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. 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	Lida Marcela Franco Pérez
Project title	Dispersal, population genetic differentiation and kinship of an ancient marsupial (*Dromiciops gliroides*) in a highly fragmented landscape.
RSG reference	8910-1
Reporting period	December 2012 – June 2014
Amount of grant	£6000
Your email address	lidamarcelafranco@gmail.com
Date of this report	June 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. Abundance of *D. gliroides* in uninterrupted forest and fragmented forest			X	We achieved this objective and find that the abundance of *D. gliroides* was higher than expected, principally in fragmented forest.
2. Communal nesting in *D. gliroides*			X	Our results suggest that communal nesting is more related to parental care associated with kin selection than to thermoregulation.
3. Genetic analyses		X		
Collect DNA samples of different areas surrounded by different use-land			X	We obtained 258 samples of seven different areas. Those samples were obtained along different years including those supports by this grant in particular.
DNA extraction			X	We were able to extract and successfully amplify both microsatellite and mitochondrial DNA markers. [on-going]
Isolation of polymorphic microsatellites loci for the species			X	Two next generation sequencing were carried out, after a DNA library preparation of fragments between 400 and 2000 bp. A total of 434965 lectures were generated. Within these lectures, we conducted a microsatellite mining of different motifs, with a final result of 228 microsatellites motifs. Of those, we find 12 polymorphic loci.
Genotype of individuals from different populations		X		Because the time consuming and expenses involved into the isolation of microsatellites loci, we were unable to genotype until today all the 258 samples obtained from the different areas.
D-loop gene sequencing			X	We were able to correctly amplify a fragment of 401 bp of the D-loop gene for a total of 221 samples. Because low quality DNA of some samples, we were not able to amplify 37 samples.

Landscape genetics and Population genetic analyses		X		Because we have just carried out population genetic analyses with the mitochondrial DNA, we have not accomplished until now this objective. However we are looking to finally amplify the 12 microsatellite loci for all the individuals and carryout the landscape genetic analysis.
Kin relationships	X			This objective will be fully achieved when we complete the genotype of all individuals
4. Conservation strategies			X	The results obtained in this research were showed and integrated in local initiatives through a process participating that include the interaction with local communities, government entities and people of different education levels

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

Because the lack of specific microsatellites loci for the species, we evaluated two approaches to overcome this problem. One was to search for specific microsatellites loci into the genome of the species and the other one was to try cross-species amplification. Because we think this species presents a real conservation problem, we decided to search and described microsatellites for the species. Because this has take a longer time than expected and was more expensive than budgeted, we are currently genotyping samples and therefore landscape and kinship genetic analyses have not been done until now.

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

From objective 1: We found significantly greater body mass and Body Condition Index for females than for males, suggesting different energetic strategies during the period of capture. Our results reveal that population characteristics of *D. gliroides* are closely linked to reproductive period, which extends across the austral spring and summer. Adults were more abundant in spring and early summer. The abundance of *D. gliroides* was higher than expected. However, it was higher in fragmented forest (18-22ind/Ha) that in uninterrupted forest (6-7ind/Ha). The results suggest that in fragmented forest the individuals are isolated and confined to small fragments, with scarce or null connectivity due to fragmented landscape of native forest bordered by exotic plantations and grasslands. This, together with little ability to disperse result in population confined to fragment isolated with few possibility of dispersal. Although genetic results showed a different pattern, we explained below these results.

From objective 2: Communal nesting occurred during summer and early fall, but torpor by single individuals and small groups was increasingly frequent during winter. Communal nesting could be a key behavioural strategy affecting survival. Given the greater frequency in warm seasons and groups composed of post-reproductive females and juveniles, our results suggest that communal nesting is more related to parental care associated with kin selection than to thermoregulation. In this sense, in *Dromiciops gliroides* is more efficient the torpor as energetic saving strategies than huddling. Finally, the continuity of this goal since the first Rufford, allowed us to conclude that the communal nesting is a strategy of parental care necessary for the survival of the offspring during the summer.

