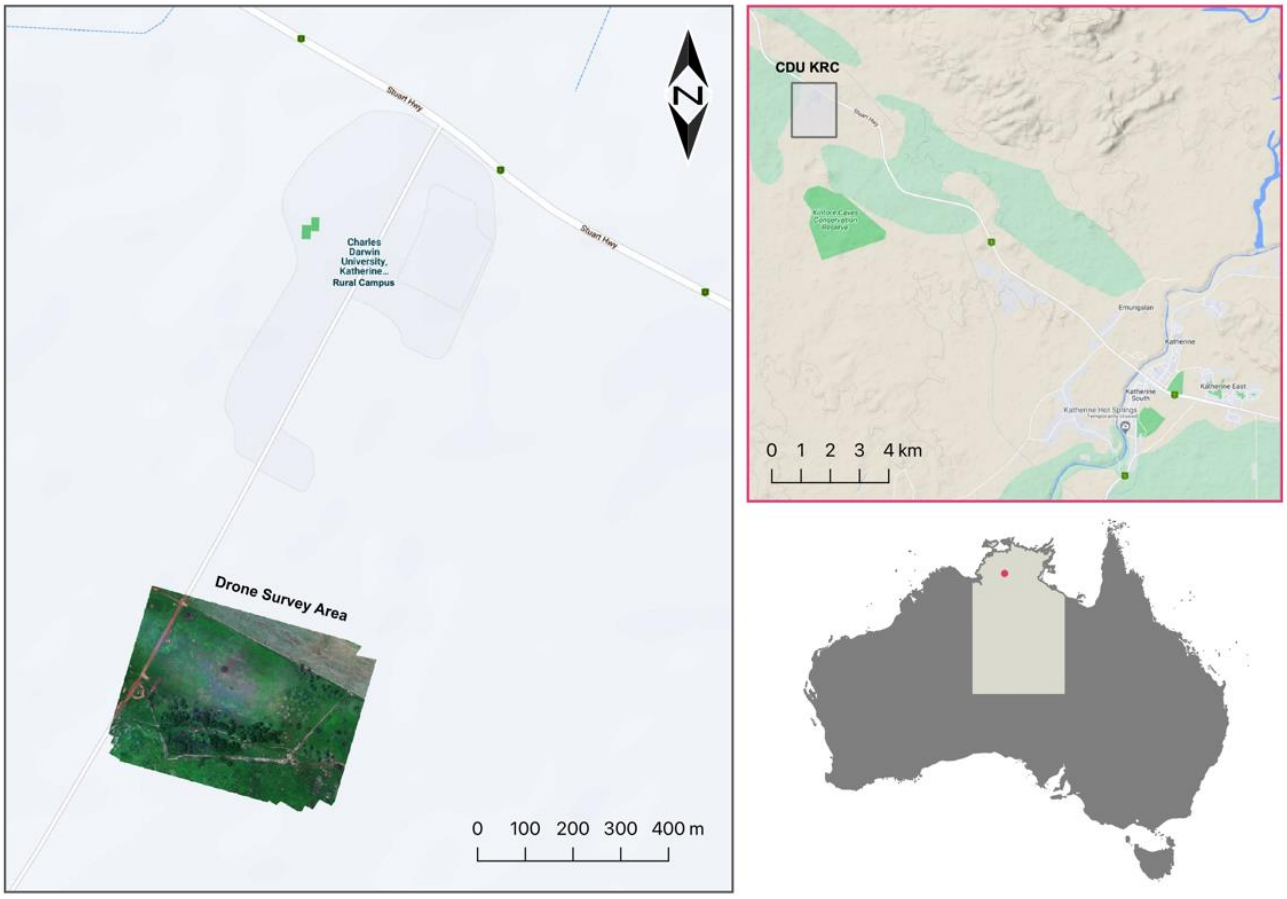

Figure 16: The drone survey area on the CDU Katherine Rural Campus (KRC) is approximately 20 km north of the town of Katherine.

In the original application, we proposed to derive feral pig abundance estimates through t3e use of camera traps and occupancy modellings. However, a pilot study undertaken on the property found high diurnal pig movements over a broad area, which resulted in low confidence limits in the pig density estimates. We, therefore, opted for an alternative method to determine feral pig abundance in the survey area with high accuracy. To enable assessment of feral pig detectability using aerial thermal imagery, it was important to have a known abundance of feral pigs and to know their exact location in the survey area when the drone was overhead. This was achieved by attaching GPS-based satellite collars to feral pigs, building a large pig-proof fence around the aerial thermal survey area (23 acres), and then

placing several captured pigs inside the fence. Thus, feral pig abundance was identical for all surveys. We determined the geographical location of the pigs inside the enclosure during the survey by attaching GPS-based satellite collars (Fig. 17).

Figure 17: a) Feral pig proof fence with electric wire constructed around 23 acres of paddock and mixed woodland; b) Purpose-built feral pig collars with location fixing, ambient temperature, and body temperature recording and data-logging; c) Feral pig released inside the fenced paddock and fitted with locating and body surface temperature recording collar.

A wire mesh fence with a barbed top wire, mesh skirt, electrified bottom wire, and high gates were constructed around 23 acres of savanna woodland (Fig. 17a). In February 2021, nine adult feral pigs (n = 5 male, n = 4 female) were captured using baited traps at other areas of the property. Each pig had a GPS-based locating device (Fig. 17b) attached to a heavy-duty collar. The device logged the geographical location of the feral pig to a resolution of less than

2 meters on a minute-by-minute basis and timed to coincide during periods when the aerial surveys were being undertaken. Sensors embedded into the collars also measured ambient air temperature and the temperature on the back surface of the pig every 30 minutes. Once the collar was attached, the pigs were released into the fenced area and allowed to roam freely within the enclosure for 3 weeks before the aerial surveys being undertaken (Fig. 17c). They were fed a diet of corn via an automatic hopper during the acclimation period.

All work was completed under CDU Animal Ethics Committee Approval – A20031, and Authority to release collared feral pigs was provided to Professor Hamish Campbell by the Northern Territory Government Minister for the Environment.

Aerial thermal photography

We trialled two remotely piloted airframes for this study: a quadcopter (DJI Matric DJI 210) and a fixed-wing aeroplane (Sensefly eBee X). The thermal camera used was a FLIR VUE 640, and a Zenmuse RGB camera was dual mounted so that optical and thermal imagery could be taken of the same area simultaneously. Due to the broad area that required aerial surveying, pilot trials deemed the quadcopter not suitable. Thus, the main study was undertaken with the fixed-wing aeroplane (Fig. 18).

