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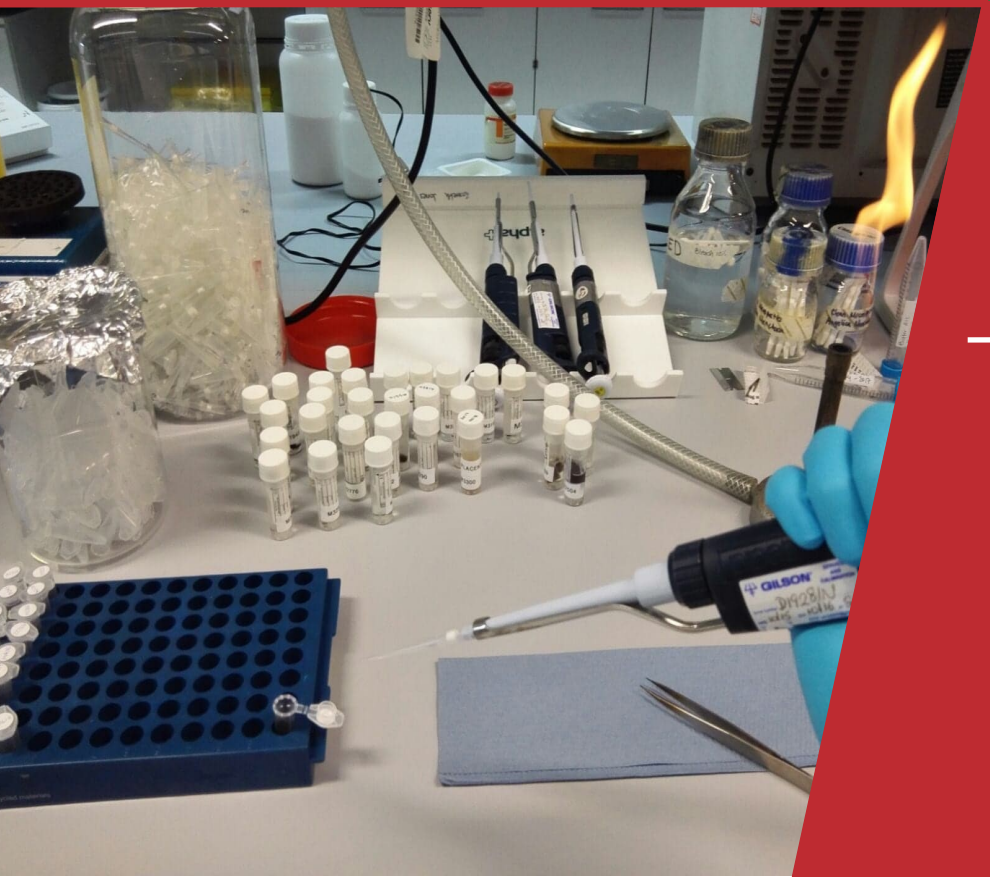

INTERIM REPORT MARCH 2019

Creating a genetic pedigree in captive Livingstone's fruit bats (*Pteropus livingstonii*)

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Creating a genetic pedigree in captive Livingstone's fruit bats (Pteropus livingstonii)

A University of Bristol PhD project, in association with Bristol Zoological Society & Jersey Zoo, supported by the Alice McCosh Trust and Experiment.com crowdfund campaign.

January 2018 - December 2019

<u>Background</u>

Livingstone's fruit bats are Critically Endangered (IUCN 2018), found only on the Comoros islands (see Figure 1) and are thought to be one of the rarest bats in the world. Due to declining population numbers, from human-driven deforestation and sporadic tropical cyclones (Mickleburgh et al. 1992), ten male and two female Livingstone's fruit bats were relocated to Jersey Zoo, British Isles, in 1992 to create a captive breeding programme as a safeguard from extinction (Young et al. 1993). In 1998, two males and five females from the Jersey Zoo population were moved to Bristol Zoo Gardens, UK to create two reproductively isolated groups. Within the captive breeding programme, there are now 68 Livingstone's fruit bats but, as paternity is often cryptic (many males may mate with the same female and it is uncertain who the father is), little is known about the genetic health of these populations.

Figure 1 - Position of the Comoros islands, relative to Madagascar and Central Africa, the native home of Livingstone's fruit bats

My work will unravel the genetic framework of captive Livingstone's fruit bats, identifying who is reproducing by creating a family tree (genetic pedigree) and allowing zoologists to make better informed decisions on future breeding to ensure the genetic health of the species is maintained.

As a self-funded student, the large expenses associated with molecular work of this type would have been prohibitive for me, meaning this vitally important project would not be accessible and would likely never occur. Through crowdfunding (experiment.com/batonthebrink) and the kind grant award from the Alice McCosh Trust, I managed to raise enough money to take on this work, for which I am very grateful.

<u>Progress (green = completed, orange = in progress, red = future work)</u>

Extract DNA from wing tissue biopsies

⇓

⇒ Wing tissue samples have been collected by the vet departments of Jersey and Bristol Zoo during routine health checks, since 2012.

⇒ DNA extraction from tissue involves digesting tissue with enzymes and washing off unrequired components until only pure DNA remains.

