

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	Gyanpriya Maharaj
Project title	A comparative study of colour mediated foraging by passion vine butterflies at <i>Lantana camara</i> : field behaviours to genomics and their conservation implications
RSG reference	15656-1
Reporting period	July 2014-April 2015
Amount of grant	£4989
Your email address	gpriyaj@gmail.com
Date of this report	11 May 2015

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
Describe the natural foraging environments of <i>Heliconius melpomene</i> , <i>H. sara</i> and <i>Dryas iulia</i> at species and individual levels.			X	I was able to use the micro spectrometer purchased with the Rufford grant to measure the spectral reflectance of flower petals of all feeding plants. In addition I have also measured the petals of non-foraging plants and I am currently carrying out data analyses to compare these readings in order to fully characterise the feeding environments of my study species.
Illustrate the temporal change in colour and reward of <i>L. camara</i> .			X	Although many studies show that wild-type <i>L. camara</i> system exhibit three main successional color phases i.e., yellow, orange and red; closer examination of these phases shows that they can be subdivided into nine stages defined by temporal variations in these colours. Measurements of sucrose concentration and volume by colour stage showed substantial variability and a significant difference among stages (Wilk's λ_{suc} con'c, $F=3337.855a$, $p<0.05$), (Wilk's λ_{suc} vol., $F=78.057a$, $p<0.05$). A multiple linear regression model predicting flowering stage from its sucrose volume and concentration showed that 55% of the total variability were explained by concentration and volume (ANOVA $F=137.945$, $p<0.05$).
Correlate the effect of inflorescences size and reward to pollination visitation rates.			X	A chi-squared test was performed and a significant relationship was found between species and the frequency of visits to treatments. <i>H. melpomene</i> visited Large Mixed, Large Red (χ^2 (2, $N = 823$) = 7.520, $p = 0.023$), 50/50 and Unrewarding only treatments (χ^2 (2, $N = 1054$) = 70.434,

			<p>$p < 0.000$) more than Small Yellow, Control and Rewarding only treatments. <i>D. iulia</i> visited Small Yellow, Large Mixed, Control and Rewarding only treatments more than Large Red, Unrewarding only and 50/50 treatments. A logistic regression was performed to ascertain the effects of species and treatment on the likelihood that butterflies visited single inflorescences or multiple inflorescences i.e., how species and treatment affects long and short distance attraction. The logistic regression model was statistically significant. Butterflies visiting a Small Yellow and Large Mixed inflorescences ($\chi^2 (3) = 41.23, p < 0.0001$), 50/50, Rewarding only and Unrewarding only treatments ($\chi^2 (3) = 60.954, p < 0.0001$) have increased odds of visiting multiple flowers, thus there were more attractive at short distances. Whereas butterflies visiting Large Red and Control inflorescences usually visited only one flower, thus these treatments were more attractive at longer distances. Overall, it is seen that <i>H. melpomene</i> are more likely to visit treatments with red flowers while <i>D. iulia</i> frequent inflorescences with yellow and orange flowers.</p> <p>*<i>H. sara</i> was not included in these data due to very small number of visits in both field seasons.</p>
<p>Demonstration project for growing larval and adult food plants in destroyed rainforest habitats to mediate butterfly species extirpations</p>		<p>X</p>	<p>Of our new transplants a few of them have survived and have shown increase in foraging activity at their locations in terms of abundance, however most of our plants were killed by leaf- cutter ants.</p>

