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	George Olah
Project title	Development of novel genetic techniques for conservation studies of large macaws
RSG reference	11007-2
Reporting period	1st February 2012 – 1st October 2016
Amount of grant	£5868
Your email address	george.olah@anu.edu.au
Date of this report	1st October 2016

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
Developing highly variable genetic markers for scarlet macaws*			x	* This objective was carried over from our 1st RSG as we could only partially achieve this objective in that project. In this present project we fully achieved this objective and not only for scarlet macaw but also for red-and-green macaw as well. We have published these markers in Conservation Genetics Resources.
Assessing the feasibility of our novel non-invasive genetic tagging technique*			x	* This objective was carried over from our 1st RSG as we could only partially achieve this objective in that project. In this present project we fully achieved this objective and not only for scarlet macaw but also for red-and-green macaw. The technique has been accepted for publication in Conservation Genetics Resources.
Documenting the structure of macaw populations among areas with recent anthropogenic fragmentation and natural fragmentation			x	We analysed the population genetic structures of two large macaw species from samples collected in different parts of the landscape. We found high genetic diversity for both study species over large geographic scales. Two manuscripts of the results have been submitted to peer-reviewed journals.
Applying our techniques to specific			x	We used landscape genetic techniques to compare macaw

conservation issues				populations between Tambopata and the Candamo Valley biodiversity hotspot isolated by the foothills of the Andes. We found significant differentiation that indicates the need for extra conservation in the Candamo region. Our novel landscape genetics approach has been submitted for publication in Landscape Ecology.
Identifying individual scarlet macaws from the feathers they drop in the study area using genetic tagging			x	We used the highly variable genetic markers to construct full genotypes of each feather sample found during our study. We demonstrated the power of the genetic tagging by recovering full genotype matches among different locations. We have also validated this technique for red-and- green macaw.
Using individual identities from genetic samples to study individual movements and to estimate home range size				We used the genotype matches in traditional capture-mark-recapture modelling to estimate population sizes in our study area. We have based all these estimates on non-invasively collected moulted feather samples. Results are included in my PhD thesis and has been submitted to peer-reviewed journals.

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

In our 1st Rufford Small Grant the field research phase of our project was supported in order to collect samples from large macaws in Tambopata for subsequent genetic analysis. In this 2nd Rufford Small Grant we carried out the laboratory analysis of the collected samples, however this took longer than expected due to slow administration issues in Peru. We applied for the genetic access permit but due

to unforeseen circumstances in the local administration had to find an alternative way to speed up the progress of our project. We consequently set up collaboration with a local university in Peru with adequate laboratory equipment to conduct the necessary DNA extractions and laboratory analysis. Unfortunately, we could only do DNA extractions in this lab, and project completion was significantly delayed.

Another unforeseen difficulty arose when the primary researcher (George Olah) became seriously ill with a mosquito borne cutaneous Leishmaniasis, a severe tropical disease in the study site. He received an intense treatment of intravenous chemotherapy in the Cayetano Heredia Hospital in Lima for 3 months, but full recovery took 6 months in total.

3. Briefly describe the three most important outcomes of your project.

First, we developed new hyper-variable genetic markers (microsatellites) based on the full genome of the scarlet macaw. We designed species-specific primers to amplify these markers and tested these on macaw DNA acquired from the wild. We also tested these markers on red-and-green macaw samples and proved their cross-species transferability (see our publication in Conservation Genetics Resources).

Second, we validated the use of the new microsatellite markers for individual identification via non-invasive feather samples. The use of microsatellite markers for individual identification is a widely used technique in human forensic science. This same technique has also been adopted in conservation genetics and is called genetic tagging. However, our project is the first to adopt genetic tagging for birds. We have specifically applied genetic tagging on macaw feathers collected in tropical field conditions. Our manuscript with these results was accepted for publication in Conservation Genetics Resources.

Third, we also used the genetic data acquired in this study to investigate the general population genetic structure of macaws from the lowland Peruvian Amazon. We showed high genetic diversity of both scarlet macaw and red-and-green macaw in the Tambopata region, which indicates genetically healthy populations. We also found that the several hundred sampled individuals over the landscape probably belong to a single metapopulation of the Peruvian Amazon.