From objective 3: Genetic Analyses. After analysing a total of 221 samples from seven different localities we have find a high genetic diversity for the mitochondrial DNA. Among the 221 samples, 22 haplotypes were revealed and a haplotype diversity (hd) of 0.8865. This high genetic diversity can be explained by several nonexclusive reasons. One of those reasons is that not enough time has elapsed since the start of logging at the first decades of the twentieth century in our study area. Thus, is probably that populations are still great enough to maintain this genetic diversity. This last combined with not enough generations since the forest reduction, can explain the retention of a high number of haplotypes. Finally, in previous studies, it has been reported a low mobility for the species, this last link with short generation times, can result in constant effective population sizes in our study area.

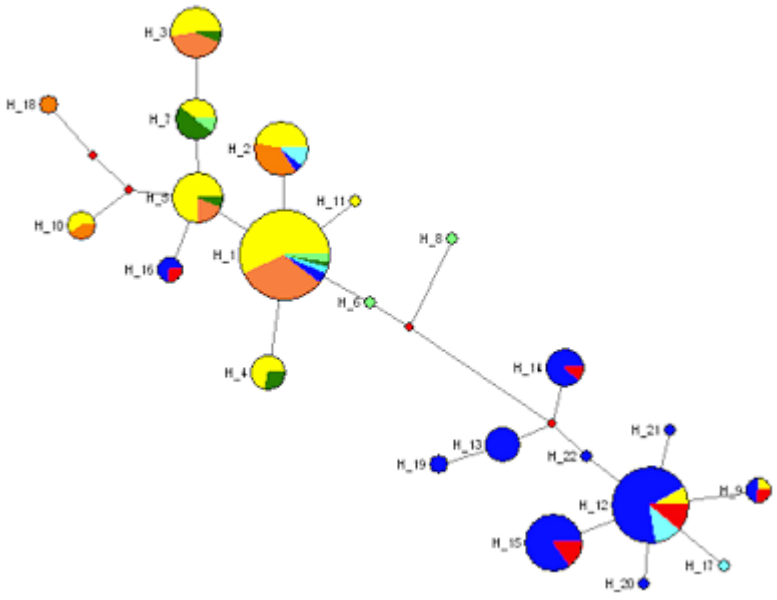
- A fine scale population structure was found among the different areas of our study. Pairwise comparisons between the seven areas revealed that both areas in Oncol Park and both areas in San Martin were not different within each other. This makes sense, because both sampling areas in Oncol, corresponds to a unique big fragment of forest separated by almost 10 km. The same happens with both areas in San Martin, where just a single unpaved road separated the forest fragment in two, with apparently none road effect in the genetic structure of the population. Interesting is that although San Martin and Oncol are separated by at least 10 km, none genetic population structure was found with a mitochondrial marker. This absence of significant differences, can be the result of a more less continue native forest and a more recently logging activity than the one occurred closely to big urban areas (such as Valdivia). Significant differences were found between both areas of San Martin and Fundo Llancahue, Fundo Donoso and Forestal Calle-Calle, as well as between

both Oncol areas and the previous three mentioned areas (Table 1). No differences were found among these three areas. The differences between San Martin/Oncol with the previous three mentioned areas could respond to the presence of the Calle-Calle River, which has been acting as an old barrier, structuring the population in a fine scale. The absence of significant genetic population structure between these three areas could be the result of the short distance between the three fragments. This fine scale population genetic structure can be better visualise with a haplotype network (Fig. 1). Although there are some common haplotypes for all the areas (Fig. 2, Fig.3, Fig.4, Fig.5) it is possible to distinguish two clusters, one of them with a majority of samples from Oncol 1, Oncol 2, San Martin 1, and San Martin 2 and the other one with the bulk of the samples from Llancahue, Calle-Calle and Donoso areas. Thirteen mutational steps separate both haplotype clusters. This reinforces the idea that this amount of divergence can be the result of a long separation time caused by the river.

- An interesting result of this research was the cloning of microsatellites loci for the species. Once we finished of genotype all individuals, we will have new insights about the population structure and about the kin relationships of the species.

Table 1. Φst pairwise comparisons significant differences after 10000 permutations.

