

## 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](mailto:jane@rufford.org).

Thank you for your help.

**Josh Cole, Grants Director**

Grant Recipient Details	
Your name	Carolina Laura Morales
Project title	Interspecific pathogen transmission as potential cause of ecological replacement of native <i>Bombus dahlbomii</i> by introduced invasive bumblebee species, Argentina
RSG reference	15.01.10
Reporting period	16 months
Amount of grant	£5719
Your email address	<a href="mailto:cmorales@conicet.gov.ar">cmorales@conicet.gov.ar</a>
Date of this report	14th May 2012

**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
Sampling <i>Bombus dahlbomii</i> , <i>B. ruderatus</i> and <i>B. terrestris</i>			X	We could fully achieve the proposed sampling. Furthermore, we greatly expanded our geographic sampling area and triplicated the sampling effort (number of sampling points and national parks) and replicated the samplings in a subset of sites in a second season, as explained below (point 2.).
Staining and microscope identification of parasites			X	Samples of collected bumblebees were observed under the microscope. Once the parasites were identified in any individual of any of the bumblebee species, we proceeded to extract DNA directly from the remaining samples to avoid PCR contamination. We could identify by microscope putative <i>Apicystis bombi</i> and <i>Crithidia bombi</i>
DNA isolation of parasites			X	Whole bumblebee DNA was extracted for PCR analysis from field-collected individuals. DNA of <i>Apicystis bombi</i> and <i>Crithidia bombi</i> was isolated.
PCR set-up and Sequencing runs		X		Gradient PCRs and different magnesium concentrations were used for the setup of <i>Nosema bombi</i> , <i>C. bombi</i> and <i>A. bombi</i> . We performed PCRs for different DNA regions (ITS, COI, COII and others when available). Direct sequencing was performed for some regions, but when double bands appeared they were not cloned, as no budget was anticipated for this extra lab efforts.
Non-invasive PCRs trial		X		We collected feces and specimens of exotic bumblebees in the field and preserved them until PCRs were set up. Next step will be to screen specimens for parasite presence, and then attempt to amplify parasites from their faeces.
Analysis in old samples and collections			X	We were able to recover bumblebee DNA ( <i>B. dahlbomii</i> and <i>B. ruderatus</i> ) as well as parasite DNA ( <i>Crithidia bombi</i> )

				from collections and museum samples up to 18 years old.
Writing results and reports			X	We have communicated our results in several ways: manuscripts submitted to scientific journals, technical reports and public outreach activities, congress presentations, and workshops (see below, points 4. and 6.).

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

We recorded a drastic decline of *Bombus dahlbomii* in the areas originally proposed for our samplings: Nahuel Huapi and Lanin National Parks (i.e., the northern portion of its historic distribution and the area of initial invasion of introduced *Bombus* species and of earliest establishment). Despite reporting this population collapse is *per se* a result of the project, the impossibility of finding specimens of the native species in the field precluded us to assess the current presence and prevalence of parasites for this species in that region. We tackled these difficulties expanding our sampling area southward from Nahuel Huapi National Park. Thus, we increased the number of sampling points from 10 to 36 and from two to six national parks (From North to South: Lanin, Nahuel Huapi, Lago Puelo, Alerces, Perito Moreno and Los Glaciares National Parks), covering an N-S transect of >1200 km until the southern tip of continental South America. In addition, we included a second sampling year (summer 2012) for the northern part of the distribution (Lanin and Nahuel Huapi National Parks) to increase the chance of finding *B. dahlbomii*. Furthermore, during that second season we could survey Tierra del Fuego Island, in the southern extreme of South America, thus completing the whole reported distribution range of *B. dahlbomii* in Argentina. This expanded geographical area allowed us to assess the status of *B. dahlbomii* populations along an invasional gradient and collect native individuals to investigate their sanitary condition in regions free of invasive species.

