16. Conservation genetics of Malleefowl

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Abstract

PART 1: Phylogeography and Population Genetics
Malleefowl present an interesting case in terms of their genetic variation and distribution across Australia. We investigated mitochondrial and microsatellite population structure across Australia and found relatively deep divergence between eastern and western Australia (split by the Flinders Ranges). However, further analysis suggests that both historical and contemporary factors are still influencing Malleefowl phylogeography and population structure. The full results of our study will be presented here with implications for national genetic management of this species.

PART 2: Landscape Genetics of Malleefowl
If analysis of this study is complete (due in September), I will present the findings of the effects of various land changes and land forms on gene flow and differentiation between fragmented blocks of mallee in north-western Victoria. This study is particularly relevant to management of Malleefowl on a local as well as national scale, as the factors affecting isolation of mallee populations are likely to be similar across Australia.

PART 3: Mating Systems and Relatedness of mound siblings
We present evidence to show that Malleefowl are not monogamous as previously suspected. Our study involved using new methods of sampling Megapode DNA for paternity analysis, which could be used in future studies of individual Malleefowl.

Introduction

Malleefowl have suffered from massive habitat clearance. In parts of Australia large tracts of mallee have been cleared, leading to small isolated patches. The populations of Malleefowl that inhabit these areas could be facing a large range of threats, including the effects of inbreeding and limited genetic variation. The aim of this research is to understand how genetic variation is distributed among populations of Malleefowl across Australia. In order to achieve this population level structure, gene flow and mating systems in this species were investigated. These components are discussed in the following chapters:

1. Phylogeography and Population Genetics
2. Landscape Genetics
3. Mating Systems and Relatedness between mounds.

PART 1: Phylogeography and Population Genetics

1.1 Methods

Mitochondrial ND2 gene as well as 13 nuclear microsatellite markers were studied in 117 individuals across Australia (shown in Figure 1).

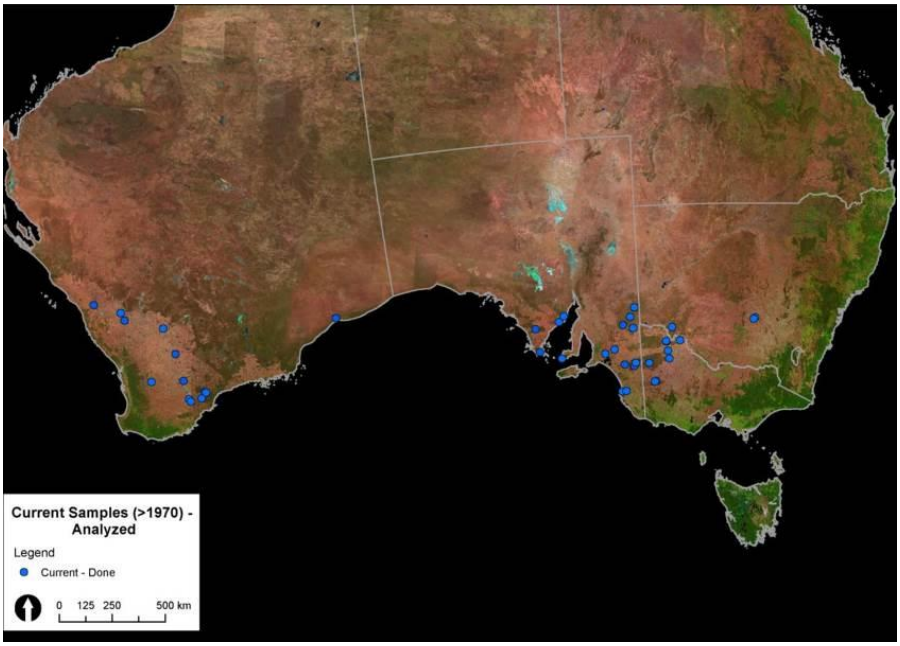

Figure 1. Sample locations of the 117 individuals used in final analysis.

1.2 Results

1.2.1 High diversity, low differentiation between populations

High levels of diversity, but low levels of differentiation between mitochondrial haplotypes were found in Malleefowl. There is no evidence of sub-species in Malleefowl across Australia. However, there is a reasonably strong split between populations of eastern and western Australia, with the Flinders Ranges (Eyrean barrier) acting as a geographical barrier between these sub-populations (as illustrated in Figure 2).