A total of 15 RPA (remotely piloted aircraft) flights were undertaken within two-hour periods from 5 am, 9 am, 2 pm, 6 pm, and 11 pm, with 2-3 replicates at each time recorded over 4 days. Due to intermittent rain throughout the study period, some surveys were unable to be completed or were paused and split into two flights. During each flight, the camera system simultaneously took optical and thermal images of the entire area within the pig-proof fencing. All flights were undertaken at a height of 70 meters, with approximately 680 overlapping images required to cover the 23-acre fenced area. Overlapping optical and thermal images were processed using Pix4D to stitch them together, producing georeferenced orthomosaics of the field for each flight.

Figure 18: RPA Pilot (Ms Rebecca Rogers) at Katherine Rural campus field. The fixed-wing aeroplane was used for the study.

Aerial thermal photography of feral pig carcasses

On the 15th of February, all collared pigs were culled by a single shot to the head. The collars remained attached and recorded location, the ambient niche temperature, and the body surface temperature of the carcass for 24 hours. Aerial surveys using RPA were undertaken as previously described. Flights were undertaken one hour after culling (9 am), 10 hours after culling (6 pm) and 22 hours after culling (6 am).

Inspection of the carcasses after 24 hours found two of the carcasses to have large chunks of flesh stripped from the abdomen. As the enclosure was fenced, these wounds and the consumption of the flesh must have been by the other feral pigs in the paddock. After 24 hours, all the feral pig carcasses were covered in large maggot masses.

Results

Feral pig behaviour

After 4 days of RPAS aerial surveys, the feral pigs were culled by a single shot to the head. The pigs were left where they lay, and a thermal survey was undertaken for a further 24 hours. The GPS-collars were then removed, and the geographical location data for each pig throughout the study was retrieved. Upon collection, it was discovered that one of the feral pigs had slipped its collar. Although we could locate the rough area where the collar was located, long grass prevented its retrieval. Thus, we collected high-resolution location fixes for 8 feral pigs every 2 minutes for 5 time periods over the diurnal cycle (Fig. 19).

Plotting the location fix data showed that all feral pigs explored the entire fenced area but had a greater preference for the canopy-covered areas compared to open grassland. There

were also discrete areas within the paddock where individuals spent a significant proportion of their time. These preferred areas were not the same area for all pigs.

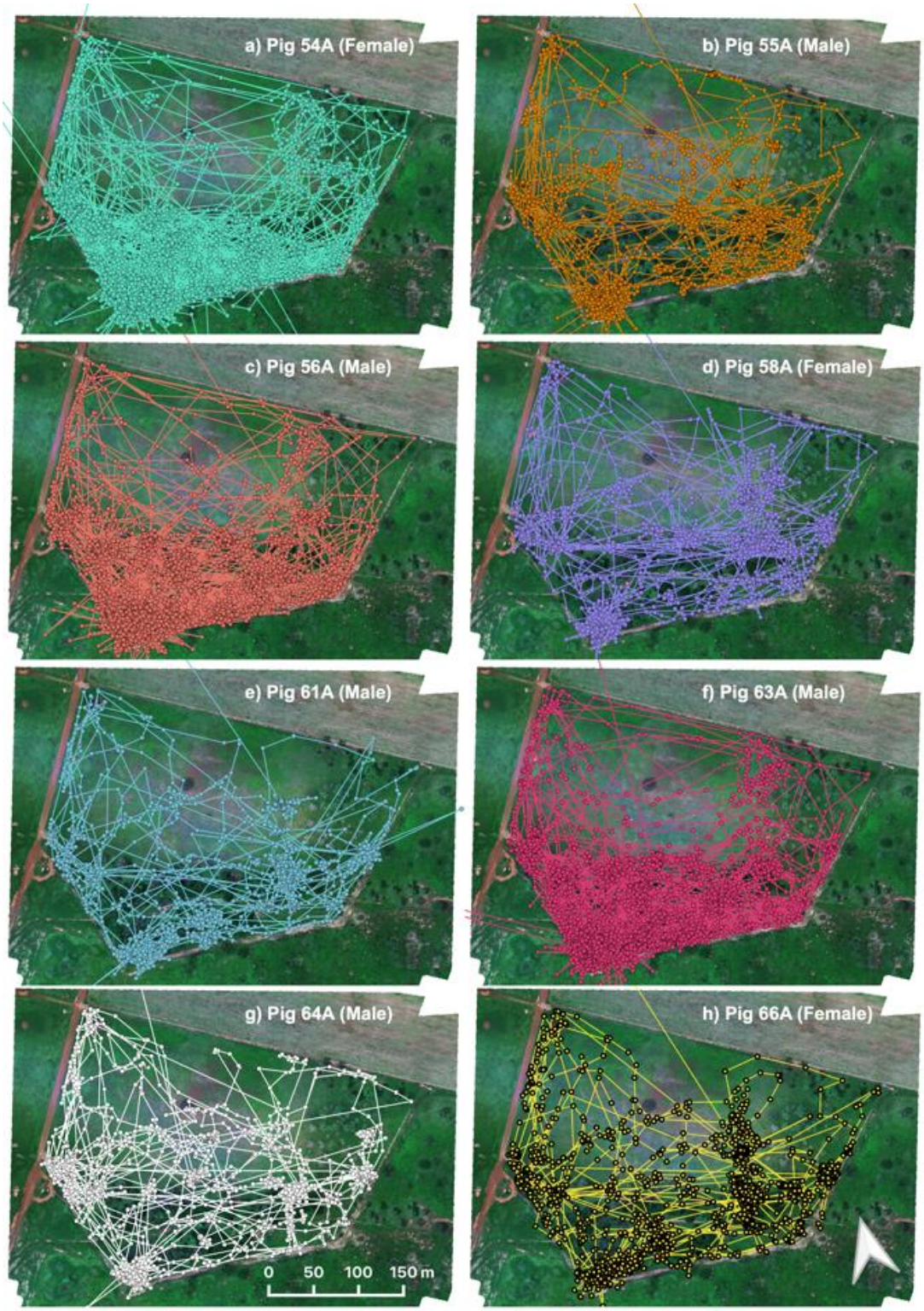

Figure 19: The geographical location fixes for eight feral pigs collected over the 4-day survey period.

From the geographical location data movement, metrics were calculated and averaged over the eight pigs. Results showed that feral pigs were most active during the crepuscular periods and had the lowest probability of being under the tree canopy during the evening crepuscular period (Fig. 20).

