The Rufford Foundation
Final Report

Congratulations on the completion of your project that was supported by The Rufford Foundation.

We ask all grant recipients to complete a Final Report Form that helps us to gauge the success of our grant giving. The Final Report must be sent in **word format** and not PDF format or any other format. We understand that projects often do not follow the predicted course but knowledge of your experiences is valuable to us and others who may be undertaking similar work. Please be as honest as you can in answering the questions – remember that negative experiences are just as valuable as positive ones if they help others to learn from them.

Please complete the form in English and be as clear and concise as you can. Please note that the information may be edited for clarity. We will ask for further information if required. If you have any other materials produced by the project, particularly a few relevant photographs, please send these to us separately.

Please submit your final report to jane@rufford.org.

Thank you for your help.

Josh Cole, Grants Director

Grant Recipient Details	
Your Name	Isabella Apriyana
Project Title	DNA Survey to Support the Conservation of the Critically Endangered Sumatran Elephant Population in Harapan Rainforest, Indonesia
Rsg Reference	18678-1
Reporting Period	December 2015 – February 2018 (Project Period Was Extended As Approved Per Email By Simon Mickleburgh)
Amount Of Grant	£4,995
Your Email Address	Isabella@Eijkman.Go.Id
Date Of This Report	28 February 2018

1. Please indicate the level of achievement of the project's original objectives and include any relevant comments on factors affecting this.

Objective	Not achieved	Partially achieved	Fully achieved	Comments
DNA extraction, genotyping, and sexing				Elephant DNA was successfully extracted from 64 faecal samples. Genotypes for identification of different individuals were achieved by multiple tube approach (Taberlet & Luikart 1999), with minimum three repetitions for confirmation. However, for several samples it was necessary to add more repetitions due to ambiguous result (thus affecting the expenditure for genotyping as indicated on the budget table below). Sex type of each elephant individual was analysed using molecular markers following Ahlering et al. 2011.
Determining minimum population size and estimating total population size				Minimum population size was obtained by analysing the genotyping results.
Determination of population sex ratio				Population sex ratio was obtained by analysing the molecular sexing results.
Determination of population age structure				We were not able to obtain bolus circumference information for all samples due to the faecal sample conditions in the field. Some samples were in such condition preventing reliable measurements, therefore we could not properly determine the population age structure.

2. Please explain any unforeseen difficulties that arose during the project and how these were tackled (if relevant).

This project is a collaborative work between PT Restorasi Ekosistem Konservasi (PT REKI) and Eijkman Institute. At the conception of the collaboration, it was agreed that PT REKI would be in charge for sample collection meanwhile Eijkman Institute would provide expertise and facilities for conducting laboratory works.

We encountered unforeseen difficulties due to delayed permit and administrative issues which prevented the transfer of samples to the laboratory as scheduled. Preparation for the fieldwork for sample collection was already started in September 2015, at the same time of grant application submitted to the Rufford Foundation. The combination of the field and laboratory work was initially planned to take up to 12 months.

The first batch of samples was collected in March 2016, with another two fieldtrips every 3 months. Part of the laboratory work was planned to start parallel with the subsequent fieldwork. Unfortunately, samples could not be transferred to the laboratory due to misunderstanding on the processing of the transfer permit to the Ministry of Forestry. The administrative issues were subsequently resolved by redoing all the permit steps, which altogether took up more than 6 months. The first batch of 30 samples was finally transferred to the laboratory on January 2017.

Another difficulty that we encountered was the small number of samples collected in 2016. After consulting with the Rufford Foundation, our proposal to extend the project period was approved. This allowed the teams to conduct another fieldwork sessions to collect more samples in December 2016 – June 2017, in order to obtain a more comprehensive population data. The second batch of samples was transfer to the laboratory on December 2017, after obtaining a new transfer permit and redoing all the paperwork. The application of the new permit was submitted before the fieldworks ended to allow enough time for the approval.

Lastly, we also encountered minor technical difficulties, in which six of a total of 70 samples sent to the laboratory had broken tubes. We decided to exclude these samples from the molecular analysis to avoid incorrect data because of the high chance of cross contamination.

3. Briefly describe the three most important outcomes of your project.
The three most important outcomes of this project are:

1. *Estimating the number of elephant individuals and its genetic diversity in Hutan Harapan*
   Based on the genotyping (microsatellite) analysis, we found seven different elephant individuals roaming the area of Hutan Harapan. We observed a rather low diversity on this population (Polymorphic Information Content/PIC = 0.2971). Due to small number of individuals and homogenous alleles, we could not distinguish if the individuals are siblings (PIDsibs of 14 markers > 0.005).

2. *Providing the information on sex ratio of elephant population in Hutan Harapan*
   By using the molecular sexing method, our findings showed that the seven elephant individuals in Hutan Harapan consisted of six females and one male. Initially, Hutan Harapan had eight elephants however two of them were killed (skulls were found in 2015) leaving only six females (E. Gemita, PT REKI, pers. comm.). The male individual found in our samples is very likely Haris, who was

translocated to Hutan Harapan from Bukit Tigapuluh population in November 2016 (A. Mossbrucker, pers. comm). When analysing the genotype results, we can also see that the male individual has unique alleles (DNA variants) not found in the female individuals, indicating that it may come from a geographically different population. The translocation of individuals carrying different variants of DNA may increase the diversity in populations which previously were rather homogenous.