	San Martin 1	San Martin 2	Calle-Calle	Donoso	Llancahue	Oncol 1	Oncol 2
San Martin 1		-	+	+	+	-	-
San Martin 2	-		+	+	+	-	-
Calle-Calle	+	+		-	-	+	+
Donoso	+	+	-		-	+	-
Llancahue	+	+	-	-		+	+
Oncol 1	-	-	+	+	+		-
Oncol 2	-	-	+	-	+	-	



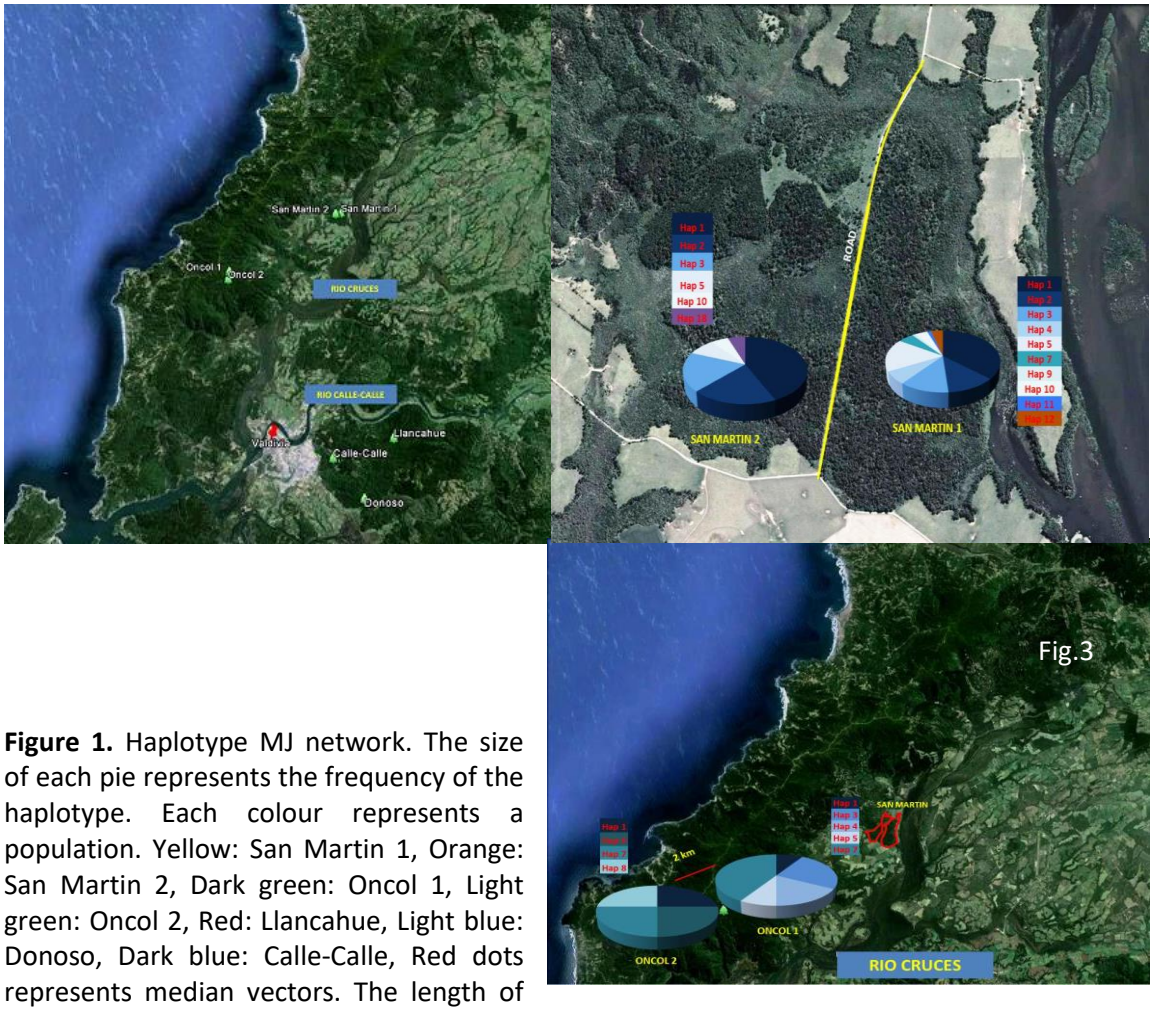
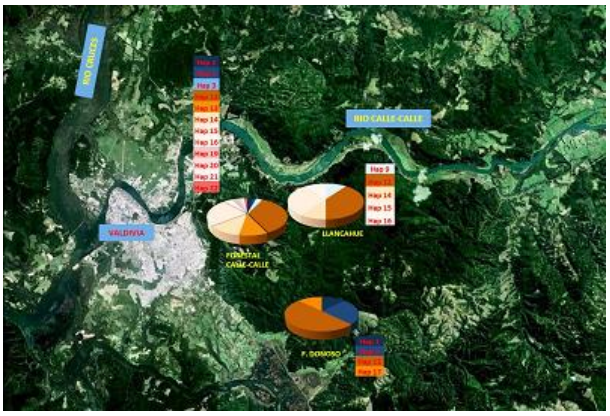


