Genetic study of the Sandy Point koala population

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TERMS AND DEFINITIONS

DNA (Deoxyribonucleic acid) encodes genetic information. DNA is made up from a great number of smaller molecules called nucleotides.

A **gene** is the basic physical and functional unit of heredity. Genes are sections of DNA that encode the instructions for the formation of proteins.

A **locus** (plural **loci**) is a fixed position within DNA and includes the position of a gene or a genetic marker (e.g. a microsatellite marker). The cells of most animals are diploid, that is, containing two copies of homologous DNA where one copy is inherited from each parent. A locus refers to the same region on each homologous copy.

An **allele** is one of a number of alternative forms of DNA at a locus that arise by mutation and are found at the same position within the DNA molecule. In sexual reproduction one allele is inherited from the mother and one from the father.

Where two different forms of DNA sequence (alleles) occur at a locus (or a gene) in a given individual, that individual is referred to as being **heterozygous** at that locus or gene. The individual may pass either of the two forms onto its offspring.

A **microsatellite** is a set of short repeated DNA sequences at a particular locus, which vary in number in different individuals and can therefore be used for genetic fingerprinting (which allows individuals to be identified).

The genetic makeup of an organism or group of organisms with reference to a single trait, set of traits, or an entire complex of traits is referred to as a **Genotype**. In the case of the twelve microsatellite markers used within this report, an individual's genotype is the set of different alleles found across all twelve loci. The genotype of an individual allows individuals to be identified as the chance that any two individuals share the same genotype is normally very small.

The **number of alleles detected (A)** provides a count of the number of different alleles (DNA variants) detected in each population averaged across the number of loci genotyped.

Allelic Richness (A_R) describes the average number alleles per locus (A), corrected for differences in sample size between populations.

The **percentage of total alleles ($A_{\%}$)** is the proportion of the alleles present in all populations, that are found within each single population.

Expected and observed heterozygosity (H_E and H_O) measures the frequency of heterozygosity (where two different alleles are present in an individual) in a population. In contrast, an individual who is homozygous has two identical alleles at a single locus. Greater heterozygosity is often associated with greater fitness.

Private alleles (P_A) are alleles that are only found in a particular population. Alleles that are only found in one population can be an indicator of genetic distinctiveness

EXECUTIVE SUMMARY

This report presents the findings of a study into the Sandy Point koala population using genetic data and DNA isolated from sampled koala scats. The study was initiated and funded by the Sandy Point Community Koala Action Group (SPCKAG).

The SPCKAG carried out a koala survey of the Sandy Point area during March 2018 and collected 22 scat samples for genetic analysis. A total of 20 scat samples provided reliable data for analysis from which 11 individual koalas (6 females and 4 males) were detected.

Bacterial infection with *Chlamydia pecorum* was not detected in any of the 11 individuals while koala retrovirus (KoRV-A) was detected in 4 individuals (36%). The rate of KoRV-A detected at Sandy Point is slightly higher but statistically similar to the average rate of KoRV-A infection within the greater South Gippsland[1] koala population (27%).

Genetic data from the Sandy Point koala population were compared to koalas sampled in South Gippsland, Cape Otway (translocated from French Island) and Raymond Island (translocated from Phillip Island). Genetic comparisons of these populations revealed that the Sandy Point koala population is a remnant of the larger South Gippsland koala population, rather than from French or Phillip Island.

Genetic diversity in the Sandy Point koala population was significantly lower than koalas sampled from South Gippsland, Cape Otway or Raymond Island. The low level of genetic diversity in the Sandy Point koala population is likely driven by its isolation and consequent lack of recent koala migration into the area, followed by successful breeding (gene flow). Inbreeding may or may not currently play a role in the population's low diversity and warrants further investigation. Questions such as whether the high incidence of sarcoptic mange in the population is related to the population's low level of genetic diversity also remain to be answered.

Continuing to improve and extend koala habitat to support the local koala population will be important for the future preservation of this koala population. The low level of genetic diversity present in Sandy Point koalas makes this population susceptible to future stochastic events (e.g. novel disease and/or changes in climate and the environment, which could, for example, influence the suitability and/or availability of food sources). Potential strategies to increase the population's genetic diversity and adaptability requires further investigation and assessment of risk.

[1] The South Gippsland koala population includes and is equivalent to the Strzelecki Ranges koala population

1. Introduction

1.1 Victorian koala populations

In Australia, extensive habitat loss and hunting post European colonisation (~1788) decimated koala populations. By the early 1900s, less than 1000 koalas remained on the Victorian mainland. Koalas were, however, introduced to French and Phillip Islands in the late 1800s and these populations flourished (Lewis 1954; Menkhorst 2008). As only small numbers of individuals were used to establish the island populations (French Island, *n*=3 and Phillip Island, *n*~10–30) genetic diversity was reduced in these populations relative to their ancestral population/s. By the 1920s, the koala populations on the islands had grown to unsustainable size. To curb population growth whilst simultaneously facilitating the re-establishment of the koala in Victoria, koalas were translocated from French and Phillip Islands to the mainland (Lewis 1954; Menkhorst 2008).

