

Final Evaluation Report

Your Details					
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Project Title	Identification of adaptive hotspots in endangered high montane ecosystems along the Sierra Madre Oriental in Mexico				
Application ID	31394-1				
Grant Amount	£5936				
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Date of this Report	February 9 th 2022				



1. 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
Evaluate the genetic resources of two keystone species for the three highest peaks within the Sierra Madre Oriental				We were only able to measure genetic diversity and structure for one of the two species proposed in the project. We did novel analyses on the missing species, the reason for this decision is detailed in the section below.
Estimate the content of adaptive variation present in the different populations				We conducted our analyses using a nuclear gene related with hydric stress.
Identify those geographical regions that house individuals with a high adaptation potential				We identified regions with unique haplotypes in high stress environments signalling a process of local adaptation despite the strong gene flow between populations.
Identify a possible relationship of ecological variables associated with adaptive hotspots on high montane areas				Based on PCA analyses we were able to identify the most important variables for species establishment.
Design new reforestation guidelines and conservation plans				Based on climatic projections and haplotype distribution we were able to design a reforestation proposal that could aid population survival under future climatic conditions.

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

The current pandemic was the first difficulty we encountered. We had the opportunity to go to exploratory expeditions on late 2020, but when we got the funds on December 2020, some of the team including my main supervisor and myself unfortunately tested positive and our field dates got delayed to early February 2021 to ensure the safety of the team and our physical condition due to the oxygen deficit on the collection sites.

For the project we were supposed to sequence and analyse genetic diversity using two candidate genes related with the species survival. Due to the rarity of genomic analyses on pine species we didn't have species specific primers available nor information about the amount of variability contained in each genetic region. From



four possible genes we had considered based on preliminary analyses, only one provided us with enough intraspecific variability for us to carry out the analyses. This took a lot of trial an error and sequencing trials.

Through our primer standardisation process, we discovered the possibility of a gene duplication on one of the two species that the project entails which made it difficult to get high quality sequences that we could use for further analyses. In order to clarify the uncertainty, we attempted to clone the PCR fragments to identify the duplication, this process took us 3 months. Based on this we designed a new set of primers that were specific for both species and sent all individuals for sequencing in October 2021, falling behind on our proposed timeline.

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

Genetic structure

We found high levels of heterocigocity for both *Pinus culminicola* and *Pinus hartwegii*. For the first species we found what we believe to be a gene duplication that we were able to confirm through a cloning process. This duplication complicated sequencing for all individuals within the populations that were sampled. Even though population sequencing wasn't achieved for this species, the possible duplication might have evolutionary implications for *Pinus culminicola*. Previous research suggests high functional genes might become duplicated to increase the tolerance of individuals against environmental stress (Bergthorsson y Katju, 2013; Bergthorsson y Katju, 2016). These results are promising as *P. culiminicola* faces according to climatic predictions analysed in the study very arid conditions in the future 35 years.

For *Pinus hartwegii*, we were able to observe through our analyses no structure between populations, which indicated that gene flow is ongoing between the three populations we sampled, and pollen dispersal is keeping populations from falling on endogamy (fig. 1) (Schmidt et al., 2008; Saenz-Romero et al., 2012; Sheng et al, 2005). This is important as it favours genetic diversity and therefore increases species survival against harsh conditions. Even the population sampled on Peña Nevada, which presented a very severe case of desertification shared haplotypes with populations found north, Sierra de la Marta and Cerro del Potosí.

Haplotype diversity

Our initial hypothesis was based on the premise that greater genetic diversity was found across an environmental gradient rather than between populations with homogenous environmental conditions. Through the analyses of haplotype distribution, we were able to conclude that 71% of the haplotypes found belonged exclusively to individuals at high of low altitudes (Fig. 2). This pattern of haplotype distribution was found in all three populations, indicating that even though gene flow is strong, local adaptation selects different genetic variants based on very specific climatic conditions (Saenz-Romero et al., 2012). These results are of high value and could aid in the design of translocation protocols based on climatic projections to move genotypes adapted to more arid areas to geographic locations where those conditions are projected for future decades.



Figure 1. Haplotype distribution on Cerro del Potosí, Sierra de la Marta, and Sierra Peña Nevada.



Figure 2a. Haplotype distribution between altitudinal gradients.





Figure 2b. Haplotype distribution between altitudinal gradients.

Translocation maps

Based on what appears to be a process of local adaptation between individuals stablished at different altitudes, we used climatic projections to analyse the possibility of translocation protocols and the establishment of seed zones that could help current and future reforestation measures (Williams y Dumroese, 2013, Castellanos, et al., 2018). We found that the climatic zones for *Pinus culminicola* and *Pinus hartwegii* will shift dramatically based on RCP 4.5 and RCP 8.5 projections in 2050. As showed in the maps below, the climatic areas showed in blue and green hues will almost disappear in the next decades (Fig. 3-8). For *Pinus hartwegii*, whose population range extends in some areas to 2500 m asl, germplasm of lower altitudes could be used to migrate upwards within the same population to where new climatic areas are projected. For *Pinus culminicola* that has a more restricted population distribution banking seeds is the more plausible option.