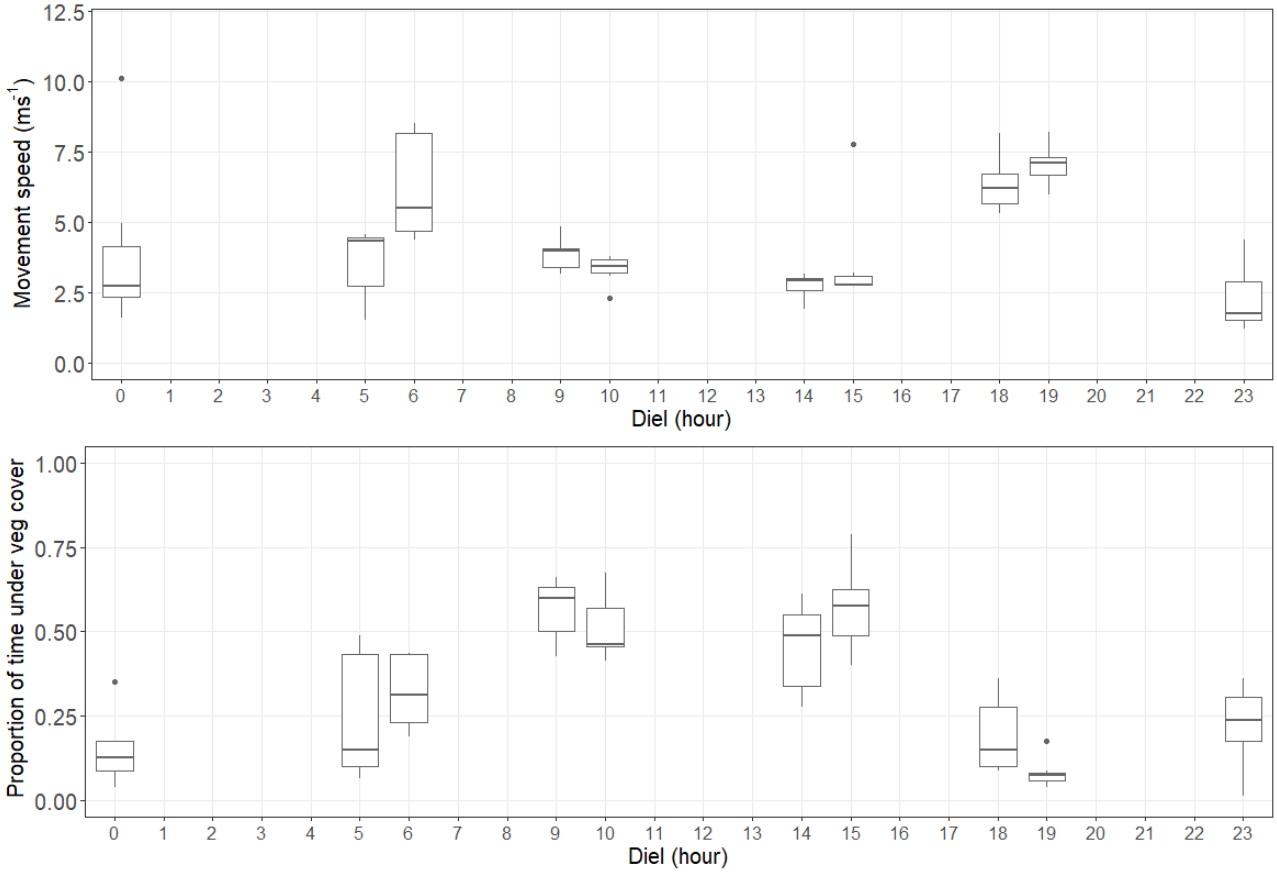

Figure 20: Upper panel shows the mean activity of eight pigs at five discrete time points over the diurnal cycle. The lower panel shows the mean proportion of time that eight feral pigs spent under the tree canopy. The box whisker plots show the mean values (horizontal line) and the outer 95% confidence intervals (box) plus outliers. Data is based on GPS collar data for individual pigs.

Feral pig visual detection from aerial thermal images

Each of the aerial surveys captured optical imagery with a corresponding thermal image. Visual interrogation of the thermal images and the simultaneously taken optical image found that feral pigs were easily identifiable when the pigs were located upon grassland or bare earth (Fig. 21). Thermal imagery easily showed up bare earth, but it was not possible to identify pig damage or wallows from the aerial thermal imagery.