A further difficulty arose as a consequence of the eruption of the Puyehue Vulcano, in June 2011. This natural catastrophe strongly impacted our city and region. Beyond the impossibility to work during a couple of weeks after the eruption because of lack of electricity and internet connection and the health risks due to ash in the atmosphere, the major constraint was that during almost eight months the local airport was closed. Thus, the deliveries of molecular reagents were delayed during those months and therefore the completion of the project was delayed as well.

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

1-Our large scale survey, which encompasses the whole continental range of *B. dahlbomii* eastward from the Andes, showed that the abundance of invasive bumblebees explained the local extirpation and geographic range reduction of the native bumblebee. This pattern is consistent with a progressive southward displacement of native by invasive bumblebee species.

2-No parasites (*Nosema bombi* or *Apicystis bombi*) were found in specimens of both the native *Bombus dahlbomii* and the first invasive species *B. ruderatus* collected before 2006, suggesting

that the Patagonia region was free from these parasites until invasion of *B. terrestris* in 2006.

3-We found *A. bombi*, a highly pathogenic parasite, in specimens of both invasive species (*B. ruderatus* and *B. terrestris*) collected after the invasion of *B. terrestris* in 2006, suggesting that *A. bombi* was co-introduced by *B. terrestris* and transferred *in situ* to *B. ruderatus* in the Patagonia region.

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

During both sampling summers we worked close together with the staff of the six National Parks we visited. We offered a workshop about the impact of invasive species on the native *Bombus dahlbomii* in El Chalten, Los Glaciares National Park, upon request of the local community and park rangers, who were already concerned about the perceived decline of the native species. In particular, park rangers are in permanent contact with the local community, naturalists and visitors to the National Parks and thus educational efforts are rapidly spread to the general public. An additional and important beneficial long term and indirect impact of this work was achieved through the technical advice provided to two different governmental agencies in charge of biodiversity conservation. Namely we advised the Wildlife Service of Neuquén Province (Departamento de Fauna de Neuquén) in relation to permits request of bumblebee collection and the Wildlife National Authority (Secretaría de Ambiente y Desarrollo Sustentable de la Nación) in relation to native bumblebee exportation. In both opportunities we could provide up-to-date information on the conservation status of the species involved and our preliminary results supported the process of informing decision making.

#### **5. Are there any plans to continue this work?**

Definitively we are planning to continue with this work. The results we obtained from this short project are highly promising, in terms of suggesting a relation between the invasion of introduced *Bombus* species, the spread of previously undetected pathogens and the collapse of the native species. However, we need more information in order to establish the cause-effect relationship among these factors in order to inform conservation measures.

In particular we plan to:

1. Analyse bumblebee feces to set up this non-invasive technique for the native bumblebee species for future samplings.
2. Screen all samples of the three bumblebee species collected along these two seasons for the presence of *Apicystis bombi*, *Crithidia bombi* and *Nosema bombi* in order to correlate the pattern of *B. dahlbomii* decline at the geographical scale with the presence and prevalence of parasites in both native and invasive bumblebees. This will allow us to disentangle the mechanisms underlying the pattern described in point 3.1.

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

We have already started to share the results in several ways and for a broad audience/readership (scientists and general public).

## Scientific communications

### Written communications:

- The manuscript “Co-introduction and spillover of parasites by invasive *Bombus terrestris*: Bumblebee's chauffer hitchhike alien parasites when traveling to Patagonia, Argentina” is now under review in the Scientific Journal *Biological Invasions* (Manuscript ID: BINV-S-12-00182), submitted on 7<sup>th</sup> March, 2012.
- Two manuscripts for scientific journals are currently in preparation. One ms. describes the ecological replacement of native by invasive bumblebees at geographical scale. The other one, in collaboration with the Lab. of Agrozoology of Ghent, Belgium, compares our Patagonian *Crithidia bombi* haplotypes with those found in Europe.
- A preliminary report to the National Park Administration (in Spanish, available upon request).