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

Local indigenous communities in Tambopata were strongly involved in the collection of samples and other logistics of the fieldwork phase. We employed assistants from the local communities to help document the feather samples and build a database

for species identification by feathers. We also trained our assistant for general genetic laboratory work in DNA extraction. All these skills can be important for their future work in field research.

Most importantly, during our extensive work with the local communities we emphasised the importance of protecting their wildlife and natural habitat. We have demonstrated that by conserving their natural resources they can also benefit from upcoming wildlife research in the region. Our work with members of native communities has motivated them to launch their own conservation education programme.

5. Are there any plans to continue this work?

Yes. In our past two projects we have developed, validated, and applied the genetic tagging on non-invasive feather samples collected from two large macaw species in a tropical environment and demonstrated its feasibility. After we have published our results, we plan to apply the newly developed markers of genetic-tagging to more endangered macaw and parrot species, or to regions where these species are more endangered (e.g. in Central America). As we demonstrated in our projects, this conservation genetic technique can be very useful for estimating population size, dispersal, genetic structure, and landscape use, among other biological and ecological attributes. In addition, for many threatened parrots the non-invasive genetic sampling of moulted feathers may be the only available DNA source and its use can also help address the ethical concerns of catching wild individuals.

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

Some results of our project have published in six peer-reviewed scientific articles, namely:

Olah G, Smith AL, Asner G, Brightsmith DJ, Heinsohn RG, and Peakall R (2016). Exploring dispersal barriers using landscape genetic resistance modelling in scarlet macaws of the Peruvian Amazon. Landscape Ecology in Press

Olah G, Heinsohn RG, Brightsmith DJ, Espinoza JR, and Peakall R (2016). Validation of non-invasive genetic tagging in two large macaw species (Ara macao and A. chloropterus) of the Peruvian Amazon. Conservation Genetics Resources doi:10.1007/s12686-016-0573-4

Olah G, Butchart SHM, Symes A, Guzmán IM, Cunningham R, Brightsmith DJ, and Heinsohn RG (2016). Ecological and socio-economic factors affecting extinction risk in parrots. J. Biological Conservation 25(2): 205–223. doi:10.1007/s10531-015-1036-z

Olah G, Heinsohn RG, Espinoza JR, Brightsmith DJ, and Peakall R (2015). An evaluation of primers for microsatellite markers in Scarlet Macaw (Ara macao) and their performance in a Peruvian wild population. Conservation Genetics Resources 7(1): 157–159. doi:10.1007/s12686-014-0317-2

Olah G, Vigo G, Heinsohn R, and Brightsmith DJ (2014). Nest site selection and efficacy of artificial nests for breeding success of Scarlet Macaws Ara macao macao in lowland Peru. Journal for Nature Conservation 22(2): 176–185. doi: 10.1016/j.jnc.2013.11.003

Olah G, Vigo G, Ortiz L, Rozsa L, Brightsmith DJ (2013). Philornis sp. bot fly larvae in free living scarlet macaw nestlings and a new technique for their extraction. Veterinary Parasitology 196(1-2): 245–249. doi: 10.1016/j.vetpar.2012.12.052

In addition, we have one manuscript under peer-review process at scientific journals, namely:

Olah G, Heinsohn RG, Brightsmith DJ, and Peakall R (2016). Non-invasive genetic tagging of large macaws in the Peruvian Amazon. Under review in Conservation Genetics

We have presented this study at the following international conferences:

X. Neotropical Ornithological Congress (Manaus, Brazil, 2015)
Ecological and socio-economic factors affecting extinction risk in parrots

26th International Ornithological Congress (Tokyo, Japan, 2014)
Ecological and socio-economic correlates of conservation status in parrots (Psittaciformes)

XVII. Congress of the Mesoamerican Society for the Biology and Conservation / VIII. Mesoamerican Symposium of the Conservation of Psittaciformes (Habana, Cuba - September 16-20, 2013)
Olah G, Vigo G, Brightsmith DJ - Estimating reproductive success in large psittacines: monitoring nests vs. counts of family groups

XVI. Congress of the Mesoamerican Society for the Biology and Conservation / VII. Mesoamerican Symposium of the Conservation of Psittaciformes (Panama City, Panama - September 17-20, 2012)

Olah G, Vigo G, Heinsohn R, Brightsmith DJ - Conservation of Scarlet Macaw (Ara macao macao) in southeastern Peru: use of artificial nests and a new genetic method

Between 2012 and 2015 we have also presented many open lectures about our study to the public in Peru, USA, Hungary, and Australia.