Although translocation of individuals from French and Phillip Islands to the mainland was extremely successful in re-establishing koala populations throughout Victoria, genetic diversity is low in both island koala populations and mainland koala populations descended from translocated island koalas (Houlden *et al.* 1996b; Lee *et al.* 2011; Wedrowicz *et al.* 2018a). Low genetic diversity can impact a species' ability to adapt to new environmental pressures such as climate change or disease (Bijlsma *et al.* 2000; Frankham 2005). Populations with low genetic diversity may be more susceptible to disease outbreaks than those with greater diversity. A lack of genetic variation is therefore of great concern for the future viability of Victorian koala populations, especially during the current period of rapid environmental change.

Although most Victorian koala populations have low genetic diversity, the koala population in South Gippsland is a remnant population that received few translocations of island stock (Martin 1989; Wedrowicz *et al.* 2017). Studies have indicated that koalas in South Gippsland have greater genetic diversity compared to Victorian island populations and those founded by island stock (e.g Cape Otway, Raymond Island, French Island, Mornington Peninsula, Brisbane Ranges and Stony Rises; Houlden *et al.* 1999; Lee *et al.* 2011; Wedrowicz *et al.* 2018a).

1.2 Sandy Point

Sandy Point is a small coastal community, with only 200 permanent residents, situated approximately 150 kilometres south-east of Melbourne. A relatively young town, which was first established during the 1950s, Sandy Point is now a popular holiday destination during the summer time, and as such the population of the town extends into the thousands during these peak periods (Wright *et al.* 2018).

The koala population is thought to have consisted of very few individuals at different times in the past. There are anecdotal reports that the Sandy Point koala population has been supplemented by individuals brought from nearby Snake Island (koalas of French and Phillip Island descent) and Walkerville and Waratah Bay (where koalas may descend from the native population; Wright *et al.* 2018), though these reports are unconfirmed.

Koalas are said to have been plentiful in the Sandy Point area in the late 1890s (Wright *et al.* 2018). Early settler of the area, Fred Pilkington noted that in around 1910 all the gum trees were dead and that koalas had become very few in number, suggesting over browsing and that the population is likely to have crashed at that time (Wright *et al.* 2018). Over browsing and population decline was also noted around this time at the neighbouring Wilsons Promontory National Park (Barrett 1939; Menkhorst 2008). Such contractions in population size can result in lost genetic diversity which is an important factor contributing to extinction risk (Frankham 2005). Low genetic diversity can also play a role in disease susceptibility, as a lack of variation within the population can mean that all individuals will have similar levels of vulnerability to certain pressures.

The koala population at Sandy Point recently suffered an outbreak of sarcoptic mange caused by infestation by the mite, *Sarcoptes scabiei*. Between 2015 and 2017, 17 koalas at Sandy Point were euthanased due to severe cases of mange. The loss of these individuals during this time may also have resulted in further losses of genetic diversity in the Sandy Point koala population which may therefore increase the risk of future extinction.

1.3 Objectives

This study was funded by the Sandy Point Community Koala Action Group to gain an understanding of the genetic health and presence of pathogens (*Chlamydia pecorum* and koala retrovirus) in the Sandy Point koala population. The genetic information obtained may be used to inform potential conservation strategies for the koala population at Sandy Point. The main aims of this preliminary study on the koala population at Sandy Point were to determine:

- The prevalence of *Chlamydia pecorum* and koala retrovirus subtype A (KoRV-A)
- Levels of genetic diversity and
- Whether the population is part of the larger South Gippsland koala population

2. Methods

2.1 Study site

The Sandy Point study site consists of five ecological vegetation classes (EVCs): Coastal dune scrub/coastal grassland mosaic, mangrove shrubland, estuarine wetland, swamp scrub and coastal saltmarsh (Appendix Figure A1). Vegetation at Sandy Point covers an area of approximately 300 hectares, though the proportion of the local vegetation that contains eucalypts is probably much less. Coastal manna gums (*Eucalyptus viminalis pyrioriana*) are common in and around Sandy Point and are likely the major feed source for koalas. A koala count was carried out in September 2017 by the Sandy Point community, where a total of 31 koalas were detected (Wright *et al.* 2018).

From Sandy Point, it is more than 7 km (north-west) to the next nearest substantial patch of eucalypt forest (Cape Liptrap Coastal Park) and almost 10 km (north) to the next nearest patches of potential habitat on private land (south of Fish Creek). Little to no tree cover exists between these habitat patches, so migration of koalas to and from Sandy Point is predicted to be low.