### Oral communications:

We presented our results in the following scientific meetings:

- Morales, C.L., Arbetman, M. y Aizen, M.A. (2011). La globalización en el mundo de los polinizadores: impacto de abejorros en la biota nativa. Simposio Impacto de especies invasoras en la Patagonia. II Jornadas de Ciencias Naturales de la Patagonia, Esquel, Argentina.
- Morales, C.L., Aizen, M.A. (2010) Reemplazo ecológico del abejorro nativo (*Bombus dahlbomii*) por abejorros introducidos (*B. terrestris* y *B. ruderatus*). 2do Taller Iberoamericano sobre degradación de hábitats y funcionamiento de interacciones planta-animal, Bariloche, Argentina.
- Arbetman M., Meeus I., Morales C., Smagghe G. y Aizen M (2010). La transmisión de parásitos patógenos entre abejorros introducidos y el nativo: un mecanismo complementario a la competencia que explicaría el colapso poblacional de *Bombus dahlbomii* en el noroeste de Patagonia. 2do Taller Iberoamericano sobre degradación de hábitats y funcionamiento de interacciones planta-animal, Bariloche.
- In the context of the Congreso Argentino de Entomología (Abril, 2012) we organised a symposium entitled “La conservación de los abejorros (*Bombus*) a escala regional y global desde una perspectiva multidisciplinaria” aimed to share experiences with colleagues from other regions, and to raise the profile of bumble bee conservation efforts and research, motivating graduate and undergraduate students to develop future studies within our research group. Prof. Sydney Cameron (University of Illinois, Urbana, [www.life.illinois.edu/scameron](http://www.life.illinois.edu/scameron)), as authority in bumblebee conservation in the United States was invited to give a keynote presentation focused on the North American situation, decline problem and perspectives. Cecilia Smith Ramirez (Facultad de Ciencias, Universidad de Chile. Instituto de Ecología y Biodiversidad, IEB) reported the conservation status of *B. dahlbomii* in south Chile.

In this symposium we presented the following lectures:

- Morales, C.L., Arbetman, M. y Aizen, M.A. (2012) Una exploración sobre las posibles causas locales y regionales de la declinación global de especies de abejorros (*Bombus*). VIII

Congreso Argentino de entomología, Bariloche, Argentina.

- Arbetman, M., Morales, C.L, Smaghe G., Aizen M. A., Meeus, I. (2012) Detección e identificación molecular de parásitos patógenos en abejorros de la Patagonia de Argentina. VIII Congreso Argentino de entomología, Bariloche, Argentina.

### **Popular communications**

In addition, we presented our results for high school students in two different events aimed to increase public awareness on the value of nature and insects: the “Entomology week” organised by the Argentinean Society of Entomology (Sociedad Argentina de Entomología,) and in the “Presentations about our Nature” organised by the Naturalist Andean-Patagonic Society (Sociedad Naturalista Andino Patagónica, SNAP,). We also had an oral interview in a local radio.

In the next future we plan to write a popular article invited by the Editorial Committee of “Macroscopia” (<http://www.nahuelhuapi.gov.ar/comunicacion/macroscopia.html>), a Technical Bulletin focused on the cultural and natural values of the Nahuel Huapi National Park. In addition we are designing educational material (brochures) for National Park visitors and local community in collaboration with the press department of the Nahuel Huapi National Park.

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

Anticipated: From January 2011 to October 2011

Actual: From July 2010 to April 2012

The extension of the length of the project beyond the anticipated time frame resulted from the following circumstances:

First, we received the funds earlier than expected (late June 2010) and despite field work started in January 2011 as anticipated we started with other activities related to the project as soon as we got the funds, in July 2010. Namely, we took advantage of the unique opportunity of collaborating with the Laboratory of Agrozoology at Ghent University, Belgium, which is in the front edge of techniques of broad range detection of bumblebee parasites by means of PCRs. Thus, we screened a subset of our samples of *B. dahlbomii*, *B. ruderatus* and *B. terrestris* from museum collections during a short visit of the project member Marina Arbetman to that Laboratory in August 2010. More important, our previous knowledge and expertise in parasite diagnosis was greatly improved as a result of this collaboration.

