Reintroducing bettongs to the ACT: issues relating to genetic diversity and population dynamics

The guest speaker at NPA's November meeting was April Suen, holder of NPA's 2015 scholarship for honours students at ANU's Fenner School of Environment and Society. This article is an edited summary of April's presentation.

A global problem

The world is currently experiencing a biological diversity crisis with one in four mammals, one in eight birds and one in three amphibians at risk of extinction globally. Species have appeared and disappeared throughout history, however the rate of animal extinctions has accelerated dramatically in the past 500 years largely due to anthropogenic activities such as land clearing and the introduction of invasive species. As such, there have been increasing efforts to protect species that are at risk of extinction and to restore natural biodiversity.

Two such mechanisms used in species rescue are animal reintroductions and translocations. Reintroductions involve the movement of animals from an area back into a location the species used to inhabit, whereas translocations are broadly the movement of animals from one location to another. Historically translocations and reintroductions have been used for species such as leopards, black-footed ferrets and lynxes. However it has been well documented that these methods have been mostly ineffective in creating long-term stable populations with far more failed attempts than successful ones. A significant reason underlying this failure has been a lack of understanding of genetic methods and centrally, the establishment of long-term management plans to monitor the reintroduced populations. A clear understanding of genetics in species rescue is central to promoting long-term population persistence by maximising genetic diversity and minimising detrimental genetic effects such as inbreeding depression and genetic drift which stem from a disequilibrium of genetic heterozygosity (genetic variability within a population).

Reintroducing bettongs to the ACT from Tasmania

Between July 2011 and September 2012, a population of 60 wild Eastern Bettongs were collected from five locations across Tasmania and translocated to the ACT into Mulligans Flat Wildlife Sanctuary (MFWS) and Tidbinbilla Nature Reserve (TNR). The founding bettongs were collected using mixed-geographic source capture technique. Animals were purposefully collected from multiple geographic sources in an attempt to capitalise on the existing genetic diversity in the Eastern Bettong population and to promote long-term survivorship of the population. The Eastern Bettong translocation from Tasmania to the ACT provides an optimal case study to assess population sustainability and integrates genetic technologies to investigate the effectiveness of using the mixed-source reintroduction model to establish a population. It is important to note that at the time of my honours research, no genetic analyses had been performed on the reintroduced bettong populations. This is centrally what this honours project aimed to investigate.

MFWS is fenced against cats, dogs and foxes. MFWS is a box-gum grassy woodland habitat, encapsulating 485 hectares of bush land in north Gungahlin. The bettong population in MFWS is classified as 'wild' as all bettongs have free range of the enclosed area enabling free mate choice and spatial distribution within the sanctuary boundaries. In contrast, the bettongs in TNR are in fenced

enclosures with 1 – 12 bettongs per enclosure, restricting access bettongs have with the rest of the population. This is a 'non-wild' population, where matings are planned. As the TNR population is not wild, there was no scope to investigate spatial auto-correlation or mating patterns. For this reason, the research questions in this thesis were primarily targeted at the MFWS population.

Research methodology

The aim of this research was to assess whether the reintroduction of Eastern Bettongs using mixed-source founders was successful in creating a genetically diverse population in the ACT. This was carried out by investigating three research questions and tested against each respective hypothesis

1. How much genetic diversity exists within and among the Tasmanian source populations?

   Hypothesis No. 1: The Tasmanian source populations will be genetically distinct from each other and also show statistically significant genetic diversity among populations.

2. Does genetic variation correlate with geographic origin? Specifically, is the MFWS population mating randomly with respect to genetic diversity?

   Hypothesis No. 2: Post trans-location, mating will be random with regard to source populations.

3. Do genetically similar individuals cluster spatially?

   Hypothesis No. 3: Genetically similar individuals will not appear to cluster together spatially in MFWS.

Additionally, this thesis also assessed the appropriateness of using Next Generation Sequencing (NGS) technologies in a reintroduction biology context to answer these research questions. NGS is a general term to refer to molecular methods capable of extremely rapid DNA through-put sequencing at a low cost and are becoming increasingly widespread in their use. The primary advantage of NGS technologies over more traditional sequencing techniques such as microsatellites is the enormous amount of data generated. More data allows for a broader scope of research questions to be asked. However a significant consideration in using this technology is that equally powerful data filtering is required to make the data useful. As such a large volume of genetic data is generated for every NGS project, it is necessary to align sequence reads, call genotypes and filter useable data.

Prior to the commencement of this project, a bettong genomic database had been generated by ANU colleagues Sam Banks and Robyn Shaw in 2014. This was generated using a NGS technique called genotyping by sequencing and had 185 samples genotyped, which was every animal captured in MFWS and TNR up to August 2014. Additionally, animals from three areas in Tasmania where animals were not taken as founders were included in the analysis to provide an independent analysis of genetic diversity in Tasmanian populations. These animal samples were collected by Kirsten Proft in 2014 and were included in the GBS bettong genomic database.

Research process

The research process was broken down into five primary phases:

Trapping – Trapping events are ongoing as part of the long-term bettong management plan. These events have occurred seasonally, with ten trapping events having occurred since the commencement of the project in 2011 in MFWS. From 2016, future trappings will occur biannually. In a typical trapping event, 92 treadle activated cage traps with a bait ball (consisting of rolled oats, peanut butter and truffle oil) are placed in known locations throughout the sanctuary in the afternoon before the night of trapping session. Trap checking then begins at 2am during the following morning.