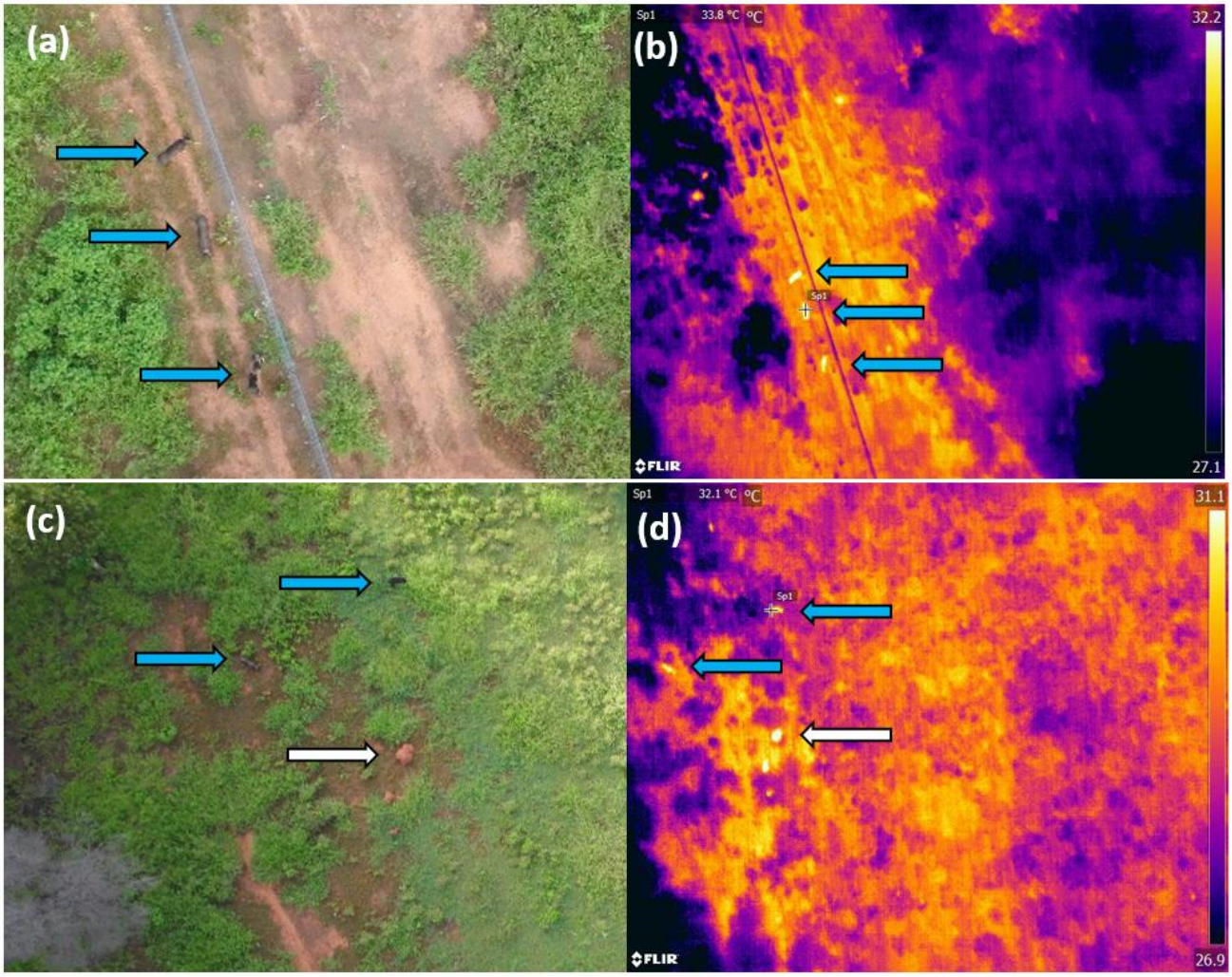

Figure 21: Simultaneous optical and thermal images taken using a dual camera mounted on a fixed-wing drone. The photos represent imagery taken between 5 am - 6 am (a & b) and between 6 pm - 7 pm (c & d). The body temperature of one of the feral pigs recorded by the thermal camera is shown in the top left of the image. The blue arrows indicate the location of the feral pigs in the optical image and their relative thermal image. The white arrow indicates the location of a termite mound in the optical and thermal image.

Aerial thermal photography undertaken at night provided a high contrast between the background and the occurrence of large warm-blooded animals (Fig. 22). However, aerial thermal photography taken during the hours of darkness was not supported by a simultaneous optical imagery, so it was not possible to ground truth the thermal signatures. Figure 22 shows a thermal signature that we know for certain from the GPS-based tracking data that it is a feral pig (right figure in image). The other two signatures (to the left) were from animals that were not GPS-collared, and we are therefore uncertain if they are un-collared feral pigs or agile wallabies, both of which were present within the fenced area (Fig. 23).

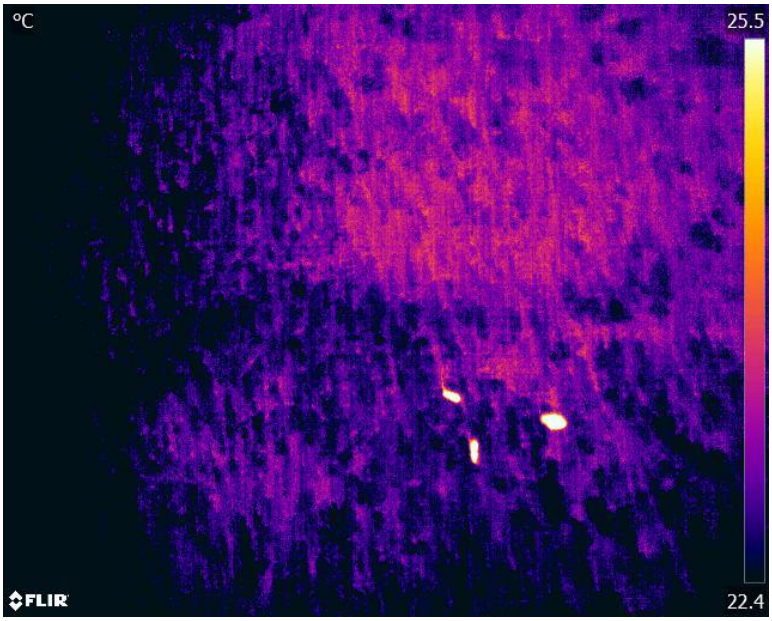

Figure 22: Aerial thermal image taken between 11 pm – 1 am of a known feral pig and two unidentifiable objects, possibly wallabies or juvenile pigs. Average ambient temperature recorded by the GPS devices at this time was 32.2° ± 1.02 SD.

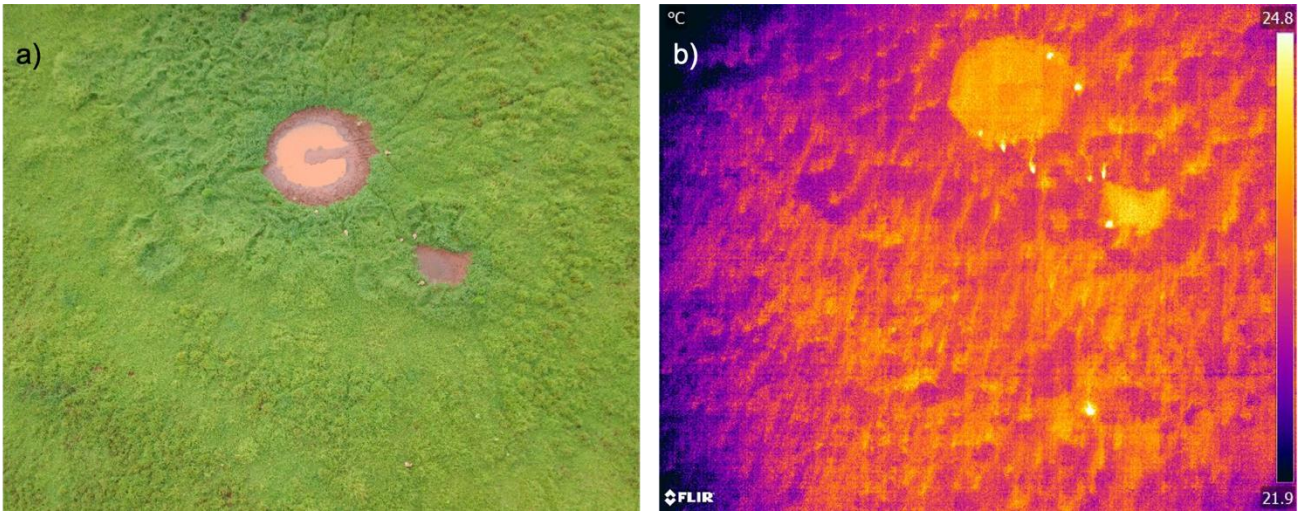

Figure 23: optical (a) and thermal (b) images taken between 5 am and 6 am, showing signals produced by a large group of agile wallabies within the survey area. Average ambient temperature recorded by the GPS devices at this time was 34.2° ± 1.03 SD.