Second, we expanded our research effort compared to the anticipated project by triplicating the number of sampling sites and National Parks during the summer season (January to March 2011), and by replicating the surveys in the northern portion of the distribution of *B. dahlbomii* in a second season (January to March 2012, see more details in point 2.).

Third, in November 2011 I had a daughter and therefore I asked for an extension of the final report deadline because of maternal leave.

Finally, the unexpected inconveniences driven by the Puyehue Vulcano eruption partly delayed the finalization of our project (see point 2.).

**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.**

See below

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

In my view, the important next steps should be:

1. Link the presence of pathogens with the effect that these may cause in the native bumblebee. In order to do so, we need to set up a breeding protocol for the native *Bombus dahlbomii* in places where we still find healthy populations, for future restoration plans.
2. Find more DNA variable regions of other parasites to identify the geographical origin, and be able to test the hypothesis of pathogens co-introduction with invasive bumblebees.
3. Extend the range of the sampling to Chile to include the whole geographic distribution of the native bumblebee species.
4. Develop specific neutral markers (microsatellites), as tools for the assessment of genetic bottlenecks and population structure of the native species.

**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, we used the RSGF logo in all instances where we presented our results and acknowledged the Rufford Small Grants in the appropriate section of submitted manuscripts. In addition, I mentioned the RSG Foundation in our radio interview.

**11. Any other comments?**

PDF copies of all materials are available upon request. This includes: power point presentations, certificates of scientific meetings, submitted manuscripts and manuscripts in preparation. However we ask Rufford Small Grants please not to make public the information contained in them until they have been published. We commit to send copies of all scientific and popular articles and other materials published in the next future as a result of this project. In addition technical reports to governmental authorities (in Spanish) are available upon request. However as the reports include confidential information about third parties (Company names and projects, etc.), this material should not be disclosed or published in the RSG webpage or elsewhere.

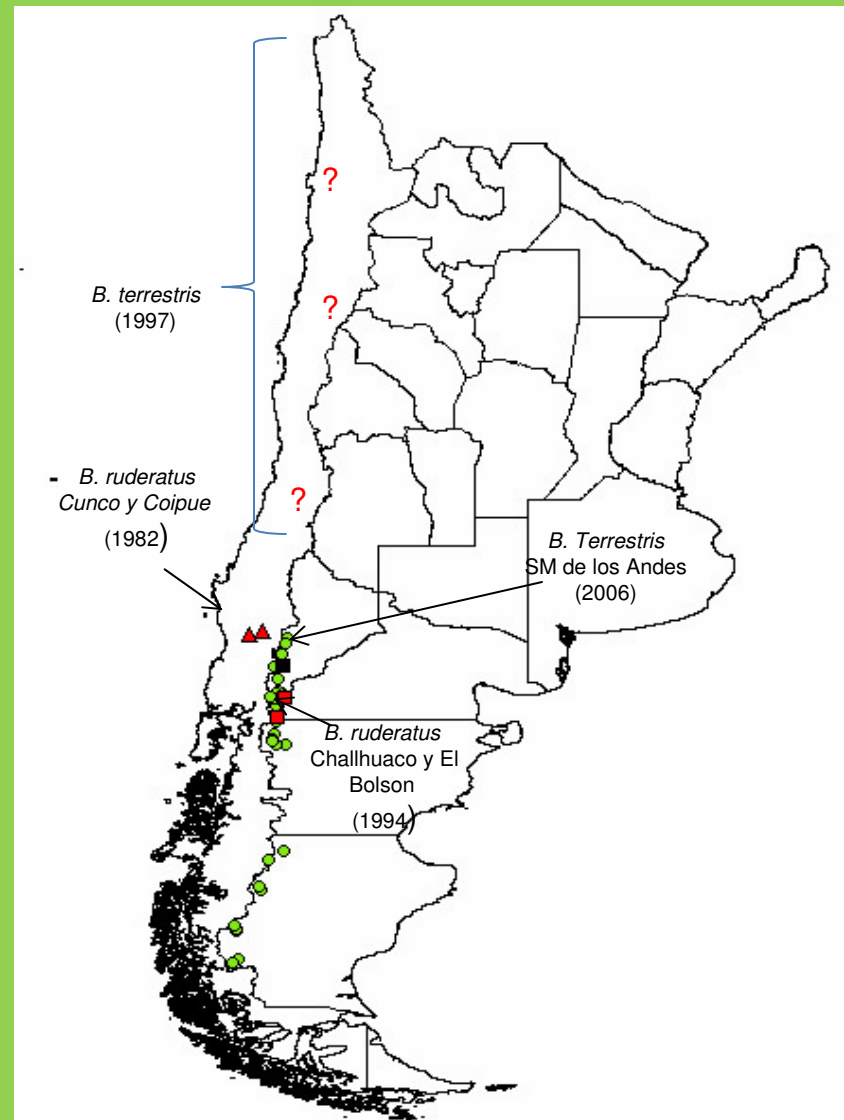
Finally, I am very thankful to the RSG for this opportunity to run this project and start this strongly conservation-focused research programme.