Figure 3. Climatic zones 1996-2015 for Sierra de la Marta and Cerro del Potosí. The polygons shown in purple represent the sampled areas (>2800 masl).



Figure 4. Climatic zones 1996-2015 for the Sierra de Peña Nevada. The polygons shown in purple represent the sampled areas (>2800 masl).



Figure 5. Climatic zones projected to 2050 RCP 4.5 for Sierra de la Marta and Cerro del Potosí. The polygons shown in purple represent the sampled areas (>2800 masl).



Figure 6. Zonas climáticas proyectadas al 2050 RCP 4.5 para la Sierra de Peña Nevada. Los polígonos que se muestran de color morado representan las áreas muestreadas (>2800 msnm).



Figure 7. Climatic zones projected to 2050 RCP 8 for Sierra de la Marta and Cerro del Potosí. The polygons shown in purple represent the sampled areas (>2800 masl).



Figure 8. Climatic zones projected to 2050 RCP 8 for the Sierra de Peña Nevada. The polygons shown in purple represent the sampled areas (>2800 masl).

Bibliography

Bergthorsson, U., & Katju, V. (2016). Gene Copy-Number Changes in Evolution. eLS, 1-11

Castellanos-Acuña, D., Vance-Borland, K. W., St Clair, J. B., Hamann, A., López-Upton, J., Gómez-Pineda, E., ... & Sáenz-Romero, C. (2018). Climate-based seed zones for Mexico: guiding reforestation under observed and projected climate change. New forests, 49(3), 297-309.

Katju, V., & Bergthorsson, U. (2013). Copy-number changes in evolution: rates, fitness effects and adaptive significance. Frontiers in genetics, 4, 273.

Sáenz-Romero, C., Mendoza-Maya, E., Gómez-Pineda, E., Blanco-García, A., Endara-Agramont, A. R., Lindig-Cisneros, R., ... & Vargas-Hernández, J. J. (2020). Recent evidence of Mexican temperate forest decline and the need for ex situ conservation, assisted migration, and translocation of species ensembles as adaptive management to face projected climatic change impacts in a megadiverse country. Canadian Journal of Forest Research, 50(9), 843-854

Sheng, Y., Zheng, W., Pei, K., & Ma, K. (2005). Genetic variation within and among populations of a dominant desert tree Haloxylon ammodendron (Amaranthaceae) in China. Annals of Botany, 96(2), 245-252.

Schmidt, P. S., SerrÃo, E. A., Pearson, G. A., Riginos, C., Rawson, P. D., Hilbish, T. J., ... & Grahame, J. W. (2008). Ecological genetics in the North Atlantic: environmental gradients and adaptation at specific loci. Ecology, 89(sp11), S91-S107.



Williams, M. I., & Dumroese, R. K. (2013). Preparing for climate change: forestry and assisted migration. Journal of Forestry, 111(4), 287-297.

4. What do you consider to be the most significant achievement of this work?

5. Briefly describe the involvement of local communities and how they have benefitted from the project.

At the beginning of 2021, a big fire affected approximately 12,000 ha of temperate forest in the Sierra Madre Oriental. The fire got close to our collection sites but stayed low in altitude and did not reach important populations of *Pinus hartwegii* or *Pinus culminicola*. Nevertheless, this was an opportunity for us to use our preliminary results and applied them to a post-fire reforestation proposal. Dr Susana Favela, project collaborator and my main thesis supervisor was part of the committee involved in the restoration plans. We both collaborated and wrote a proposal that involved climatic projections to assess the best possible sites to collect and translocate germplasm. We then took this proposal and worked closely with reforestation agencies, visited the affected sites and worked with local communities to teach them the correct guidelines to plant seedlings and prevent future fires (Fig. 9-10). This also created jobs for the locals since agencies employed them to plant and care for the reforestation sites.



Figure 9. Aerial picture of a site affected by the fire.





Figure 10. Working on site based on our reforestation proposal.

6. Are there any plans to continue this work?

Yes, we would love to continue this work, based on our results we believe there is plenty of more we could use this gene primer for. We want to explore the possible gene duplication we found on the Cembroides section of pines and its evolutionary repercussions. We would also like to analyse if this pattern of distribution of haplotypes and local adaptation persists on other species that are important for the prevalence of temperate forests in Mexico.

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

Thesis

The outcome of this project are the main results of my MRes thesis. The thesis was submitted in late December 2021 and dissertation is scheduled for March 18th, 2022.