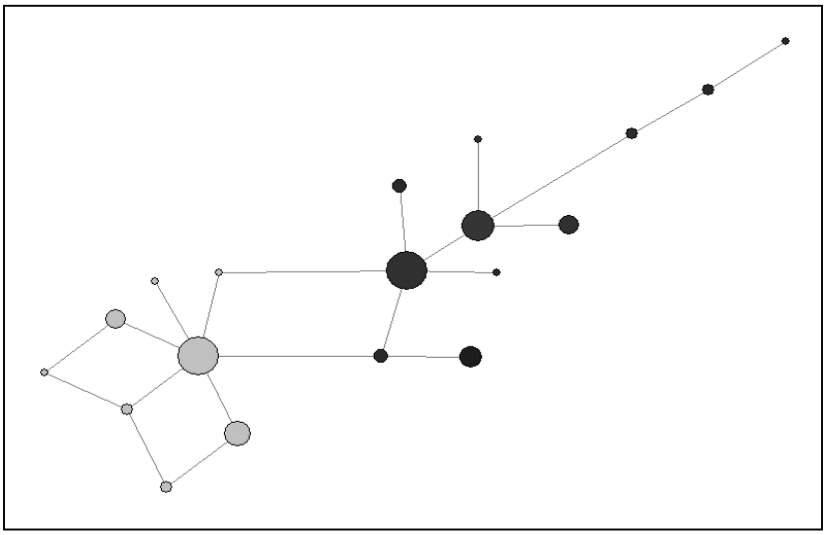

Figure 2. Mitochondrial haplotypes (variations) in Malleefowl populations across Australia. Light grey circles indicate western Australia and black circles indicate eastern Australia (split by the Flinders Ranges). The size of each circle is an indication of the number of individuals with that haplotype. Links indicate a single mutation between haplotypes.

Implications:

1. No sub-species of Malleefowl.
2. Separate populations in eastern and western Australia. Management applications of this species should reflect this.

1.2.2 Past range contractions and expansions

Secondly, Malleefowl appear to have been through population contraction and expansion but in a very ancient context. Looking at their ancestral population size, this was larger than eastern or western populations are today. Results of statistical tests undertaken during analysis are outlined in Table 1; while the results of the population size probability analysis is shown in Figure 3.

Table 1. Results of various statistical tests undertaken during analysis. Ticks indicate significant values as evidence in supporting either the constant population size or the range expansion hypothesis. Crosses indicate no significant result.

Test	Constant Population Size	Range Expansion
Raggedness	x	✓
Fu&Li's D	x	✓
Fu&Li's F	x	✓
Fu's Fstat	x	✓
Tajima's D	x	x

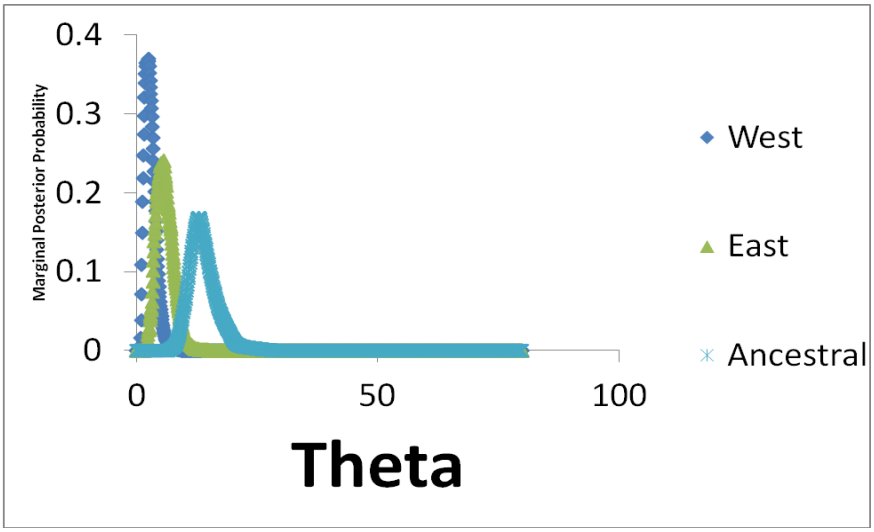

Figure 3. Results of Isolation with Migration analysis (IMa) showing the probability of population sizes for eastern, western and a hypothetical single ancestral population.

All of these tests showed a similar result, and indicated that Malleefowl have gone through gross expansions as well as declines.

The most likely time frame for these changes is during the peak of the last ice age (approximately 20,000 years ago). Instead of being covered in ice, Australia was covered in rolling sand dunes, devoid of vegetation. There were two possible refuges during that time: south-west of Western Australia, and the South Australian-Victorian area.

