Abstract

This paper investigates the genetic diversity of three separate Koala populations based on their microsatellite Genotypes. Two of the populations are Victorian Koalas at two different locations and the third are Queensland Koalas in a US Zoo. The results showed the Queensland koalas to be different from the Victorian koalas having a higher genetic diversity. The Victorian koalas were not statistically different and were nearly identical to each other. The data supported the hypothesis that the Victorian koalas and Queensland koalas would be genetically different bas3d on their microsatellite haplotypes at 13 different loci.

Introduction

Koala populations in Australia have declined heavily in the past 100 years. Due to this decline it is anticipated that their genetic diversity has also declined. The populations that were remaining we relocated and protected against the hunters driving them towards extinction. Some of these relocations included populations as small as 2 individuals resulting in massive bottlenecks and minimal genetic diversity. Due to this it’s assumed that inbreeding was very high, and heterozygosity is likely very low.

Translocation may lead to interbreeding between the northern Australian Koalas and the Southern Australian Koalas, which are considered to be two different species. One of the two population origins of the *Phascolarctos cinereus* present in the data in this experiment was Queensland which has an estimated population decline of 53% over the past three years which is expected to remain constant over the next three years as of 2016 (Adams‐Hosking et al. 2016). While hunting is a massive reason for the population decline, fragmentation may also affect the genetic diversity of subpopulations. The Queensland area is becoming highly fragmented, changing the natural population and potentially inbreeding inbreeding in these subpopulations with respect to the total population (Lee et al. 2010). Based on all of this data most would assume koalas would have had far more genetic diversity centuries ago then they have now. However, a 2012 study showed that the diversity in the mitochondrial DNA from centuries ago was nearly identical to the current diversity in mitochondrial DNA (Tsangaras et al. 2012). This shows it is highly likely that an event prior to the recent decline in the koala population reduced their genetic diversity. This could be in some way related to its very specific diet of the toxic eucalyptus.

The Koalas reliance on its diet heavily narrows the environments it can succeed in making environmental protection of the koalas homeland vital for its prolonged existence (Johnson et al. 2018). This is more important for Victorian Koalas than most subpopulations of the species. A 2017 study used mitochondrial DNA to compare the Victorian koalas of the South Gippsland area to other populations such as the Queensland population by comparing mitochondrial DNA and microsatellite genotypes. The results showed a significant difference between the genetic diversity of the South Gippsland Victorian koalas and the other koala subpopulations tested (Wedrowicz et al. 2018). The research hypothesized that the Victorian Koalas increased genetic diversity would make them more likely to survive a change in the environment, like the recent fires, than a less diverse group. Somewhat surprising is the size of the Queensland population.

The final open hunting season on koalas in the 1920s reduced the population size to approximately 20,000. 10,000 of which were located in the Queensland area, being the largest population, its lack of genetic diversity came as a surprise. Research in the year 2000 determined two hypotheses. The lack of distribution of the central phylogenetic haplotype implies a bottleneck occurred at the north gold coast, and two highly divergent haplotypes at the Moreton site indicated multiple translocation events likely occurred (Fowler et al. 2000). As new information about genetic diversity becomes available legislation can be modified to improve the koalas chances at survival and increase their numbers in the wild (Clark et al. 2000). Sexual selection may also play a role in the genetic diversity of the koalas, however little is known about this (Ellis et al. 2015). Furthermore, sexual behaviors seem to be quit constant between subpopulations (Ellis et al. 2010).

This study will focus on the genetic diversity of three different koala subpopulations. This will be determined using previously collected data from the Ruiz-Rodriguez study. The use of microsatellite markers to determine polymorphisms at different loci will determine the genetic diversity of the two groups. By using figures created in R and a t-test the number of different polymorphisms will signify if there is a difference between the genetic diversity of the three different subpopulations (Ruiz-Rodriguez et al. 2016).