Scientific papers

The results of the project are going to be used to prepare and submit two scientific papers. One for a national indexed journal that shared a general perspective of temperate forests against the future climatic conditions they will face.

"Current Situation of the Temperate Forests from the Sierra Madre Oriental under a Climate Change Scenario. Treviño-Cuéllar, Katia, Favela-Lara, Susana. Revista Mexicana de Ciencias Forestales. In prep."

We are awaiting samples to have more robust data and submit later this year a paper with all our results to an international journal, some options we are considering are Molecular Ecology and Ecology and Evolution by Wiley.



Workshops

We plan to continue collaborating with reforestation agencies as well as "Parque Ecologico Chipinque" (Natural Protected Area Park) to inform local decisions based on our final outputs from the project.

Conferences

Preliminary results of this project have been presented in one international congress and one national symposium.

International Congress

	OF PRESENTATION
K.L. Treviño-Cuéllar, S. F S. Pér	avela-Lara, E.P. Gómez-Ruiz, ez-Espona
"Identification of Adaptiv Ecosystems from the I Landscap	ve Hotspots in High Montane Northeast of Mexico using ne Genomics"
"That was presented d Conference Conservatio 2020 held on-line from	uring the 2nd International n Latin America and Caribean m 9 - 13 November, 2020"
"That was presented d Conference Conservatio 2020 held on-line from	uring the 2nd International n Latin America and Caribean m 9 - 13 November, 2020"



National Symposium



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

From February 2021 to December 2021

This is different from what we originally proposed. The project was approved for funding earlier on 2020 but we got awarded with the grant until December 2020 due to Covid restrictions. The pandemic also changed and limited our field tips and lab access at for the first half of last year which delayed our obtention of results.

9. Budget: Provide a breakdown of budgeted versus actual expenditure and the reasons for any differences. All figures should be in \pounds sterling, indicating the local exchange rate used. It is important that you retain the management accounts and all paid invoices relating to the project for at least 2 years as these may be required for inspection at our discretion.

Item	Budgeted Amount	Actual Amount	Difference	Comments
Silica Gel	320	320		We still own silica gel that hasn't been used up. It is still being used for



Dntp´s Taq Polymerase Sequencing	288 344 1440	288 344 1440	Even though we didn't sequence all individuals of <i>Pinus culminicola</i> , we did end up using all the sequencing resources because we had to do a
			lot of tests in order to standardize the primers and switched to a provider that was more expensive but delivered better quality sequences that at the end saved up sample costs.
Lodging	1000	1000	
Food	720	720	
Barometric Altimeter	72	72	We bought two GPS instead to facilitate the sampling process.
Car Rental and Gas	1752	1752	
TOTAL	5936	5936	

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

I believe that applying the data we obtained to more reforestation guidelines and management plans is crucial if we are to help forests and plant communities to survive the rapid changing climate.

One of the next steps could be to identify the priority regions as well as species, using phylogenetic diversity and evolutionary distinctiveness indices to understand the distribution of vulnerable areas across the Sierra Madre Oriental Mountain range. This would allow us to pinpoint the species that need protection and implement the methodology used in this project to study if they share the same pattern of haplotype distribution and start planning for future climatic scenarios.

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

Yes, the Rufford Foundation Logo was used in both the international and national presentations.



12. Please provide a full list of all the members of your team and briefly what was their role in the project.

Dr Susana Favela Lara

Main Thesis Supervisor. Her role was mainly the direction of the project, she also drove and collaborated in all field trips. Dr Susana Favela Lara supervised the analyses, results, and coached the project conclusions. She also collaborated with the Biodiversity and Restoration of the Cumbres de Monterrey National Park Committee to write the post-fire reforestation proposal.

Dr Sílvia Pérez Espona

Her role consisted of giving guidance and direction to the project as well. She gave instructions on the cloning protocols to follow and overviewed the quality of the sequences.

BSc Katia Lizeth Treviño Cuéllar

As project leader I was mostly responsible for the project. I designed the sampling transects. Collected and organized the samples coordinates. Extracted DNA, standardized PCR primers, and prepared samples for sequencing. I also conducted the cloning trials and eventually analysed all the data. I constructed the translocation maps and collaborated on the post-fire reforestation proposal.

BSc María Susana Sánchez Chávez

She assisted in all field trips and helped with the collection of samples, DNA extractions and with some PCR runs to prepare samples for sequencing.

Dr Emma Gómez Ruíz

Her main role was to train me in GIS to be able to construct the maps used for sample collection, PCA analyses and translocation areas.

13. Any other comments?

We would like to express our gratitude towards The Rufford Foundation for the support throughout this project. We know the pandemic circumstances have slowed down our results and truly appreciate the opportunity and understanding. We look forward to future collaborations.

All figures and pictures in this report are mine and I approve their use by The Rufford Foundation.