3. *Providing input for the management of the small population in Hutan Harapan, including the importance of translocation*
   The genetic analysis of the elephant population in Hutan Harapan provides an overview of the genetic structure of the population. In addition to the population being small and almost uniformly female, the low genetic diversity also indicates a rather grim future if no counter measures is taken. The viability and the survival of a population are greatly questioned with such proportion structure. It will be difficult for the population to grow without additional introduced female and male individuals to enrich the local gene pool. Furthermore, homogenous genetic makeup might increase susceptibility to diseases which could affect the whole population. At the same time, Hutan Harapan is one of the last resorts for the rapidly declining Sumatran elephant populations, thus strategic actions such as introducing new Sumatran elephants, especially the more genetically diverse individuals, from other populations is urgently needed.

4. Briefly describe the involvement of local communities and how they have benefitted from the project (if relevant).

We employed local people as part of the field teams who conducted the faecal sample collection. Their involvement on the first genetic-based population census in Hutan Harapan enabled them to understand different approach of studying the elephant populations. Instead of counting the elephants or tracking for elephants signs as they usually do, they also can collect faecal samples (something that was rather odd for them at the beginning) as part of their elephant conservation work.

5. Are there any plans to continue this work?

We plan to conduct mitochondrial DNA analysis on the samples in order to obtain a more comprehensive look at origins and genetic diversity. Both the genotyping and mitochondrial DNA information will serve as a supporting baseline data for expanding the small and isolated population of Hutan Harapan, for example through translocation.

6. How do you plan to share the results of your work with others?

The results of this research project will be reported to the Ministry of Forestry of Indonesia in order to provide the most updated Sumatran elephant genetic data. This data can be used as the reference for better management strategy on the

Sumatran elephant population in Hutan Harapan. We are also planning to publish the results in a peer-reviewed international journal.

7. Timescale: Over what period was The Rufford Foundation grant used? How does this compare to the anticipated or actual length of the project?

The Rufford Foundation grant was used over the period of 26 months. We understand that this is much longer than anticipated (12 months), due to issues arising regarding the transfer permit of the samples. However, we have been in consultation with Rufford Foundation grant officers about this matter and the proposal for project period extension was kindly approved through email correspondence with Simon Mickleburgh.

8. Budget: Please provide a breakdown of budgeted versus actual expenditure and the reasons for any differences. All figures should be in £ sterling, indicating the local exchange rate used.

Item	Budgeted Amount	Actual Amount	Difference	Comments
DNA Stool Extraction Kit	£600	£504	£96	
Proteinase K 10 mL	£310	£250	£60	
Multiplex PCR Kit 100rx	£236	£190	£46	
Genotyping	£2,025	£2,424	-£399	Excess due to optimization phase and additional genotyping on several samples
PCR Kit for Molecular Sexing (100 reaction)	£105	£104	£1	
dNTPs	£75	£69	£6	
Agarose gel 100 gr	£96	£164	-£68	Price for this item went up
Electrophoresis buffer	£300	£150	£150	
Thermal paper	£70	£100	-£30	Price for this item went up
Aerosol Resistant Tips	£480	£355	£125	
Microtubes	£105	£95	£11	
Gloves	£28	£27	£1	
Box Samples	£15	£12	£3	
Laboratory fee	£500	£500	£0	Covers electricity and the use of facilities, such as freezing storage for samples.
Sample transfer fee	£50	£0	£50	Paid in kind by PT REKI
Total	£4,995	£4,945	£50	Note: £1 = IDR 20,539

9. Looking ahead, what do you feel are the important next steps?

Eijkman Institute has been involved in genetic analysis of the critically endangered Sumatran elephant since 2011, covering key sites in Sumatra and currently has a database of almost 500 elephant individuals. Together with various collaborators working in elephant conservation, we aim to collect as much genetic data and build a database for all of the remaining elephant populations in Sumatra (island-wide survey). In the future, it will be important to use this data to support management strategies, for example translocating animals with high genetic diversity to create a viable population over the long term. In addition, such data can also support forensic cases, for example by quickly identifying the origin (or source population) of poached animals.

As for Hutan Harapan, the area continues to be a model for protected and sustainable forestry. It is one of the most important sites for Sumatran elephant to roam free. However, the small population lacking male individuals in Hutan Harapan area might be problematic in the future. It is important to ensure the population viability in Hutan Harapan, including by introducing more genetically diverse male individuals to increase the population size and avoid population homogeneity as currently seen.

10. Did you use The Rufford Foundation logo in any materials produced in relation to this project? Did the RSGF receive any publicity during the course of your work?

The Rufford Foundation logo will be used in reports submitted to Ministry of Forestry of Indonesia and our collaborator, PT REKI. We are also planning to use the logo whenever the result of this project is being presented (such as posters, presentations, and publications).

11. Any other comments?

N/A

Outer layer of dung bolus containing epithelial cell from elephant's gastrointestinal tract are scrapped off using sterile wooden sticks and preserved in buffer solution.


Left: A herd of elephants in Hutan Harapan. Right: Elephant faecal samples.


Laboratory analysis includes DNA extraction, genotyping, and molecular sexing, followed by data analysis.

References

Ahlering, M. A., Hailer, F., Roberts, M. T. and Foley, C. 2011. A simple and accurate method to sex savannah, forest and Asian elephants using noninvasive sampling techniques. *Mol. Ecol. Resour.* 11:831–834.

Taberlet P, Luikart GS. 1999. Non-invasive genetic sampling and individual identification. *Biol J Linnean Society* 68:41-55.