Figure 1 Koala scat sampling locations collected in and around Sandy Point. Sample numbers correspond to sample identification (ScatID) provided in Appendix Table A1.

2.2 Sampling

Koala scat survey of the Sandy Point area was conducted between the 10th and 12th of March 2018. Scats were collected using wooden toothpicks as described in Wedrowicz *et al.* (2013). The GPS location of the collection site and whether the koala was present were recorded. A total of 22 samples were collected and sent to Federation University Australia for DNA isolation and genetic analysis. The locations of sample sites are illustrated in Figure 1 and listed in Table A1 of the appendix.

2.3 Genetic testing

DNA was isolated from koala scats and the quality of the isolates were determined following methods described in Wedrowicz *et al.* (2013). DNA isolates of sufficient quality were genotyped with a suite of twelve microsatellite markers: K2.1, K10.1, Pcv6.1, Pcv6.3, Pcv24.2, Pcv25.2, Pcv30, Pcv31 (Cristescu *et al.* 2009), Phc4, Phc13 (Houlden *et al.* 1996a), Phci2 and Phci10 (Ruiz-Rodriguez *et al.* 2014), using three or four replicates (depending on the quantity of DNA obtained; Wedrowicz *et al.* 2013). Sex specific markers were included to determine the gender of sampled individuals (Wedrowicz *et al.* 2018a). Individuals were also tested to assess infection with *Chlamydia pecorum* and KoRV-A using methods described in Wedrowicz *et al.* (2016).

2.4 Analysis of genetic data

To make comparisons between the Sandy Point koala population and other Victorian koala populations, Sandy Point genotype data were compared to data obtained from other Victorian populations, including the remnant koala population in South Gippsland (*n*=90) and populations founded from French Island (Cape Otway *n*=50) and Phillip Island (Raymond Island *n*=30) koalas. These population data were used to asses 1) genetic structure 2) genetic differentiation and 3) genetic diversity.

A range of genetic statistics that describe genetic diversity were also calculated using the *diveRsity* (Keenan *et al.* 2013) and *poppr* (Kamvar *et al.* 2014) packages in the R software environment (R Core Team 2014). These included the mean number of alleles detected per locus (A), allelic richness (A_R), the proportion of total alleles ($A_{\%}$), private alleles (P_A) and expected (H_E) and observed (H_O) heterozygosity (see front matter for a list of definitions).

To confirm genetic distinctiveness between different populations analysed, genetic structure was investigated using Discriminant Analysis of Principal Components (DAPC) in the *adegenet* package (Jombart 2008) with the genetic data classified by sampling location.

Genetic differentiation is a measure of how different populations are compared to one another. Values of genetic differentiation range from zero to one, with a value of zero indicating no genetic difference between populations and increasing values indicating greater amounts of genetic difference between populations. The R package *diveRsity* (Keenan *et al.* 2013) was used to calculate estimates of genetic differentiation.

3. Results

3.1 Sampling success

Koala scats collected by the Sandy Point Community Koala Action Group were of high quality, with 21/22 (95%) of samples submitted passing quality control. Genetic testing was also high, with 20/21 (95%) samples producing reliable DNA profiles (Table 1).

3.2 Individuals sampled

From the 20 DNA profiles generated, genetic analysis indicated that 11 individuals had been sampled (Table 1; Fig 2), where one individual had been sampled seven times, whilst three others had been sampled twice (Table 1). Molecular sexing was successful for 9/11 samples, revealing that six females and three males had been sampled (Table 1).

3.3 *Chlamydia pecorum* and KoRV

Chlamydia pecorum was not detected in any of the eleven identified individuals (Table 1). Koala retrovirus (KoRV-A) was detected in four (three females and one male) out of eleven sampled koalas (36%; Table 1).

Table 1: Summary of genetic results koalas sampled at Sandy Point, Victoria, including the number of loci successfully genotyped, each individuals gender and whether *Chlamydia pecroum* and KoRV-A were detected. Genotypes matching at all scored loci are assumed to be the same individual. Unique individuals sampled are listed in the Individual ID column. NA: not applicable, QC: quality control.

Scat ID	Individual ID	Loci scored	KoRV-A	*Chlamydia pecorum*	Sex
SP001	SP-IND-01	11	Detected	Not detected	Female
SP002	SP-IND-01	11	Detected	Not detected	Female
SP003	SP-IND-01	11	Detected	Not detected	Female
SP004	SP-IND-01	12	Detected	Not detected	Female
SP005	SP-IND-01	12	Detected	Not detected	Female
SP006	SP-IND-01	12	Detected	Not detected	Female
SP011	SP-IND-01	12	Detected	Not detected	Female
SP007	SP-IND-02	12	Not detected	Not detected	Male
SP008	SP-IND-03	12	Detected	Not detected	Male
SP010	SP-IND-03	12	Detected	Not detected	Male
SP009	SP-IND-04	12	Detected	Not detected	Female
SP013	SP-IND-05	11	Not detected	Not detected	Male
SP015	SP-IND-06	12	Not detected	Not detected	Fail
SP016	SP-IND-07	12	Detected	Not detected	Female
SP017	SP-IND-07	12	Detected	Not detected	Female
SP018	SP-IND-08	11	Not detected	Not detected	Fail
SP019	SP-IND-09	12	Not detected	Not detected	Female
SP022	SP-IND-09	12	Not detected	Not detected	Female
SP020	SP-IND-10	12	Not detected	Not detected	Female
SP021	SP-IND-11	12	Not detected	Not detected	Female
SP014	Failed	0	Fail	Fail	Fail
SP012	Failed QC	NA	Fail	Fail	Fail