If a bettong was found in the trap, it was removed from the trap and handled in a soft polar fleece trapping bag before being examined for physical health checks. If the animal has not been caught previously (a 'clean skin'), a microchip was inserted under the skin and a 2mm circular tissue punch from the ear was also collected and stored in 70% ethanol as a source of DNA. Following from this procedure, all bettongs had head, tail width, left and right foot and weight measurements taken. Physical condition was also assessed and if the bettong was female, pouch young were also assessed before the bettong was released back into the sanctuary.

In my thesis a total of 258 bettong ear punch samples had been obtained.

Laboratory work – Bettong ear tissue punch samples were used as a source of DNA in this experiment. DNA was extracted using an ammonium acetate precipitation method. Only half of the ear punch tissue sample was used for each extraction. This was to ensure there was a sample backup for every animal if any extractions or sequencing runs failed.

Sequencing – GBS (the same technique used by Sam and Robyn to establish the bettong database) was attempted on two occasions in this project. Unfortunately both attempts were unsuccessful and it is hypothesised that the most likely reason for this was because of highly variable DNA quality and quantity in the bettong samples. Consequently a second NGS method was attempted. Genotyping in thousands was performed in this project. GT-SEQ was chosen as library preparation was feasible in the remaining time period of this research. GT-SEQ required minimal sample volume to be used (an enormous amount of DNA had been consumed through GBS), the reagents were easily obtainable and hundreds of samples could be sequenced simultaneously. This method was successfully performed once and samples were sequences at JCSMR, however upon sequencing it was discovered that the read quality was too low for most of the samples to infer meaningful information.

Data filtering – Consequently the original bettong database established by Robyn and Sam in 2014 was used in this project for all analyses. This database had been sequenced, however the data had not been filtered. Using GBS, it is essential to filter the data to a manageable size and to eliminate poor sequence reads. This process was carried out the bioinformatics pipeline TASSEL integrated with UNEAK, and then was further filtered with integrated R and Perl scripts.

Data analysis – Comparative genetics statistics, principal components analysis, AMOVA, spatial analysis, structure analysis were performed on the filtered GBS database.

Research results

Hypothesis 1: AMOVA – was also supported by genetic statistics which indicated that each Tasmanian population had high genetic diversity and there was no strong evidence that either reintroduced population was inbred. This hypothesis was upheld.

Hypothesis 2: The analyses suggested that mating was random in MFWS post trans-location. This created highly genetically diverse and non-inbred reintroduced populations which supports this hypothesis. The PCoA works on a molecular distance matrix to visually shows if individual genotypes tend to cluster with populations from similar geographic origin, revealing molecular assortment trends in the reintroduced populations. Under random mating, it is expected that there would be a stochastic distribution of principal coordinates in MFWS. If there is some underlying mating structure it would be expected that the reintroduced populations' principal coordinates might cluster in a distinct pattern. The PCoA shows that each founder population had relatively clustered principal coordinated (genetic components) with some overlap with geographically close Tasmanian populations.

Hypothesis 3: No significant relationship between genetic distance and geographic distance among individuals within MFWS was detected. This conclusion supports hypothesis 3.

Conclusions

It was concluded that the reintroduction of Eastern Bettongs to the ACT was successful in creating the foundations for a genetically diverse and sustainable population. The Tasmanian populations had significant genetic diversity among populations. Additionally, it was found that the source populations showed distinct genetic clustering according to geographic region, and the MFWS population was mating stochastically with respect to genetic diversity. Genetic similarity did not significantly correlate with geographic distance in MFWS. Despite both GBS library preparations and the GT-SEQ sequencing being unsuccessful in this research, NGS technologies do have the capacity to be an effective and useable tool to answer biological research. This was demonstrated by the use and extent of genetic analyses performed on the data generated from the first two GBS runs performed in 2014.

As far as limitations and recommendations, this project was significantly limited by the fact that the NGS was unsuccessful and subsequently an updated bettong genomic database could not be generated. Future directions for this project include creating a current genomic database of all bettongs sampled within MFWS and TNR to assess current genetic diversity, spatial and mating patterns. Similarly it is planned for 2016 that parentage analysis will be performed on the current population to further investigate spatial-autocorrelation and mating trends. It is highly recommended that additional bettongs are translocated from Tasmania into the reintroduced populations and that animals are exchanged between reintroduced populations to maintain genetic diversity. As far as logistics in laboratory techniques, it is recommended that two ear punch samples are taken with each newly caught animal to ensure a tissue backup is available for all animals. Additionally, it was hypothesised that a possible cause for a non-significant spatial autocorrelation result in this analysis was that there possibly had not been enough time for spatial-genetic patterns to emerge in the

population. As such, it is highly recommended to repeat this analysis with an updated bettong genomic database and current trapping records.

As of August 2014, it was concluded that the reintroduction of Eastern Bettongs to the ACT was successful in creating the foundations for a genetically diverse and sustainable population. The outcomes of this study directly inform the management plans of the bettong populations both within MFWS, TNR and for future bettong reintroductions across Australia. More broadly, this study provides a detailed assessment of genetic considerations when performing animal reintroductions for biodiversity and animal conservation management.

A 'thank you NPA' from April

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