Figure 2 - Rasputin, the Livingstone's fruit bat, enjoying the sun at Bristol Zoo Gardens

Replicate whole genome to increase DNA yield

⇒ Unfortunately, due to the age of some tissue samples and historical storage, some of the DNA yields from extractions were lower than expected, so this additional step was added

Optimise polymerase chain reactions (PCRs)

⇒ PCR replicates target DNA many thousands of times, allowing comparison of target DNA between individuals for assessing relatedness.

⇒ PCRs must be optimised before use, which is a very time consuming and labour intensive trial-and-error method of changing recipes minutely repeatedly.

⇒ For example, in figure 3, doubling the concentration of magnesium chloride increased the intensity of bands seen in gel agarose, indicating it is the best recipe.

Run PCRs for all individuals

⇒ Once all of the PCRs are optimised, all 100 individual bat samples will be processed.

⇒ This totals 3,000 PCR samples.

Send PCR products away for sequencing

⇒ PCR products will be sent to a sequencing lab, who will convert the small tubes of clear liquid into a digital DNA signature.

Use digital sequence data to produce family tree

⇒ The DNA signatures will be compared in bioinformatics software GENEIOUS, allowing assessment of relatedness, creating a family tree.

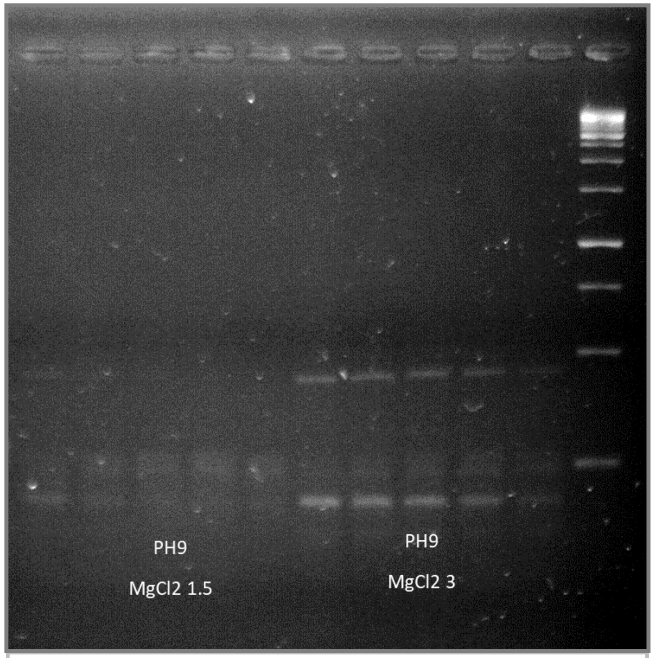

Figure 3 - Gel electrophoresis photo showing optimisation of primer PH9 with manipulation of $MgCl_2$ levels (the clearer/brighter the bands the better)

Table 1 - Overall cost breakdown of project, with Alice McCosh grant contribution in green

PCRs	£4,900
DNA sequencing	£4,100
DNA extractions	£400
Lab consumables	£600
Total	£10,000

The project is about half-completed now, with all DNA extracted and amplified, and PCRs optimised. The next step is to run all PCRs, send products off for sequencing and analyse the results.

Project implications

Compare wild and captive bat genetics to see how captive life affects genetic diversity

Mitigate inbreeding in captivity by relocating individuals between collections

Assess the genetic component of heart disease in captive bats, acting to prevent this disease being passed on

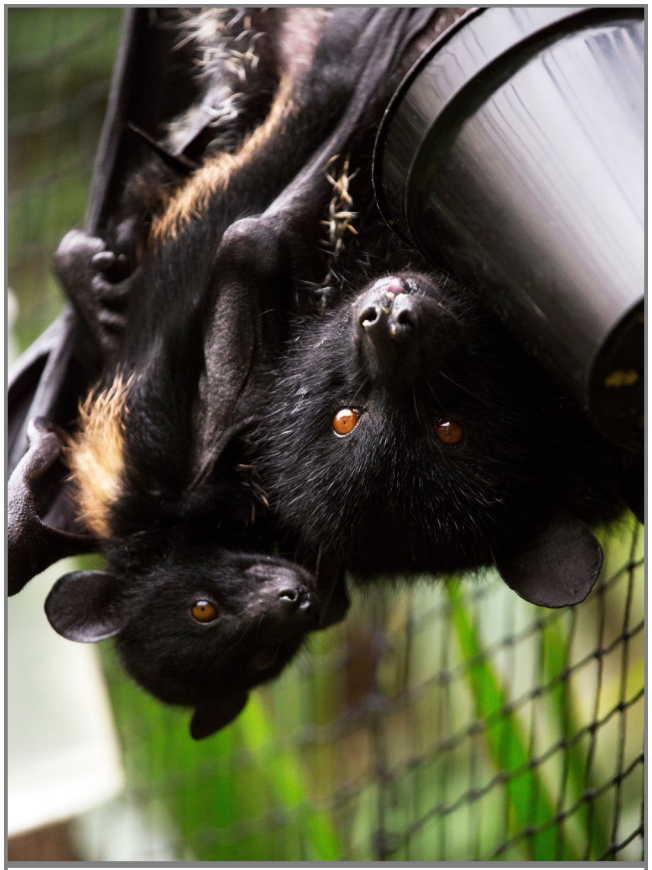

Figure 4 - Claudia and baby Ben at Jersey Zoo

Acknowledgements

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