In attempting to answer the question of which refuge the Malleefowl were confined to, research that has been undertaken on 1080 resistance in Australian birds was investigated. This research looked at a number of our native birds resistant to 1080 and found that Malleefowl have very high tolerance to 1080 (King *et al*. 1996). Furthermore, Gastrolobium, the family of plants that produce the active ingredient (and key toxin) of 1080 (Monofluoroacetate), has high endemism in south-west Western Australia, with all but two of the 62 known plants in this family confined to south-west Western Australia (Twigg *et al.* 1996).

If Malleefowl were primarily restricted to the South Australian-Victorian area and then spread west, once the sand dunes retreated you'd expect high mortality of the birds from the east when they come in contact with the plant as they had not yet developed a resistance. However what was found was that all birds across Australia have the same resistance to 1080, indicating that Malleefowl were once confined south-west Western Australia refuge for a significant period of time, enough to develop a resistance to the toxins produced by these plant species, and spread east.

1.2.3 Isolation by distance

Another key result was that Malleefowl populations have an isolation-by-distance structure. Meaning that individuals that are geographically closer end up being genetically more similar, as neighbouring populations are more likely to interbreed.

Implications:
1. Management of current populations should concentrate on corridors between habitat fragments.
2. Translocations should be undertaken on a local scale with neighbouring populations.

1.2.4 Mitochondrial vs. Microsatellite (nuclear) markers

Different patterns in mitochondrial and nuclear DNA of Malleefowl were observed. The mitochondrial data indicates a deep split between eastern and western Australia. However, the microsatellite markers show no evidence of population structure.

There could be several reasons for this difference, including a) male biased gene flow; b) differences in introgression rates between nuclear and mitochondrial markers; or c) insufficient time since isolation between eastern and western populations to show evidence in the microsatellites.

Our analysis of isolation-with-migration suggests that there is no migration from eastern Australian to western Australia. However, there is evidence of some very low, but significant, amounts of migration from western Australia to eastern Australia. Unfortunately the software doesn't tell us at what point this migration happened, so it is not clear whether migration occurred two generations ago or 20,000 years ago.

The majority of Australian species that are separated or impacted by the Eyrean barrier show an eastern expansion (moved from west) (Schodde 1982). As discussed in Section 1.2.2, there is additional support for this in the resistance of many Australian species to 1080 (King *et al.* 1996, Twigg *et al.* 2003).

1.3 Conclusions

The isolation by distance analysis indicated that translocations should ideally be undertaken between geographically close populations, i.e. from NSW to NSW populations rather than from WA to NSW. Maintaining corridors between habitat fragments are also very important, as this allows birds to come in contact with their closest relatives.

With evidence of past population collapse (on a massive scale) and low corridor movement, Malleefowl populations have changed significantly over time (from 100s to 1,000s back to 100s). Ensuring Malleefowl have the habitat to allow them to spatially expand will therefore be important to ensure the long term persistence of the species.

PART 2: Landscape Genetics of Malleefowl

One of the main objectives of the Malleefowl Recovery Plan was to undertake genetic investigation of populations (Benshemesh 2000) so that management decisions can be made. Populations of Malleefowl have been subjected to extensive land clearance leading to fragmentation and isolation of a once continuous population. The severity of the impact of this fragmentation and isolation has only limited understanding, but Malleefowl are known to be reluctant fliers and do not disperse readily across open country (Frith 1962; Benshemesh 2000). Understanding if and how Malleefowl move between remnant patches of mallee will be important in aiding management decisions, especially relating to the need for habitat corridors between remnants.

2.1 Methods

The landscape genetics component of this study concentrates on the factors that influence gene flow between isolated fragments in south-east South Australia / north-west Victoria. Microsatellite markers were used to determine whether any environmental factors (e.g. patch size, distance between patches, time since last burn, corridor type and quantity) are influencing population structure.

2.2 Results

Preliminary analysis of landscape genetics has been undertaken, however more analysis is needed to provide any degree of certainty in the results. Initially, there has been no evidence of measured environmental factors influencing the genetic structure of Malleefowl. There also appears a pattern of isolation by distance, whereby geographically close reserves are also genetically similar. However the results of this preliminary analysis should be interpreted with caution, as the analysis is not yet complete.