Figure 2 Locations of unique individuals identified from the sampled Sandy Point koala population. Individual koala labels (koala ID) correspond to those provided in Table 1. Circles denote females and squares males.

3.4 Genetic diversity

Genetic diversity in the Sandy Point koala population was found to be quite low (Table 2). The eleven individuals sampled had on average only 1.7 different alleles per microsatellite locus (A; Table 2). This is in contrast to averages of 3.3 alleles in the Cape Otway (French Island descendants) population, 3.3 alleles per locus in the Raymond Island (Phillip Island descendants) population and 6.5 alleles per locus in the greater South Gippsland koala population (Table 2). The mean number of alleles per locus can, however, be affected by differences in sample sizes (as greater sample sizes provide a greater likelihood of sampling more alleles).

Table 2 also shows estimates of allelic richness (A_R), which is the mean number of alleles (A) corrected for differences in sample size. Allelic richness was significantly lower in the Sandy Point koala (A_R: 1.7) population compared to Cape Otway (A_R: 2.8), Raymond Island (A_R: 2.9) and greater South Gippsland (A_R: 4.0).

Private alleles are alleles that are unique to a single population group. Sandy Point had no private alleles which means that all the alleles present in the Sandy Point population are also present in the South Gippsland koala population. Conversely, the greater South Gippsland population was found to have 113 alleles that were not found in the Sandy Point, Raymond Island or Cape Otway koala populations.

These results are also reflected in the proportion of total alleles ($A_{\%}$) which is the percentage of alleles detected in a single population from the alleles detected in all populations (Table 2). 98% of the alleles found in all four populations are found in South Gippsland koalas, while only 29% of the total alleles are found in the Sandy Point koala population. Results for $A_{\%}$ can, however, also be influenced by differences in sample sizes between groups.

Heterozygosity measures the frequency of loci where two different alleles are present. Greater heterozygosity is often associated with greater fitness (Frankham *et al.* 2012). In comparison to other Victorian koala populations, heterozygosity in the Sandy Point koala population is critically low at 0.17. This means that it is more common for Sandy Point individuals to have two copies of the same allele at a particular locus (Appendix Table A3).

Table 2 Genetic statistics for the Sandy Point koala population compared to the South Gippsland, Cape Otway (French Island descendants) and Raymond Island (Phillip Island descendants) populations.

	Sandy Point	South Gippsland	Raymond Island	Cape Otway
N Number of individuals sampled	11	90	50	30
A Mean number of alleles detected per locus	1.7	6.5	3.3	3.3
A_R Allelic richness (95 % confidence interval)	1.7 (1.5 - 1.7)	4.0 (3.5 - 4.5)	2.9 (2.6 - 3.2)	2.8 (2.5 - 3.1)
$A_{\%}$ Proportion of total alleles	29	98	56	56
H_E Expected heterozygosity	0.18	0.59	0.50	0.45
H_O Observed heterozygosity	0.17	0.58	0.50	0.45
P_A Private alleles	0	113	2	0

3.5 Genetic structure and differentiation

Discriminant Analysis of Principal Components (DAPC) was used to visualise genetic structure in the Sandy Point koala population in comparison to reference populations. Genetic structure of Sandy Point koalas compared to reference populations is illustrated by the DAPC plot in Fig. 3. Each point in Fig. 3 represents the genotype of an individual koala. Points are distributed according to how genetically similar or different the sampled individuals are. Individuals sampled at Sandy Point are shown in red and can be seen to be separate from the two island derived populations, but to overlap with the South Gippsland koala population. This suggests that the Sandy Point koala population is (or was historically) part of the larger South Gippsland koala population. If translocations of island koalas to the area have occurred in the past, these have not largely contributed to the local gene pool.

