

The Rufford Small Grants Foundation

Final Report

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

We ask all grant recipients to complete a Final Report Form that helps us to gauge the success of our grant giving. 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. 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	Maria Clara Arteaga Uribe
Project title	Conservation genetics of endemic Yucca and Yucca Moths on the Baja California peninsula
RSG reference	13704-1
Reporting period	June 2013- December 2014
Amount of grant	£5995
Your email address	mariaclaraarteaga@yahoo.com
Date of this report	December 19 th 2014

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
1. Sample collection along the distribution range of each species, <i>Yucca valida</i> , <i>Y. capensis</i> , and <i>Tegeticula baja</i>			x	We visited 23 populations of endemic yuccas across the peninsula. We collected leaf tissues of 12 populations of <i>Yucca valida</i> and 11 populations of <i>Yucca capensis</i> . To obtain yucca-moth pollinators, we collected fruits from 45 trees in 15 localities. In order to contribute to knowledge of ecology and reproductive biology of these species, we also explored morphological variation and describe demographic aspects of species in their geographical distribution, measuring phenotypic features of 297 trees.
2. Geographic distribution and the level of genetic diversity of the two <i>Yucca</i> and the <i>Tegeticula</i> species. Identification of evolutionary units and corridors of population gene flow		x		We genotyped 100 individuals of yuccas for five nuclear microsatellites and for three chloroplast regions. We also genotyped 60 larvae collected inside the fruits, using two mitochondrial markers. Regarding the yuccas, we found high allele number in microsatellites, most of them shared between the two species, and the chloroplast regions show low variation. The mitochondrial markers used in yucca-moths have few polymorphic sites. In the beginning of the next year, the genetic data will be completed and they will be reanalysed.
3. Intra and interspecific genetic structure in both <i>Yucca</i> species to assess if genetic data support the morphology-based hypothesis of two separate species or if the genetics suggests further divisions		x		The analysis carried out with the genetic data obtained until now allow to explore partially this question. The endemic yuccas share the few polymorphism found in chloroplast. However, the nuclear markers indicate a separate gene-pool for each species, despite share some alleles between them. At intraspecific level, there is not clear genetic structure among populations or geographical regions. The yucca-moths collected in fruits of both yucca species, also share the polymorphism found in mitochondrial markers. An unexpected and very interesting result is that inside the fruits

				collected, there were larvae of other species than <i>Tegeticula</i> , indicating that <i>Yucca</i> 's fruits are host for other invertebrate species in the ecosystem. The analysis of morphological data collected of 297 trees indicates a high phenotypic variation within and among populations of each species and between the two endemic yuccas.
4. Characterisation of environmental features such as soil type and climatic variables for each location sampled to explore how these variables are related with genetic connectivity among <i>Yucca</i> populations		x		The environmental database describing 19 climatic variables of the 23 sampled sites is ready. When the genetic information of nuclear microsatellites of yucca will be complete (in the next 4 months), the analysis to explore the influence of environmental features on genetic connectivity will be carried out. The quality and soil characteristics in each site is highly variable in a small scale. For this reason, this information was not collected.
5. Assessment of the reproductive isolation of the two <i>Yucca</i> species by analyzing for patterns that would indicate hybridization between them		x		We analysed three populations present in the boundary of geographical distribution of both species. Morphological measures of vegetative features of these adults are similar to <i>Y. valida</i> , while floral and seedling features are similar to <i>Y. capensis</i> . Assignment analysis using nuclear microsatellites indicates that these populations share the gene pool of <i>Y. capensis</i> , which is a very interesting result since the morphological features are intermediate between both species. This result suggest an incomplete reproductive isolation between the two species.
6. Analyse the effect of fruit and pollen dispersal on genetic structure in <i>Yucca</i> populations, and male and female behavioral effects on genetic structure of <i>Tegeticula</i> populations.			x	The gene flow mediated by pollen apparently is high because there is no genetic structure among yucca populations assessed through nuclear microsatellites. However, because the chloroplast markers show few polymorphism, is not possible to conclude about the effect of fruit dispersion affecting the genetic structure. The mitochondrial data of yucca-moths suggest a high gene flow among populations of their host.