The ability to detect warm-blooded animals depends on the contrast between the background ambient temperature and the emission temperature from the animal in the image. Comparing ambient and animal temperatures from the aerial imagery throughout the day showed that the largest temperature difference occurred in the early morning, and early evening, with thermal overlap between 9 am and 4 pm (Fig. 24).

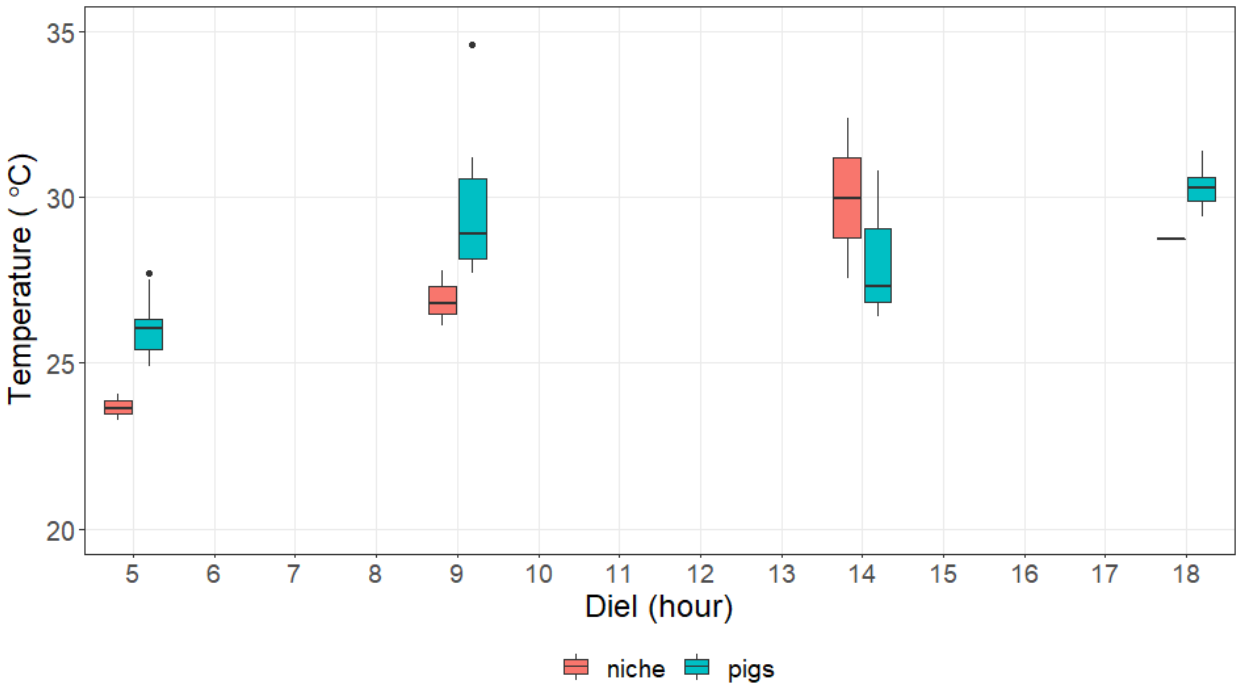

Figure 24: The averaged background temperature in the aerial imagery and the body surface temperature of the feral pigs as detected by the thermal camera. Data show the mean and 95 % confidence limits for all images per flight (n = 680) and averaged over the number of replicate flights (n = 3). Night flights were excluded as it was not possible to stitch these images together to estimate niche temperature.

The likelihood of detecting a feral pig using aerial thermal imagery was assessed at four time periods throughout daylight hours. Locations of feral pigs were determined by visual interrogation of the thermal imagery, and then each heat signature was verified by consulting the corresponding optical imagery. Individual images taken by the aircraft were used to detect pigs as their movement behaviour made them difficult to identify in the stitched images for the entire field (orthomosaic). The estimated detectability of feral pigs was conservative because only heat signatures where identification could be confirmed with GPS locating collars were used in the final analysis (Fig. 25). The results show that surveys carried out around 6 pm produced the highest probability of visually detecting a feral pig from the aerial thermal photography. However, even at this time, the detection probability was not greater than 60%. Detection probability was extremely low (< 15%) in the mid-afternoon and morning. Mid-afternoon pigs spent a significant amount of time undercover where they were undetectable in aerial imagery (Fig. 20). Additionally, on average, their body surface temperature was lower than the background temperature detected by the thermal camera, making heat signatures more difficult to distinguish in thermal images (Fig. 24). The reason for low rates of detection in the morning was less clear but may be explained by high rates of movement and moderate amounts of time spent undercover (Fig. 20).