The genetic difference between the Sandy Point koala population and the Cape Otway (French Island descendants) and Raymond Island (Phillip Island descendants) populations is further supported by the estimated levels of genetic differentiation between them (Table 3). Genetic differentiation can be estimated using a statistic called F_{ST}. Lower values of F_{ST} indicate a closer genetic relationship while higher values indicate that the relationship

between groups is more distant. Table 3 shows estimates of genetic differentiation between the Sandy Point koala population and the included reference populations. These results show that Sandy Point is significantly differentiated from the Cape Otway and Raymond Island populations but not significantly differentiated from the South Gippsland koala population. The Sandy Point population was most different to the Cape Otway population (F_{ST}=0.09), followed by the Raymond Island population (F_{ST}=0.07) and least different to the greater South Gippsland koala population (F_{ST}=0.04, though this value was not significantly greater than zero).

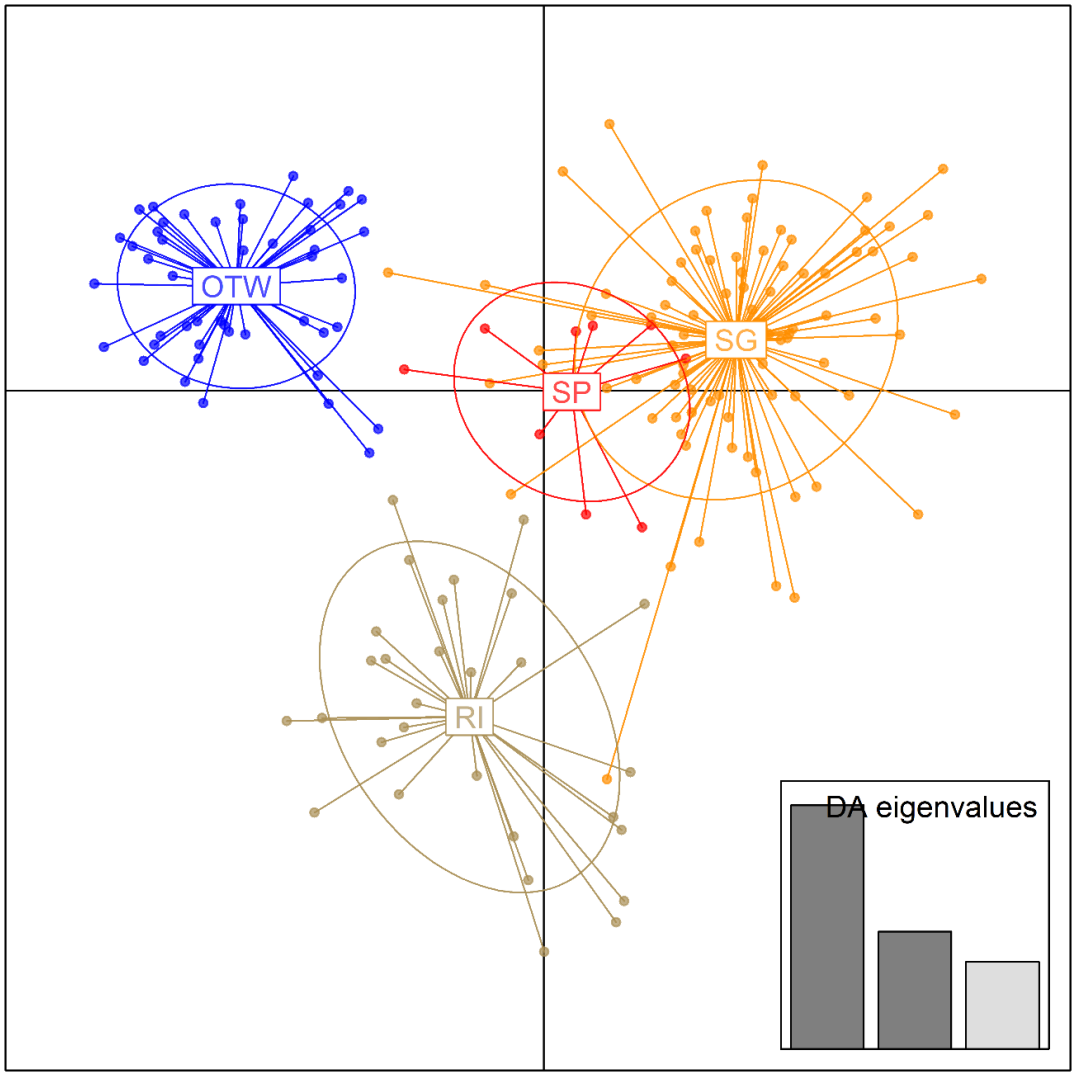

Figure 3 Genetic structure of the Sandy Point koala population in comparison to the South Gippsland, Cape Otway and Raymond Island koala populations (SP: Sandy Point, SG: South Gippsland, RI: Raymond Island, OTW: Cape Otway)

Table 3 Genetic differentiation (F_{ST}) between the Sandy Point population and other Victorian koala populations. Significant differences between populations are shown in bold and marked with an asterisk.