PART 3: Mating Systems and Relatedness of mound siblings

The understanding of genetic variation within a population, as well as the variation in genetic contribution of individuals to future generations, is essential for conservation and management of species (Quader 2005). Biased reproductive success can limit populations by reducing genetic variation (Lacy 1987). Malleefowl have been noted as generally monogamous, although polygamy has been recorded (Weathers *et al.* 1988). In most bird species the social mating system is often a poor reflection of genetic parentage (Birkhead & Moller 1996).

3.1 Methods

The aim of this paper was to analyse paternity in Malleefowl mounds. Mounds at Wandown (Victoria) were sampled, as well as mounds throughout the riverlands in South Australia in collaboration with the Department of Environment and Natural Resources, South Australia (DENR). A permit was required in order to take eggs from mounds, but was restricted to no more than 20% of the eggs from a nest, on average 6 eggs, and a proportion of mounds within each reserve had to be left undisturbed. Each mound is monitored for activity by the national monitoring programme. During excavation, each egg was numbered and a map drawn indicating the location of each numbered egg (shown in Figure 4). All eggs were visually inspected in the field to determine the age of the embryo, using a candling technique adapted from Jessica van der Waag. Eggs were placed into “stubby holders” and incubated in Brinsea

incubators at specific temperature and humidity requirements (shown in Figure 5). Once the chick hatched, a blood sample was taken along with the egg membrane for use in genetic analysis. After drying out for a minimum of 6 hours, each chick was released at their natal mound.

Figure 4. Carefully numbering each egg uncovered in a Malleefowl mound.

Figure 5. Left – specially adapted Brinsea incubators. Right – eggs in stubby holders with a chick hatching in the centre.

Attempts to catch adult birds were unsuccessful. Subsequently non-invasive genetic sampling was undertaken. The males spend the majority of their time tending the mound, and fight aggressively with any intruder that comes near their mound. It is therefore highly likely that feathers on the mound belong to that male. All feathers on a mound were collected over two days. The feathers collected on the second day (fresh feathers <1 days old) were primarily used to extract DNA.

Molecular sexing techniques (on large, medium and small sized feathers, as shown in Figure 6) were undertaken to determine which feathers resulted in the best quality DNA, as well as whether the feather was from a male or female from that mound. A large difference was observed in the quality of the DNA extracted from small to medium sized feathers compared to large feathers, as they were shed more frequently, and feathers known to be <1 day old consistently produced the best quality DNA.

Figure 6. Feathers found next to a Malleefowl mound. Left – large sized feather. Right – medium sized feather.

The collected feathers, blood from the chicks hatched in the incubators and the chorioallantoic membrane (as shown in Figure 7) collected from the mounds were analysed. A total of 13 microsatellite markers were used for analysis of paternity and each feather sample analysis was repeated at least three times to ensure accuracy of results.

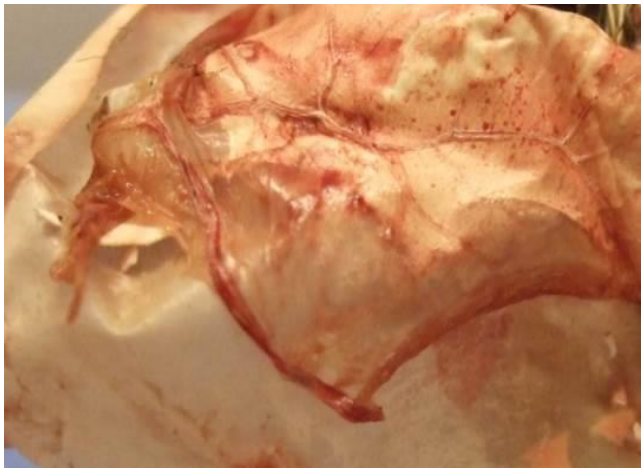

Figure 7. Chorioallantoic membrane (inner membrane) containing fresh DNA from the chick.

3.2 Results

Evidence of monogamy was found in the majority of mounds sampled. In a "monogamous" Malleefowl mound, all sampled eggs belonged to the male and female that regularly tended that mound. There was evidence of extra-pair paternity in a smaller proportion of mounds sampled (ranging from two to four different sires) and evidence of egg dumping (where an offspring failed to match with the female feather collected at the mound) in two mounds. An example of a sampled Malleefowl mound is shown in Figure 8.

These results should be interpreted with caution as not all offspring within a mound were able to be sampled due to permit restrictions. A “monogamous” finding in this case is a conservative label as any of the unsampled chicks could potentially have different parentage.