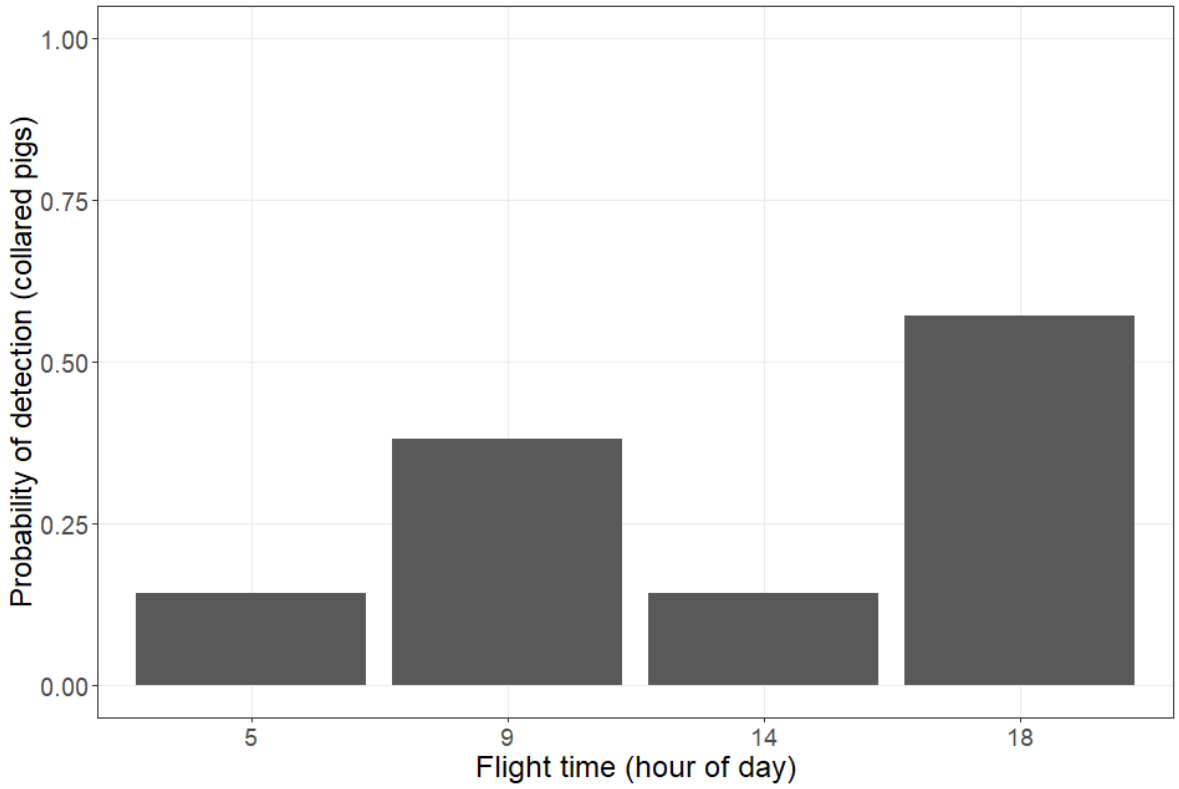

Figure 25: The probability of detecting a feral pig within the survey area at 4 time periods during daylight hours from aerial thermal photography. Reduced detection probability was due to canopy cover.

Measuring body temperature from free-ranging pigs by aerial thermal photography

The surface body temperature of feral pigs was measured from the aerial thermal imagery and simultaneously by a thermal sensor embedded in the attached collar (Fig. 26). At all survey time-periods when optical imagery data was available (5 am – 7 am, 9 am – 11 am, 2 pm – 4 pm, 6 pm – 8 pm, 11 pm – 1 am), body surface temps measured by the thermal camera were consistently lower than body surface temps measured by the data loggers on the collars. This may be because the data logger only recorded the temperature at the pig neck, whilst the thermal camera was an averaged temperature across the whole surface of the animal. This would have included the cooler extremities. The surface body temperature recorded by aerial thermal photography was less variable between individuals than that recorded by the data logger.

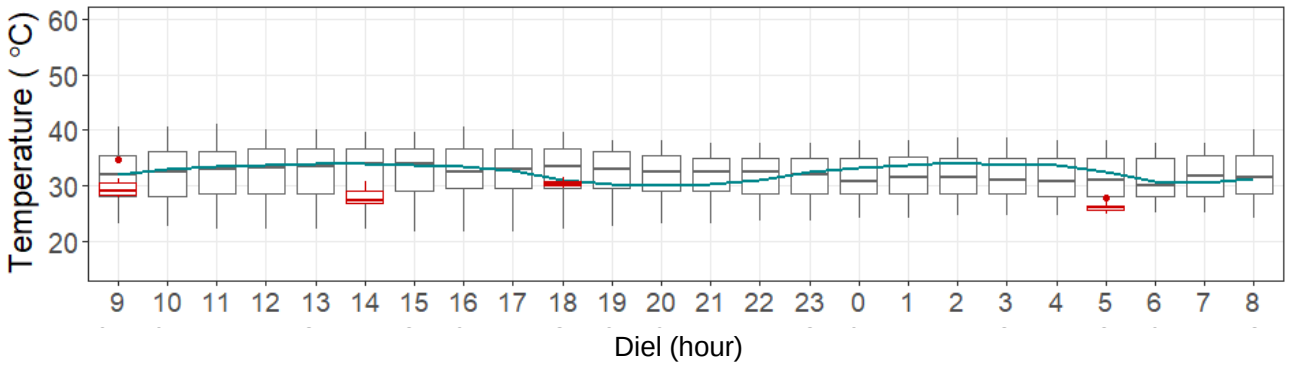

Figure 26: The ambient niche temperature around the feral pigs (green line n = 9) recorded by a temperature logger embedded in the collar but a few cm away from the bodies' surface, and the body surface temperature recorded on the back of the pig (grey box whisker plots, n = 9) by a temperature logger under the collar and adjacent to the skin. Red box plots show the pig body surface temperature recorded by aerial thermal imagery.

Detecting feral pig carcasses with aerial thermal imagery

After the feral pigs were culled, a total of 3 fixed-wing RPA (remotely piloted aircraft) flights were undertaken at 1 hour, 10 hours and 22 hours post-cull. One hour after the pig cull, it was easy to visually identify the feral pig carcasses against the ambient thermal background in the orthomosaic (stitched images) and the individual images (Fig. 27).

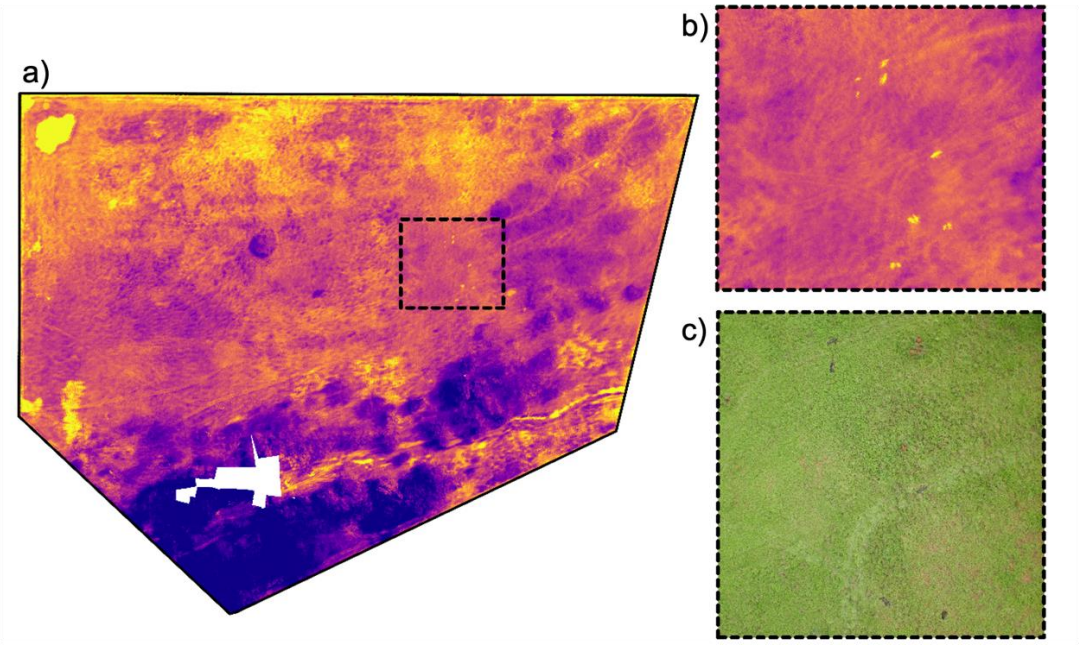

Figure 27: a) Orthomosaic of the thermal data across the entire field, b) thermal image, and c) optical images taken of feral pig carcasses 1 hour after death (9 am). These images show that the pig carcasses were clearly identifiable in the stitched imagery.