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

- i) The populations of each of my study species, although they were considered common butterflies at the beginning of my research decrease dramatically due to ongoing land clearing for agriculture in the area and as such I had to carry out an additional field season (December 2014- January 2015) to collect supplementary data on my study species. With the extra field season I was able to carry out further inflorescence manipulation work, which then provided sufficient data for significant statistical analyses. The populations of *H. sara* continued to dwindle and although data was collected on this butterfly in both field seasons numbers were too low for meaningful analyses. Additionally, I stopped all specimen collections for all of my study species owing to their low abundance in the area. I have decided to use butterflies provided by the Butterfly House, Missouri, St. Louis, USA, for future genetic work.
- ii) After transplanting both nectar and larval food plants into areas that were not under threat from logging and clearing for agricultural purposes most plants thrived for a few days before losing their leaves. Subsequent to shedding their leaves, the new leaves which began to grow on all of our transplants were frequently attacked by leaf-cutter ants (*Atta cephalotes*) during night time. After returning to our site a few months later for the second field season we had lost most of our transplants. However, the surviving ones bloomed and were fed on by butterflies in the area, resulting in a general increase in butterfly feedings in the transplanted area. In the first field season after discussion with the neighbouring owner of a mixed farm, who was involved in ongoing land clearing in the area, we had asked him to allow the growth of *L. camara* and *Wulffia baccata* bushes on his farm scattered among his plants to allow for open and shaded feeding patches. On returning for our second field season in this area we noticed a significant increases in butterfly abundance and diversity on his farm and the surrounding habitats.

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

- 1) Describe the foraging environments of *H. melpomene*, *H. sara* and *D. iulia*.
- 2) Establish the relationship between inflorescences size and reward and pollinators visitation rates
- 3) Train field assistants to take spectrometry and behaviour data.

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

During this project I was able to train undergraduates from the University of Guyana and the University of Missouri, St. Louis to use the micro spectrometer, take sucrose readings and collect

butterfly observational behaviour data. I also worked with one undergraduate student to carry out a butterfly survey of the area to compare diversity and abundance of habitats that were used for agriculture versus untouched areas. He carried out his first survey in December 2014 - January 2015 and will continue this work in the upcoming summer 2015.

5. Are there any plans to continue this work?

- i) I am already conducting experiments at the butterfly house at Chesterfield, Missouri USA, I have received a Biology Graduate Student Grant from the University of Missouri to buy all additional materials, to investigate the innate colour preferences of my study species and the effect of colour patterns of inflorescences on foraging choice. I plan to compare these data with foraging results from the field. All colour measurements will be carried out by equipment purchased by the Rufford grant.
- ii) I also intend to investigate traplining in butterflies, specifically focusing on interplant movement of *H. melpomene* and *D. iulia* under natural conditions (no changes to site) and interplant movement patterns when experimental treatments; a) involve plant exclusion and subsequent reintroduction to feeding circuits and b) competitor absence/change in competitor behaviour at foraging site. I will only be focusing on *Heliconius melpomene* and *Dryas iulia*, because they still have sufficiently high numbers. Specifically, my research project sheds light on how changes in the behaviour of territorial competitors' and food plant availability (disappearances/reappearances), both effects of habitat destruction, will affect the feeding behaviours and ultimately the survival of my study species. For this work I have applied for Lewis and Clark, Idea Wild and Webster Grove Grants.

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

The data that I have collected during this project will be used in the second and third chapters of my PhD dissertation, entitled *Correlations between pollinator feeding and interspecific mating preferences and Honesty signalling and pollinator attraction: Butterflies and L. camara, respectively*. In addition to my dissertation, the information generated from this study will be widely distributed through publications in tier one journals. I plan to submit these two manuscripts by April 2016 to the following journals: The Journal of Experimental Biology, Animal Behaviour and Journal of Pollination Ecology. In addition, I plan to disseminate my results via national and international platform and poster presentations and as a current member of staff at the University of Guyana, Department of Biology and the Centre for the Study of Biological Diversity, Guyana (CSBD) this information will be presented at the CSBD seminar series when in Guyana. The Rufford Foundation will be acknowledged in my dissertation and all publication resulting from the use of this grant.

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

My Rufford grant was used from July 2014 - January 2015, instead of the July - September 2014 timeline previously stipulated. This extra time was needed to carry out an extra field season which allowed for the collection of additional data from my inflorescence manipulation experiments. Owing to this extra field season my Chesterfield butterfly experiments started in February 2015 instead of September 2014, however I have started my genetic work (RNA extraction) on the specimens that have been collected in July 2014 in September 2014. I also continue to use the equipment from this grant for my experimental work at the Chesterfield Butterfly House.

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.