	Cape Otway	Raymond Island	South Gippsland
Raymond Island	**0.072***		
South Gippsland	**0.098***	**0.046***	
Sandy Point	**0.088***	**0.067***	0.049

4. Discussion and recommendations

4.1 Marker resolution for individual identification

Eleven individuals were identified from 20 koala scat samples collected. Low diversity can, however, make it difficult to confidently identify individuals as the probability that two individuals will share the same twelve marker genotype may be insufficiently low. In the greater South Gippsland koala population, the chance that two individuals will share the same 12 marker genotype by chance (probability of identity, P_{ID}) is more than 1 in 200,000,000 while the probability that siblings will share the same genotype (probability of identity between siblings, P_{IDsibs}) is around 1 in 4,000. In contrast, the probability that two Sandy Point koalas will share the same genotype was calculated to be 1 in 470 (for twelve successfully genotyped loci) and 1 in 150 (for eleven successfully genotyped loci). Where two individuals are siblings the probability is even lower, 1 in 20 (for twelve successfully genotyped loci) and 1 in 12 (for eleven successfully genotyped loci). There is therefore a chance that two different individuals within the Sandy Point sample set could share the same genotype and be identified as the same individual. Further analysis using a greater number of markers would be needed to confirm that all matching genotypes putatively assigned to an individual do represent the same individual rather than different individuals with the same genotype.

4.2 Presence of *Chlamydia pecorum* and KoRV

Chalmydia pecorum is a bacterial infection afflicting some koala populations that may result in disease of the urogenital tract and decreased reproductive output in females (Obendorf & Handasyde 1990; Martin & Handasyde 1999). *Chlamydia pecorum* was not detected in any of the eleven individuals sampled for this study. Given the small sample size further sampling may be required to confirm this. If the Sandy Point population is found to be free of this pathogen, its appearance may affect the population greatly, since animals previously not exposed to *Chlamydia pecorum* may be more susceptible to severe infections (Martin & Handasyde 1999).

Subtype A of the koala retrovirus (KoRV-A) was detected in 4/11 (36%) of the Sandy Point individuals sampled. The impacts of KoRV on koala health are unclear, although there does appear to be a greater tendency for sick and injured koalas entering shelters to be infected with KoRV-A (Wedrowicz *et al.* 2018b). Future testing for *Chlamdyia pecorum* and KoRV in the Sandy Point koala population would be useful to identify changes in the prevalence of these infections which may put this population at risk of decline.

4.3 Genetic diversity

The Sandy Point koala population has significantly lower levels of genetic diversity (on average 1.7 alleles per locus) compared to other Victorian populations included in this report. It was also more common for individuals in the Sandy Point koala population to have two copies of the same allele at any given locus (high levels of homozygosity / low levels of heterozygosity). A lack of past genetic data for Sandy Point koalas makes it difficult to determine whether the genetic diversity in the population is currently increasing from lower levels having occurred in the past or decreasing from higher past levels of diversity.

The Sandy Point koala population is likely a small isolated fragment of the greater South Gippsland population (see section 4.3). Genetic diversity is easily lost from small isolated populations. Given the lack of tree cover (and koala habitat) connecting Sandy Point to other local areas of koala habitat it is unlikely that gene flow into or out of the Sandy Point population occurs at a substantial level.

Increasing the genetic diversity in the Sandy Point koala population may be important for the continued survival of the population. Increasing the amount of koala habitat in and around

Sandy Point would be an ideal first step towards increasing diversity in the population. This is because populations that are larger in size are less susceptible to loss of genetic diversity than small populations. The development of habitat corridors between Sandy Point and other patches of koala habitat in South Gippsland may also help to encourage natural movement and gene flow into and out of Sandy Point. This would be likely to have a positive effect on genetic diversity in the Sandy Point koala population. Potential negative effects would also need to be considered, such as the unintended introduction of pathogens not currently present in the local resident population (e.g. *Chlamydia pecorum*, but this requires confirmation / further work).

Another way to increase genetic diversity in the Sandy Point population might be the periodic assisted migration of individuals to the area, however, more research would be required to ascertain the feasibility of such a solution. A thorough assessment of potential risks would also need to be conducted. For example, introduced individuals may have little chance of establishing themselves in the area if most habitat is taken up by resident koalas (i.e. habitat may already be at or exceed carrying capacity). It would need to be determined whether the amount of presently available habitat in Sandy Point is sufficient for some population growth, which if not, may result in further problems such as over-browsing of feed trees (which can, in turn, lead to population decline). The introduction of pathogens from outside the Sandy Point area could also drive the extinction of koalas at Sandy Point if a majority of individuals were susceptible to the pathogen. There is also a risk that introduced individuals could contract pathogens from the resident population to which they are susceptible, resulting in a decreased chance that they will successfully contribute new genes to the resident population.