The background ambient temperature was still relatively low in the morning compared to the pig body surface temperature. After ten hours, the pig carcasses could still be easily identified visually (Fig. 28). These images were taken around 6 pm, and most of the carcasses still stood out against the warm background because they heated up in the sun throughout the day.

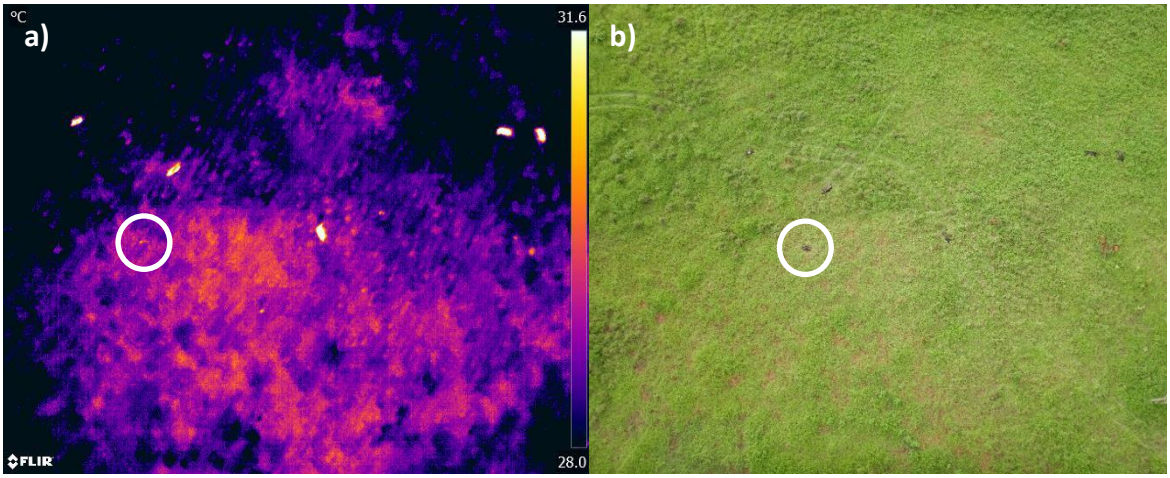

Figure 28: a) thermal and b) optical images taken of feral pig carcasses 10 hours after death (6 pm). These images show that most pigs are still easy to identify. However, one of the pigs (circled) is no longer detectable from the background signal, likely due to the carcass being consumed.

By 22 hours post-cull, the feral pig carcass temperatures were not significantly elevated over the background temperature and could not be visually identified (Fig. 29).

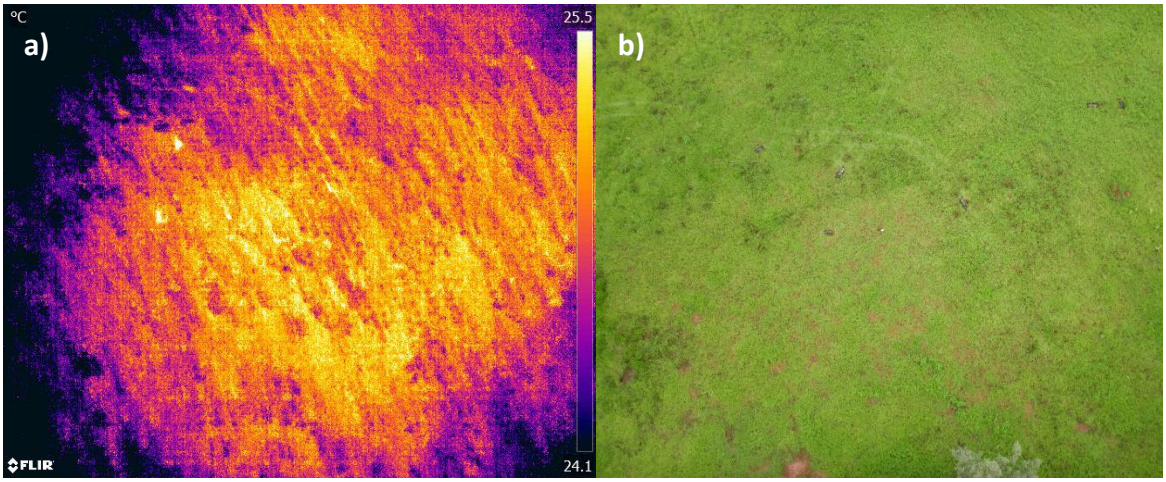

Figure 29: a) thermal and b) optical images taken of feral pig carcasses 22 hours after death (6 am). These images show that most pigs are no longer identifiable in the thermal data.

The body surface temperature of the carcasses measured from the thermal imagery at 9 am (1 hour post-cull) were 3 to 4°C higher than measured by a thermal datalogger at the body surface. Thermal imagery taken at 6 pm (10 hours post-cull) produced carcass temperature recordings 2 to 3°C greater than recorded by a temperature device on the pigs' body surface. By 6 am (22 hours) the following morning, the aerial thermal photography recorded a carcass temperature that was 2 to 3°C lower than recorded by the temperature devices on the body surface of the carcasses (Fig. 30).

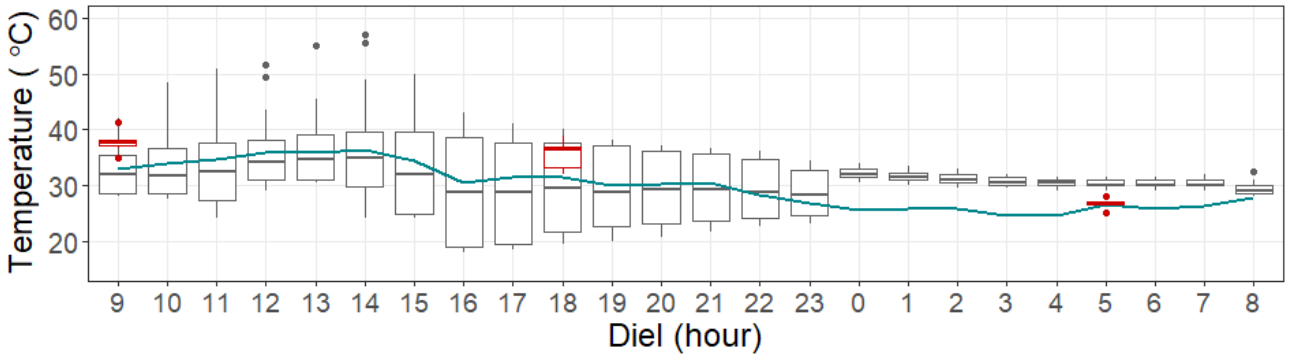

Figure 30: The ambient niche temperature around feral pig carcasses (green line n = 9) recorded by a temperature logger embedded in the collar but a few cm away from the bodies' surface, and the body surface temperature recorded on the back surface of the carcass (grey box whisker plots, n = 9) by a temperature logger under the collar and adjacent to the skin. Red box plots show the pig body surface temperature recorded by aerial thermal photography.