1 GBP (£ sterling) = 1.65998998 USD

Item	Budgeted Amount	Actual Amount	Difference	Comments
EPA research permit	105	105	0	No difference
Labelling tape	12	15	-3	Brand bought was more expensive than budgeted for
Cryogenic vials 1 bag of 50	24	35	-11	Purchased bag of 100 to accommodate collection of specimens, although specimens will not be collected from Guyana due to low numbers I will be collecting specimens from the Butterflies House in Chesterfield Missouri to finish up my genetic work
Cryo-Glove, 1 pair	75	0	75	Bought by Bourne lab
Liquid Nitrogen	100	13	87	Due to low numbers of butterflies we were only able to collect once for the field season thus we only needed to purchase liquid nitrogen one time while in Guyana
Cryo Express CX 100 – dry shippers	600	0	600	We did not buy this as we transported our few specimens in the Lab owned Cryostat
Portable Spectrophotometer	1680	2015	-335	The portable model needed for this study was more expensive than a lab based model.

Microscope slides with slip covers 1 box of 72	6	4	2	Brand bought was less expensive than budgeted for
Fine tip permanent markers 3 @ 3 ea.	9	0	9	Bought by Bourne lab
Flagging tape	3	0	3	Bought by Bourne lab
Tube Micro-Cal Microcapillary tubes	17	0	17	Bought by Bourne lab
Digital caliper	12	7	5	Brand bought was less expensive than budgeted for
Ultraviolet flash light	12	9	3	Brand bought was less expensive than budgeted for
Extra heavy duty puncture resistant clear reclosable zipper bags box of 36	9	0	9	Bought by Bourne lab
Ziploc Slider All Purpose Gallon Storage Bags 20	5	0	5	Bought by Bourne lab
Rite in rain notebooks 4	20	0	20	Bought by Bourne lab
International Travel - Roundtrip Air Coach STL-GEO (Summer 2014)	700	564	136	I travelled to New York first and was able to get a cheaper ticket than was budgeted for
Extra baggage	100	15	85	Due to airline choice I only needed to pay for one extra bag
Ground travel	100	10	90	Family and friends helped with my transportation needs while in Guyana thus I only needed to pay for a few taxi trips around Georgetown.
Per diem to cover room and board for researcher and Guyanese counterpart (Field season 1)	1400	1400	0	No difference
Chemglass CLS-4768-G01 Green	0	21	-21	Used to store specimens in cold storage refrigerator as I use them for genetic work

Polycarbonate Cryostore Storage Box for 1.2 and 2.0mL Cryo Vials 5-1/4" Length x 5-1/4" Width x 2-1/16" Height, 100 Posit				
Cryoflex tubing	0	68	-68	Used to store specimens in cryostat to prevent breakage of vials
PST-MP3500-I Battery for spectrometer	0	111	-111	Used with spectrometer so that readings can be taken in field without use of electricity
International Travel - Roundtrip Air Coach STL-GEO (Winter 2014-2015)	0	644	-644	Used for second field trip
Per diem to cover room and board for researcher and Guyanese counterpart (Field Season 2)	0	361	-361	Used for second field trip
Total	4989	5397	-408	I have covered the difference of this research with personal funds

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

I think it is essential that we try to protect our replants from herbivory and ascertain the actual improvement in abundance and possible diversity at sites where we have replanted nectar and larval plants by carrying out a small survey. I also think it is imperative that we work with farmers to discuss the importance of planting and keeping nectar and food plants on their farms to support native pollinator populations.

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?

I have used the Rufford Foundation logo in my poster presented at the Graduate Symposium, University of Missouri, St. Louis (UMSL) and will use it in papers published as a result of this work. In addition to word of mouth where I have encouraged a number of my colleagues to apply for at the grant at UMSL, there was an article published in the UMSL Daily, EP/22/2014,

Myra Lopez, and this was republished by a local Guyanese newspaper (Guyana Times, 11/30/2014) describing my work and the Rufford Grant received.

11. Any other comments?

I am grateful to the Rufford Foundation for their support as it allowed me to carry out conservation efforts and my research in my own country with fellow Guyanese students which is especially meaningful to me as this is one of the main reason that I am currently pursuing my PhD and it is the job that I hope to return to once I have completed my studies and return to Guyana.