Materials and Methods

73 separate koalas were studied with the use of 4 microsatellite markers and 13 primer pairs. Three separate groups of koalas were studied from the following areas: Brisbane Ranges in Victoria, Stoney Rises in Queensland, and a US Zoo in which the koalas were originally from Queensland. The PCR set up and algorithm used in this study was set up by (Ishida et al. 2011) and used on African Elephants. In this research done by Ruiz-Rodriguez, some of the microsatellite markers were being used for the first time including Phci21, Phci23, Phci24, and Phci30 (Ruiz-Rodriguez et al. 2016). The data collected included the sampling locations, the state of origin, the microsatellite markers, and their microsatellite genotypes in microsoft excel.The excel file was read into R software using readxl package. By creating averages for each genotype based on its location and microsatellite markers we can determine which populations had the most and least genetic similarity. R software was then used to make visual representations of this data, this was done via the ggplot2 package. Lastly the same software was used to statistically compare the data sets, with the use of a t-test to see if the difference is large enough to be deemed statistically significant. Three separate T-tests were ran the first between the Brisbane Ranges and Stony Rises genotype data, the second between the Brisbane ranges and US Zoo genotype data, and the last between the Stony Rises and US Zoo data.

Results

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Figure 1- Average Genotypes of the Koalas from Three Different Locations at Thirteen Different Loci

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Figure-2

Avereage of Microsatellite Genotypes at 13 different Loci for the Brisbane Ranges Population

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Figure-3

Avereage of Microsatellite Genotypes at 13 different Loci for the Stony Rises Population

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Figure-4

Avereage of Microsatellite Genotypes at 13 different Loci for the US Zoo Population derived from Queensland

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Figure-5

Avereage of Microsatellite Genotypes at each of 13 different Loci for all Three Populations

The average value the microsatellite genotypes were 172.7 for the Brisbane Ranges, 173.2 for the Stony Rises, and 182.6 for the US zoo. Three T-tests were done using averages created at all 13 loci. The independent T-test for the Brisbane Ranges and Stony Rises resulted in the following data: t = -0.11031, df = 23.5, p-value = 0.9131. Implying that there is not a significant difference between the two data sets. The independent T-test for the Brisbane Ranges and the US zoo resulted in the following data: t = -1.5755, df = 19.233, p-value = 0.1314. Implying that there was a significant difference between the two data sets. Lastly, the independent T-test for the Stoney Rises and the US zoo resulted in the following data: t = -1.4309, df = 20.977, p-value = 0.1672, this data also implies a significant difference.

Discussion

By using the averages. The figures, and the T-test it came clear that the US population was statistically and genetically different from the Brisbane Ranges and Stony Rises population. This makes sense considering the Stoney Rises and Brisbane Ranges are both Victorian Koalas and the US Zoo koalas were brought in from Queensland. It also matches the results found by Ruiz-Rodriguez interpreting similar data (Ruiz-Rodriguez et al. 2016). However, it was surprising that the Queensland Koalas were more genetically diverse than the Victorian Koalas given previous research by Fowler (Fowler et al. 2000). It is possible that the controlled breeding in captivity promoted increased diversity and skewed the data compared to wild Queensland Koalas.

In the figure the US data at Phci 15 appears to be an outlier, however this was one of the highest values for all three genotypes and it was quite consistent though all of the koalas in that population. The low genetic diversity between the wild Victorian koalas further supports their need for environmental protection in the wild. Environmental change like the recent fires will be very difficult for the wild koalas to endure given their lack of genetic diversity so protecting them and saving their environment could be very important. Also, the data supports the success of human interference in breeding. The US koala’s high genetic diversity may imply that similar programs may help the remaining koalas by introducing more genetic diversity back into the wild. In conclusion, the Queensland Koalas of Stoney Rises and Brisbane Ranges had lower genetic diversity than the US koalas and were not statistically genetically differ. The Queensland koalas in the US showed a significant genetic difference from the two sets of Victorian koalas and increased genetic diversity.

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