Ambient niche temperature and body surface temperature decreased between 2 pm and 9 pm. The inter-individual variation in body surface temperature was greater in the carcasses than in the live pigs. This was likely caused by the pigs' body size and thermal latency of heat from the carcass, with larger pigs retaining more heat than the smaller animals. From 10 pm until 8 am, this inter-individual variability in body surface temperature significantly decreased and was much more similar than recorded between live pigs. During this period, the surface body temperature of the carcasses remained 3 to 4°C greater than the ambient niche temperature.

Discussion

As far as we are aware, this was the first empirical ground-truthing of the application of aerial thermal photography to detect and measure body temperatures in free-ranging feral pigs. Supporting previous work, the study showed that aerial thermal photography can detect feral pigs but the probability of detecting a feral pig within the survey area changes throughout the diurnal cycle due to feral pig behaviour. The probability of detecting a feral pig is very low (<15%) in the morning and early afternoon because individuals tend to remain in dense

vegetation and under the tree canopy and are obscured by aerial survey. Feral pig detection probability (60%) was highest in the late afternoon (after 6 pm) as, during this time of the day, they tended to be out from under the canopy and moving around in a more open country. It should be noted that not one of our aerial surveys detected all the feral pigs present in the survey area. We would expect these findings to be similar in other northern Australian landscapes, although the exact probabilities would vary depending on the proportion of canopy cover in the landscape. Thus, we recommend that any aerial feral pig survey be conducted in the late afternoon, and some type of detection probability correction factor be applied to provide a more accurate measure of feral pig numbers in the landscape.

We also found a high incidence of false positives in the thermal images in northern landscapes due to the high occurrence of wallabies and termite mounds. We believe that it would be challenging for any automated image analysis program to distinguish between terminate mounds, wallabies, and feral pigs from the thermal images alone. We, therefore, recommend that thermal imagery be accompanied by optical imagery, with the two types of photographs being taken simultaneously. Using a combination of these two data types would negate the persistence of false positives from the thermal images and improve detection specificity. Consequently, thermal aerial surveys undertaken during the hours of darkness are likely to return a high number of false positives and overestimate feral pig numbers.

Thermal aerial photography was effective at detecting feral pig carcasses for up to 12 hours post cull. The ease of detecting pigs in the stitched imagery is significant because it reduces the effort involved in manually going through the large number of images captured during a survey. A remotely piloted aircraft could be deployed after a cull, survey a broad area (+ 900 hectares) and then the imagery stitched together to create a single image of the cull area. Carcasses did not show up 24 hours post cull, so this may also be an effective means for counting the number of dead pigs and detecting any surviving feral pigs after a cull.

We aimed to test if aerial thermal imagery could be used to detect exotic disease infected feral pigs under the premise that they would show an elevated body temperature. However, measuring the pigs' surface temperature at a distance is probably not possible because only an average temperature across the whole body is possible by using aerial thermal photography. This can be subject to change due to the angle and position of the pig's body relative to the camera. Body surface temperatures recorded by the aerial thermal photography were between 2 to 4°C different than recorded by a body surface-mounted temperature datalogger. Further work would be required to calibrate these measurements.

The traditional aerial survey methodology to survey feral pigs is to count from a helicopter or small aeroplane manually. These types of aircraft usually cost more than $1500 per hour, plus

the cost of the standalone thermal camera $50,000 and the salary costs of the spotter. The fixed-wing drone used in this project cost $24,000 to purchase, and the thermal camera was $14,000. The hourly running costs were virtually zero and the only costs were for the pilot (~$50 per hour). The size of the survey area covered per unit of time would likely be greater for manned compared to remote aircraft, but this gap will narrow in the coming years due to technological advancement. Air safety restrictions around flying remote aircraft beyond visual line of sight limited the survey area, but these restrictions are also likely to ease and be overcome in the future. The fixed-wing drone in this study covered an area of around 400 acres in 60 minutes. One advantage in using the fixed-wing drone over a crewed aircraft is that the flight route and imagery are recorded and can be scrutinised at a later date, reducing the risk of human error.

Key findings

- Morning and early afternoon aerial surveys only detected a small proportion (> 15%) of the feral pigs present within the survey area due to the pigs' behaviour during this time of the day.
- Feral pig detection probability by the aerial survey was highest (>55%) in the early evening (6 pm – 8 pm).
- The night-time aerial thermal survey returned a high number of false positives due to wallabies and termite mounds.
- To reduce the likelihood of false positives, aerial survey of feral pigs in northern landscapes requires thermal and optical imagery to be taken simultaneously
- Recording free-ranging feral pig body temperature by aerial thermal photography as a means of detecting ASF infected pigs has potential, but further research is required to better understand the inter-individual variability.
- Aerial thermal photography was very good (100%) at detecting feral pig carcasses up to 10 hours post-cull.

Best practice to maximise feral pig detection with aerial thermal survey from remotely piloted aircraft

- Equipment recommend: a fixed-wing drone fitted with an optical and thermal camera that can take both sets of images simultaneously.
- Undertake flights in the late afternoon as this has the highest detection time for feral pigs during daylight hours because they tend to be out from under tree canopy.

- Do not take flights at night, as it was not possible to collect optical imagery and require simultaneous optical images due to the high number of false positives (termite mounts & wallabies) in northern Australian landscapes.
- Flights can be taken from 100 meters elevation. Flight speed can be set to maximum, with minimal image overlap required.

Future work and operationalisation of study findings

This research was undertaken in the mid- wet season under very hot and humid conditions, and it would be valuable to repeat the study under dry cool conditions to assess if detection probabilities would be the same over the diurnal cycle.

Further work should explore the application of autonomous image analysis methodology using a combination of both thermal and optical imagery. Here we have provided imagery for autonomous analysis to the DAWE funded automated thermal imagery analysis project run by Dr Peter Adams at WA DPIRD. At the time of writing this report, this analysis was still ongoing as the algorithms developed in this project were only applicable to the thermal images and not a combination of thermal and optical imagery. Our recommendation is that future AI development uses these two types of imagery for determining feral pig presence in northern landscapes.

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