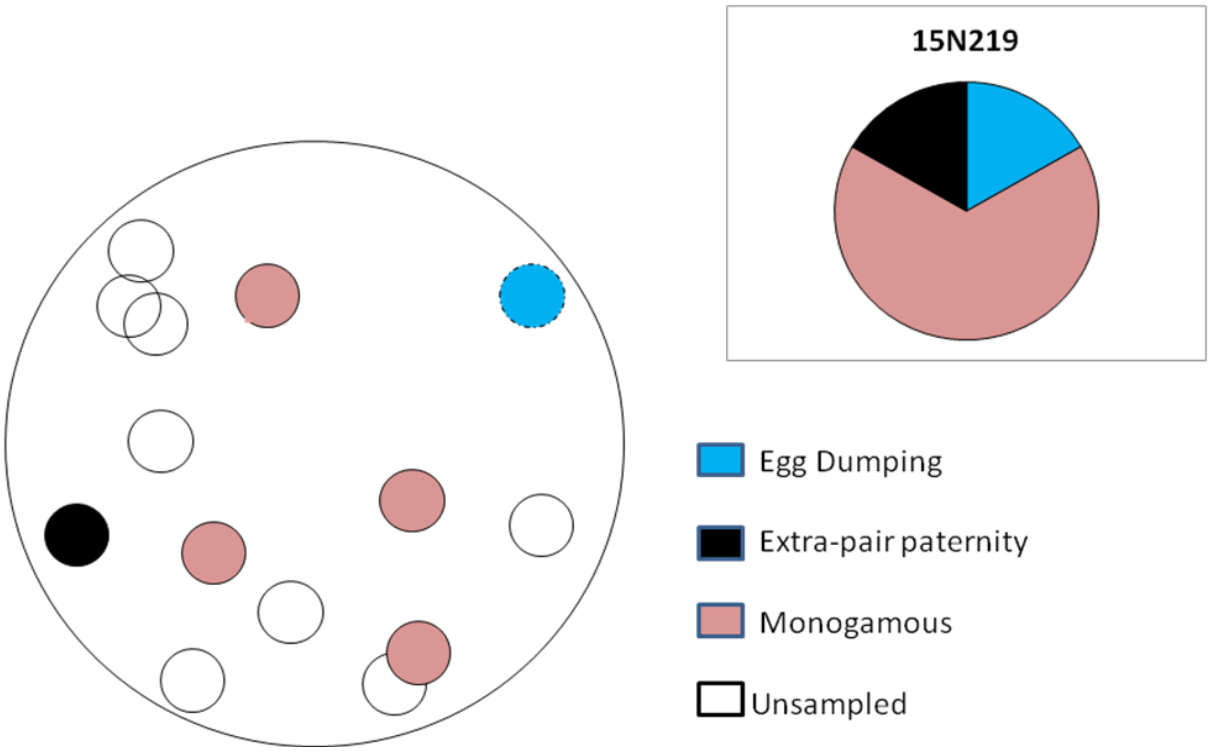

Figure 8. An example of a sampled Malleefowl mound. Each small circle represents an egg as found during excavation. In this particular mound, four of the sampled eggs belonged to both the male and female that tended the mound (eggs shown as pink). One offspring did not match the mound-tending male (egg shown as black) and another offspring did not match the mound-tending female (egg dumping - shown as blue).

The results of this study suggest that Malleefowl are not genetically monogamous, which is the norm in paternity studies of birds (Birkhead & Moller 1996).

Implications:

1. Non-invasive sampling is a successful method of sampling Malleefowl.
2. Small, fresh feathers contribute the most useable DNA out of the feather samples.
3. Offspring can be sampled by digging up freshly hatched membrane from Malleefowl mounds.
4. Our study is the first to undertake paternity analysis in Malleefowl and suggests that captive breeding programmes should consider the need for extra pair parentage to increase the genetic diversity of populations, or at the very least to simulate natural behaviours as found in the wild.

4.0 Future Studies

In the near future it will be possible to analyse the entire genomes of individuals at a reasonably affordable rate. This could open up explorations into adaptations of Malleefowl to local conditions, evidence of any immune system variations, as well as further defining the population structure and interactions between populations.

Tracking individuals (particularly chicks) over a long period of time would help to understand local movements and survival rates / recruitment within various reserves.

Understanding interactions between individuals could help to understand the social and genetic mating systems of this species in more detail. For example, which individuals are coming into contact and for how long? What are the implications of this for reproductive success of individuals?

There are a lot of questions to be investigated if the funding was available.

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