4.4 Population origins

The Sandy Point koala population is more closely related to the greater South Gippsland koala population than to populations derived from French and Phillip Islands. This suggests that the ancestors of the koala population at Sandy Point were part of the greater South Gippsland koala population. This does not rule out the possibility that some koalas were brought from neighbouring areas (e.g. Snake Island, Walkerville and Waratah Bay; Wright *et al.* 2018). Individuals potentially brought from Walkerville and Waratah Bay were likely part of the South Gippsland koala population (rather than descending from island koalas) so their introduction is not likely to have had a large effect on the genetic makeup of the Sandy Point

koala population (assuming that the Sandy Point population was originally part of the South Gippsland koala population).

The introduction of small numbers of individuals from Snake Island (population established by French and Phillip Island koalas) may have had little effect on the population if these introduced individuals did not successfully breed and contribute to the larger population at Sandy Point. Similarly, if there were only a small number of individuals translocated from Snake Island (and there was a much larger number of resident individuals of South Gippsland origin), then the effect of alleles from Snake Island would be very small (the alleles would be 'diluted' amongst the alleles from the larger population). The results of this work show that the Sandy Point koala population is (or was historically) most likely part of the greater South Gippsland population. Given the low level of genetic diversity detected and the populations current isolation due to a lack of tree cover and habitat linking Sandy Point to other regions, it seems unlikely that gene flow into the area would be currently occurring. The isolation of this small population puts it at an increased risk of reductions in genetic diversity, population decline and potentially extinction.

5. Conclusion

Resampling of the Sandy Point koala population over time would be useful to get an idea of whether genetic diversity is decreasing or increasing. The use of additional methods to ensure that individuals can be confidently identified despite low levels of diversity will be an important consideration for future work. Changes in the presence of *Chlamydia pecorum* and KoRV could also be monitored. This would allow for potential strategies to be devised as soon as potential problems are detected.

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Appendix

Table A1 Koala scat samples collected for DNA analysis, their location and whether the koala was present.

Scat ID	Easting	Northing	Location	Koala present
1	425461	5701306	Site E	NO
2	425465	5701362	Site E	NO
3	425513	5701297		NO
4	425680	5701156	Zone K	NO
5	425680	5701156	Zone K	NO
6	NA	NA		NO
7	**426334**	**5700499**	**Inlet roundabout**	**YES**
8	426331	5700485	Inlet roundabout	NO
9	**426253**	**5700571**	**Roy Henderson Track Zone L**	**YES**
10	426259	5700532	Roy Henderson Track	NO
11	425316	5701314	Area C	**NO**
12	424929	5701220	Tip Track plantation	NO
13	**423948**	**5702185**	**Gyndanook**	**YES**
14	**423948**	**5702185**	**Gyndanook**	**YES**
15	424841	5702488	Ennisvale	NO
16	424537	5702273	Ennisvale	NO
17	**424537**	**5702273**	**Ennisvale**	**YES**
18	424844	5702490	Ennisvale	NO
19	**424709**	**5702280**	**Ennisvale**	**YES**
20	**424615**	**5702279**	**Ennisvale**	**YES**
21	**Est**	**Est**	**Ennisvale**	**YES**
22	**Est**	**Est**	**Ennisvale**	**YES**

Table A2 shows the frequency at which different alleles were detected in the Sandy Point koalas sampled. Three loci were fixed for a single allele – Pcv30, Pcv31 and Phc4. Five loci consisted of only two alleles (K10.1, K2.1, Pcv25.2, Pcv6.3 and Phc13), with one of the two alleles dominating in the population (the frequency of one of the alleles being greater than 80%). Sandy Point koalas sampled for this study also had two alleles at locus Pcv6.1 but in approximately equal proportions. Two loci were found to have three alleles (Pcv24.2 and Phci2) and one allele (211) tended to dominate at locus Pcv24.2. Locus Phci10 had four alleles, with alleles 201 and 209 base pairs in length the most common.

Table A2 Allele frequencies by locus for the Sandy Point population

Locus name	Allele size	Allele frequency (%)
K10.1	130	95
	132	5
K2.1	164	14
	172	86
Pcv24.2	211	82
	213	9
	217	9
Pcv25.2	170	9
	178	91
Pcv30	204	100
Pcv31	230	100
Pcv6.1	217	59
	233	41
Pcv6.3	297	17
	303	83
Phc13	113	86
	127	14
Phc4	111	100
Phci10	201	36
	205	5
	209	55
	213	5
Phci2	147	68
	159	23
	165	9