We have also filmed a documentary movie about this research that is now broadcasted worldwide with the title of "The Macaw Project - Biologists, Ecotourists and Local Communities for the Amazonian Rainforest". Official website: www.macawmovie.com

The full content and results of this research was accepted as PhD thesis by The Australian National University in May 2016.

Media articles and appearances:

New study reveals extinction dangers for parrots - https://news.mongabay.com/2016/05/exceptional-beauty-exceptional-risk-new-study-revealsextinction-dangers-parrots

Feather forensics - http://news.mongabay.com/2014/0617-devitt-macaws-olah.html

Video abstract of our latest article - https://youtu.be/7Ab1ESyTmFs

Candamo Expedition - https://vimeo.com/157747586

A Third of Parrot Species Face Extinction - http://www.takepart.com/article/2016/02/25/a-third-ofparrot-species-face-extinction

Australian parrots need more protection - http://www.anu.edu.au/news/all-news/australianparrots-need-more-protection

Using Artificial Nests to assist and study Macaw Parrots in lowland Peru - http://fennerschool.anu.edu.au/news-events/using-artificial-nests-assist-and-study-macaw-parrotslowland-peru

The Macaw Project on Freshpreneur - http://www.thefreshpreneur.com/thursday-recap-pop/themacaw-project

The Macaw Project on Indiegogo - http://igg.me/at/macawmovie

The Macaw Project official website - www.macawmovie.com

George Olah's website - http://georgeolah.weebly.com

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

This grant was approved on the 12th January 2012, but the grant was not used until actual lab work started in August 2012. We used the grant for expenses of DNA extraction in 2013. Due to the difficulties described under question #2 we did not use the other half of the grant until mid-2014 for PCR when full genotypes were constructed from the genetic samples. We used the entire grant by the end of 2014. We analysed the data and published the genetic results in 2015 and 2016.

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 extraction (kits, reagents)	1467	1623	+156	More samples for extraction
Laboratory consumables (plastics, tubes, tips, etc.)	893	910	+17	
PCR kits	1786	1550	-236	We used less DNA samples for PCR analysis
Lab assistant (for 5 months)	1722	1900	+178	We employed lab assistant for 6 months
Total	**5868**	**5983**	**+115**	

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

In the long term, we feel that an important step is applying the genetic tagging method developed here for the conservation genetics of macaws and parrots with

high conservation concern. The genetic markers designed in this study are already being used in other parrot species, for instance at the critically endangered blue-throated macaw in Bolivia (T. Wright personal communication), and also at the critically endangered swift parrot in Australia (G. Olah unpublished data). These markers can also be useful in conservation genetic studies of scarlet macaws in Central America, where their populations have declined drastically.

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 was used in our documentary movie called The Macaw Project (www.macawmovie.com). The logo was also used in our poster presentations and lectures in scientific conferences and public talks (see question #6).

The Rufford Small Grant Foundation was fully acknowledged in all our peer-reviewed scientific articles, will also be acknowledged in our manuscripts resulted from this study, and in the PhD thesis (see question #6). The foundation has received publicity via our international conference presentations, open lectures, and largely via our documentary movie.

11. Any other comments?

Unexpected difficulties, tropical diseases, and administrative delays around biology field research in third world countries often happen. However, these unforeseen difficulties should not be discouraging for researchers to conduct their study as originally planned. With enthusiasm, lots of dedication to the problems, and patience one can overcome the most difficult challenges in international research endeavours. These developing countries contain the largest biodiversity of our planet and hence provide most work for us field researchers. We would like to encourage future studies to do similar efforts to overcome complications under such conditions. We are grateful to the Rufford Small Grant Foundation for their flexibility, understanding, and continuous support during our project.