Figure 1. Haplotype MJ network. The size of each pie represents the frequency of the haplotype. Each colour represents a population. Yellow: San Martin 1, Orange: San Martin 2, Dark green: Oncol 1, Light green: Oncol 2, Red: Llancahue, Light blue: Donoso, Dark blue: Calle-Calle, Red dots represents median vectors. The length of each branch is proportional to the mutational steps (1 or 2), except for the longest branch which represents 13 mutational steps.

Figure 2. Figure 2,3,4,5. Representation of haplotypes in each locality. (2) Spatial configuration of the localities sampled, (3) San Martin 1 y 2, (4) Oncol 1 y 2, (5) Calle-Calle, Donoso y Llancahue.

From objective 4: Conservation strategies. Results obtained from the first and second Rufford Small Grant provide scientific support and insight of different ecological, behavioural and genetics characteristics of *D. gliroides.* At this point this has provided us with enough information to propose conservation and management strategies that guarantee the conservation of this species and its habitat. Considering this, the results obtained in this project were shared with local people, regional and rural schools and representatives of environmental government entities (CONAMA) guaranteing a promissory future for this small mammals at least in the areas of study. Taking into account the genetic results it has to be highlight that although we find a high genetic diversity and almost no genetic population structure at a fine scale range but differences between two great areas *i*) Oncol/San Martín and *ii*) Llancahue, Donoso and Calle-Calle, we recommended at a conservation strategy to implement a corridor between areas of the first cluster with the aim to maintain genetic flux among them, the same is recommend for the areas of the second cluster. Also, taking into account the great divergences between both clusters, we recommend to maintain each other separate as different evolutionary significant units (ESUs).

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



The results obtained in this research were showed and integrated to propose conservation strategies, through a participating process that includes the interaction with the local communities, government entities, and people with different educational level (e.g. rural inhabitants, gamekeepers, researchers. teacher schools, secondary school students and elementary school students). We elaborate educative materials showing the ecological role of *D. gliroides* and importance of monito del monte as key species for the conservation of the temperate rainforest ecosystems.

This material was distributed in the community and in local and regional secondary and elementary schools with the aim to implement thrusting initiatives together with environmental and educational programme that raising consciousness and permit minimise the deforestation of the native forest. However, it is necessary to continue working to create consciousness and sustainable management strategies highlighting the importance of the temperate rain forest as a model of rural development for

the this region of southern Chile. The integration of the local community in this project has been of great importance. It was a multi-year process, but we achieve the goal that the community and rural people knew about the monito del monte and its importance to the ecosystem. It can be anecdotally mentioned that in certain occasions when secondary forest trees are cut, the people find monitos del monte hibernating (somewhat rare). After this and because the education process of our project, the woodcutters already know the importance of the species and try to save the individuals involved in their activity, usually with great success. In this second RSG, a total of eight primary, secondary and rural schools were visited. During the visits, we implemented workshops about the species and the importance of the rainforest for its conservation. Within these workshops we delivered educational material, theoretical and dynamic discussions around *Dromiciops* and the different components of its ecosystem. The local and rural selected schools had an average of 20 and 40 students each. Finally, we conducted an activity together with the EXPLORA Project of CONICYT (Comisión Nacional de Ciencia y Tecnología de Chile), in which there was an interesting exchange of experiences and information about *Dromiciops*.