<p>7. Identification of specific regions with highest conservation priorities for yucca and yucca-Moth species, using a hotspot map of genetic diversity</p>		x		<p>In our filed work we observed that the region south of <i>Yucca capensis</i>'s distribution is suffering a rapid change of land use because tourism activities. These populations are in high risk to disappear in few time. Across its distribution, populations of <i>Y. capensis</i> are very spread in the landscape and each one is composed by less than 15 individuals, which is another factor increasing the extinction risk. In the other hand, population densities of <i>Y. valida</i> are higher and they are present in the landscape in a more continuous patch. To identify regions with highest conservation priority is important to have the complete genetic database, and we hope to get this result in the next months.</p>
<p>8. Seeds collection of endemic yucca species across their geographical distribution. Germination success and early establishment of Yucca seedling and their relation with genetic diversity and anthropogenic impact on population</p>			x	<p>We collected fruits from 45 trees in 15 localities. We extracted the seeds. Germination experiments are carrying out in the greenhouse since the beginning of this year. Measure of growing of seedling are taking every month, and they will be related with genetic diversity levels of populations where they come from.</p>

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

The standardisation of protocols to amplify the molecular markers in *Yucca* species was a very difficult process. After many months of work, we finally obtained good results in the amplifications, but the sequences of the three chloroplast regions selected showed low genetic variation. From the nine nuclear microsatellites for yuccas, seven work very well, but a problem of contamination in the lab delayed the advance and we could not genotype the complete sample size. We did not obtain the sample size proposed of *Tegeticula* larvae, because there were not fruits in all populations, neither all individuals with inflorescence, had fruits after some months.

Currently we are working to solve the contamination problem in the lab and in a few months we will have the complete data set of eight nuclear microsatellites for 200 trees (not 300 as was proposed). We established collaboration with others researchers interested in these species and with them, we will looking for other markers (from chloroplast and nuclear DNA) that can be useful to disentangle

the evolutionary history of *Yuccas*. In the next year, we will standardise the nuclear microsatellites to yucca-moths, as was proposed in the project.

During our fieldwork, we took several morphological measures of reproductive and vegetative features in order to contribute to knowledge of ecology of these species. We explored morphological variation and describe demographic aspects of species in their geographical distribution. We found very interesting patterns that will address our next steps in this project.

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

1. Nuclear data support the morphology-based hypothesis of two separate species of yucca in the peninsula. We found a high phenotypic variation across the distribution of each species, probably related with environmental features where the individuals are growing. This information is very relevant to conservation task because the planning should consider a representative sample of variation and diversity of each species.
2. Populations of the boundary of the geographical occurrence of both species show evidence of hybridisation. This pattern is supported with molecular and morphological data. The biology of these populations will help us to understand the evolutionary history of these endemic yuccas, such as the time of separation between both species and the effect of weak reproductive barriers, probably related to share the same pollinator.
3. The low genetic structure of yucca populations detected with nuclear markers, probably correspond to the pollen movement in the landscape. During the 2 years of sampling, we observed that the population fruit-set along the distribution of both species is very irregular. Because the maintenance of yucca-moth populations depends of fruit presence, the irregular production of fruits probably cause the movement of yucca-moth among yucca populations. Mitochondrial data of *Tegeticula* indicate that there is no genetic structure among individuals collected in different yucca populations, and nuclear data should supported this finding. This result suggest that yucca-moths probably use the resources available in a scale bigger than we expected, and the strong interaction is maintained despite the unpredictable pattern of inflorescence

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

N/A

5. Are there any plans to continue this work?

Certainly. This study is the beginning of a long-term project in my investigation career. In the next months the data will be completed to reach the objectives proposed in this study, and we will advance in the new questions that appeared during the progress of it. I hope that our work help to increase the ecological and evolutionary knowledge of these species and to understand the importance of these endemic yucca and *Tegeticula* in the peninsula ecosystems. Currently, there are undergraduate and graduate students involved in this project, as well as researchers of this region.