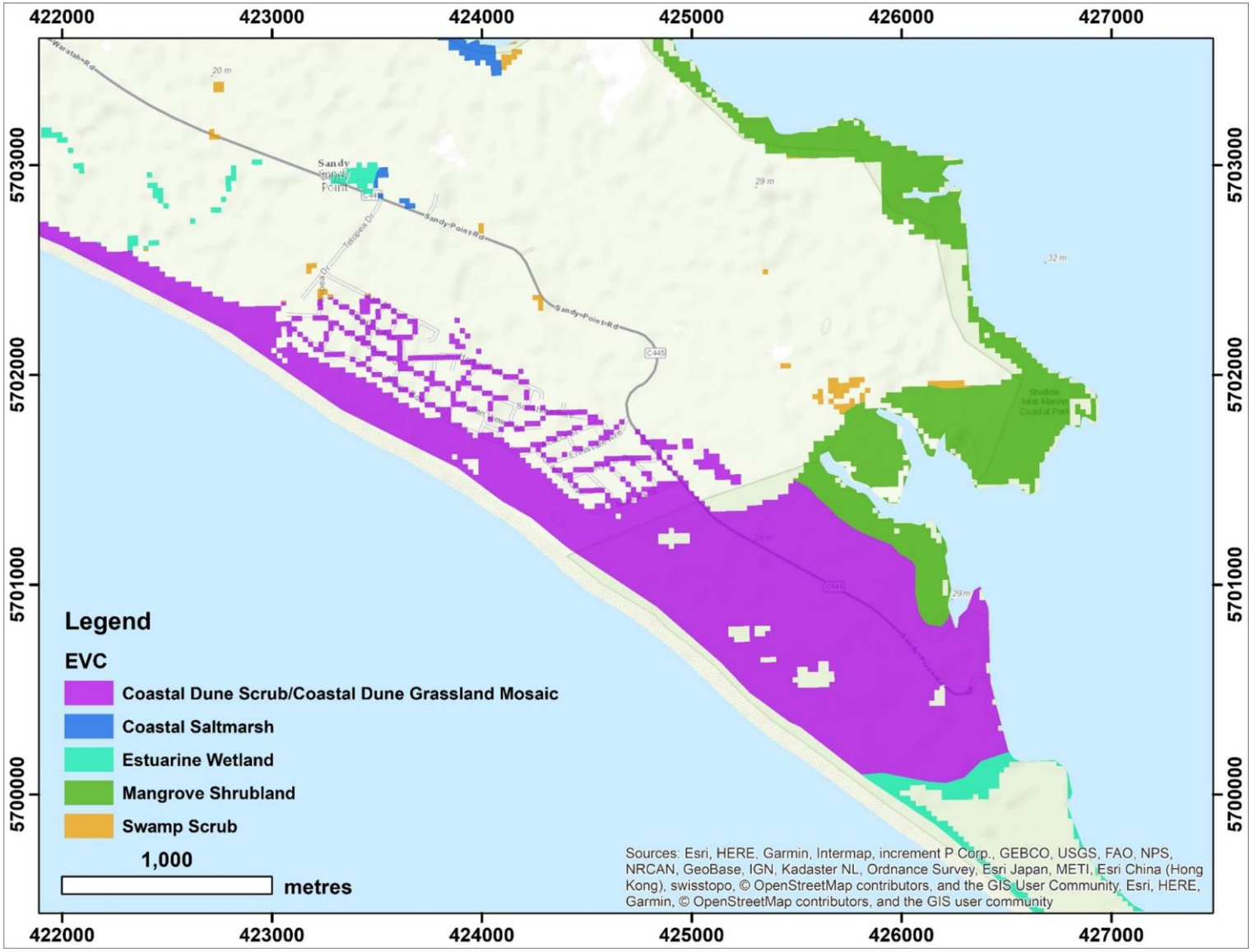

Figure A1 Map of the Sandy Point Township and surrounds showing Ecological Vegetation Classes (EVCs) of the vegetation present.

Table A3 Count of individuals with certain numbers of homozygous loci. Values indicate the number of individuals that are homozygous for particular numbers of loci.

Population	Number of homozygous loci[1]										
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Sandy Point[2]							1	3	3	1	2
South Gippsland	1	4	8	17	27	15	12	4	2		
Raymond Island				4	10	5	7	3	1		
Cape Otway			2	3	8	9	15	11	2		

[1] Only ten loci that were common between all four populations were used here for this analysis (K2.1, K10.1, Pcv6.1, Pcv6.3, Pcv24.2, Pcv25.2, Pcv30, Pcv31, Phc4, Phc13)

[2] One extra Sandy Point individual sampled in 2015 was included in this summary

Compared to reference populations, Sandy Point individuals tended to have a greater level of homozygosity (having two copies of the same allele at a locus rather than two different allele versions). Of the ten Sandy Point individuals included in this analysis, two were homozygous at all ten loci, one was homozygous at 9/10 loci, three at 8/10 loci, three at 7/10 and one was homozygous at 6/10 loci.

In the South Gippsland and Raymond Island populations, most individuals were homozygous at 4/10 loci (and heterozygous at 6/10 loci), while in the Cape Otway population most individuals were homozygous at 6/10 loci. In contrast, Sandy Point individuals had the highest amount of homozygosity, with most individuals homozygous at seven to eight loci.