5. Are there any plans to continue this work?

Yes, we are currently working on the lab analyses with the aim to genotype all the samples with the 12 microsatellite loci described. With this, we aim to carry out the landscape and kinship analyses. Also, we are planning to include in a short future more samples from other populations with the aim to carry out a complete population genetic analysis.

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

We are working on two papers to publish in peer-reviewed journals. One of them describing the microsatellites loci and the other one about genetic population structure of the species in the area we worked. Also, we have for other two papers, once the complete multi-locus genotypes were obtained.

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

The RSG was used from December 2012 to June 2014. Because the lack of specific microsatellites loci for the species and that we think this species presents a real conservation problem, we decided to search and describe microsatellites for the species. However, this has taken a longer time than expected and was more expensive than budgeted, we are currently genotyping samples and therefore landscape and kinship genetic analyses have not been done until now. Likewise, another aspect that extended the time projected for the completion of this research was the capture period of *D. gliroides,* which is linked to reproductive period and extends across the austral summer. Then, was necessary wait each year to spring and summer to make campaigns capture. Finally, the first years the captures were lower than expected and we could not have the number of individuals necessary for the analyses, so it was necessary to maximize the capture time in the second year.

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.

Budget. Funding were requested for two season of field work during the reproductive period of *D. gliroides* (November 2012 - March 2013, November 2013 - March 2014), and during the hibernation season (May to August 2013). Funding was also requested for field equipment (sleeping bags, tent) and food during the trip days. The budget included the total cost of travel and subsistence to three persons required to successfully complete the field work and laboratory during genetic analyses within the allotted time. In addition, was requested material of laboratory for these analyses, to be conducted during the last 2012 and 2013.

Item	Budgeted Amount	Actual Amount	Difference	Comments
1. Travel of field work. Vehicle rental and gas (5 travels (5 days/travel) 374£ per travel to each localities)	1870 £	1870 £		
Subsistence for 3 people (25 days per five travel, £24 per day)	600 £	450 £	150£	The budget for subsistence was less than expected
Camping equipment: (2 tent for 3 people 267 £, water proof clothes 160£ and 3 sleeping bags 280 £) **Subtotal:**	703 £ **3173** £	703 £ **3023 £**		
2.Educative materials: Illustrative poster (200 poster)	330 £	330 £		
Adhesive picture (150 adhesive) and broadcast talks **Subtotal:**	630 £ **960 £**	430 £ **760 £**	200 £	The budget for illustrative poster was less than expected
3. Laboratory analyses of 221 samples of tissues of *D. gliroides:* -Next-generation sequencing. -Fluorescent labelled and not fluorescent markers. -Sequencing and genotyping **Subtotal:**	1867 £ **1867 £**	 1714 £ 932 £ 785 £ **3431 £**	1564 £	Due to the lack of specific microsatellites loci for the species, we decided to search and describe microsatellites. However, this has taken a longer time than expected and was more expensive than budgeter.
Total	**6000 £**	**7214 £**	**1214 £**	

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

We think the next step is to finish the genotyping of all the samples and after that conduct the landscape genetic analyses. Once we finish that, it will be very interesting to conduct a global population genetic diversity and population genetic analyses (including samples from different localities among the species distribution range). With these results, we can evaluate if the population is structured or if it is just a historical differentiation as showed by the mitochondrial DNA. After that, we aim to delineate possible connectivity corridors among populations and propose them to the government authorities.

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?

Yes, below are the main logos (in Spanish language) of my "Conservation of the Monito del Monte

Campaign". Left: poster allusive to monito and its parental care (show a mother and her pup); right: two different adhesives allusive to *Dromiciops* and finally, a banner showing

Yes, I highlighted the support from RSG in all cases.

11. Any other comments?

We are very grateful for the second support for our project, which permit us carry out successfully the proposed objectives. Without these important funds, our research will be inconclusive. I hope that this report will be fully satisfactory for you and your RSG team. Our principal interest is follows with the genotyping and landscape genetic analyses in the studied populations. Finally, we want to wish success and congratulate RSG for the great job that has been doing with the aim to conserve species and ecosystems around the world.