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

- The first scientific paper was accepted the last October.
Title: Phenotypic variation of flowering and vegetative morphological traits along the distribution for the endemic species *Yucca capensis* (Agavaceae)
Authors: Maria Clara Arteaga, Rafael Bello-Bedoy, Jose Luis León de la Luz, José Delgadillo, Reymundo Dominguez
Journal: Botanical Science. It will be published in the volume of 2015.
- We will work in two scientific manuscripts and one diffusion paper.
- We will present the results in academic meetings, two national and one international.

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

We started to use the RSG as well as this was available in my bank account. Our first field trip was in July 2013. Because it was not possible to do soil analysis we still have some budget and we are planning other trip in order to collect fruits from different populations and obtain yucca-moths and seeds.

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.

When I received the money, the local exchange rate used was approximately £ 1.00 Sterling Pound = \$18.2 Mexican Pesos. £5995.91 = \$109,354.65 **Amount received: \$109,078.34 mexican pesos (with bank discounts and commissions)**

Item	Budgeted Amount	Actual Amount	Difference	Comments
<i>Personnel</i> 1 field assistant £12.8/day/40 days	£512	£512		
<i>Equipment</i> GPS Garmin eTrex 30 (358.97); 1 Tree pruner 12ft (51.28); 1 Liquid nitrogen container (717.94); 2 Tubular soil sampler (51.28)	£1179	£1179		
<i>Materials and Supplies</i> 500 plastic bags (102.56); 500 tubes falcom of 15 ml (153.84)	£846	£257	-£589	The quality and soil characteristics in each site is highly variable in a small scale. For this reason, this data was not collected. We don't bought soil sample bags and not pay by soil analysis. The money will be used to do a field trip in order to

				collect fruits from different populations and obtain yucca-moths and seeds.
<i>Travels</i> Transportation among field sites (including car and fuel) £41.02/day/ 40 days (1641.02); Living stipends £20.5/day/40 days/2 persons (1641.02)	£3282	£3282		
<i>Other material to field and lab</i> flags, markers (51.28); alcohol and laboratory material (125)	£176	£176		
TOTAL	£5996	£5406	-£589	

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

Yucca populations are exposed to different kind of threats in the Peninsula of Baja California; *Yucca valida* and *Yucca capensis* occurring in areas where land use are changing hastily for agriculture and tourism. We need to work close to governmental entities in order to provide scientific information that help them to take decisions over land use changes considering the ecological services of the yuccas. Also, we need to prevent the increase of direct human exploitation over the endemic yuccas. *Yucca shidigera*, a species distributed in the north of the peninsula, is a resource highly exploited as a source of phytochemicals. We need to study the genetic and ecology of this species and assess the effect of human exploitation over genetic variation of its populations. It is a hard work to avoid the use of this resource by human communities, since the economy of the rural people depend of this activity. However, there is no a planning that establish exploitation rates of yuccas and we can work to get it.

Also, we can work to preserve the yucca populations (of three species) distributed within the areas of national parks and ecological reserves, because currently the rural people extract trunks of yucca from these population without any regulation. In this way, we need to establish a direct link between our research and regional governmental entities, in order to provide them our findings and work together to conserve the populations of the three *Yucca* species and their pollinators.

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

I have not used the RSGF logo, but I hope to do it in the next year when I present my work in scientific meetings. I have used the foundation name. In my webpage, I describe my project and I indicate a Rufford Foundation as the financial source of it.

In the acknowledgements section of the recent accepted paper, we thank the Rufford Foundation for the financial support to develop the study. (Title: Phenotypic variation of flowering and vegetative morphological traits along the distribution for the endemic species *Yucca capensis* (Agavaceae); Authors: Maria Clara Arteaga, Rafael Bello-Bedoy, Jose Luis León de la Luz, José Delgadillo,

Reymundo Dominguez; Journal: Botanical Science; Accepted: October 2014. It will be published in the volume of 2015)

11. Any other comments?

I really appreciate the support and trust of the Rufford Foundation because I began a study that now is a long-term project in my research line. The possibility to study endemic species of this region and work for their conservation is a noble and rich task. Indeed, the involvement with other researchers that share the same interest is very valuable. Finally, the information that result of our study can be used to know more about the history of biota from the peninsula, and also can help to governmental entities to designed more accurate preservation measures for the species.