<b>Essential Equipment and materials for Field Work:</b>	Budgeted Amount (£)	Budgeted (ars) (anticipated rate: 1£ =6.23ars)	Amount approved by RSG (£)	Amount delivered by RSG (USD)	Amount received (ars) (rate: 1USD =3.886ars)	Actual expenditure (ars)	Difference (ars)	Actual expenditure (£) (using anticipated rate: 1£ =6.23ars)	Difference (£)	Justification difference
Vehicle (acquired with previous funding <sup>(1)</sup> )										
Gasoline (0.048£ /km x 200 km x day x 20 days)	192	<b>1196.16</b>				1962.29	-766.13	314.97	-122.97	expanded sampling effort and > 50% rise in gasoline prices
Vehicle Maintenance = (0.16£/kmx 4000 km)	642	<b>3999.66</b>				3999.66		642		
Nets, flasks, vials, consumable-plastics	250	<b>1557.5</b>				884.64	672.86	142	108	difference reassigned to other expenses
Alcohol etilic 96% = (£ 1.5/lit x 10 lts)	15	<b>93.45</b>				327	-266.55	52.49	-37.49	more samples collected and stored
Portable Field Freezer-Refrigerator	500	<b>3115</b>					3115		500	covered by other funds, difference reassigned to other expenses
Field Technical Assistance= £ 20/day x 20 days x 2 assistants	800	<b>4984</b>					4984		800	covered by other funds, difference reassigned to other expenses
Other expenses (meals during field work for two persons)	120	<b>747.6</b>				1287.74	-540.14	206.70	-86.70	expanded sampling effort and rise in overall prices
GPS= £ 200	200	<b>1246</b>					1246		200	covered by other funds, difference reassigned to other expenses
<b>Equipment and materials for Laboratory Work:</b>										
Staining/microscope filter = other funding <sup>(1)</sup>										
DNA extraction (KIT + reagents)	1450	<b>9033.5</b>				7153.87	1879.63	1148.29	301.71	difference reassigned to other expenses
Travel expenses for bumblebee DNA analyses (in Lab. Agrozoology, Ghent, Belgium)						6099.01	-6099.01	978.97	-978.97	Travel expenses(reagents and lab costs covered by the Host Institution)
PCR reactions and cleaning products = collaborations <sup>(3)</sup>										
Big Dye + sequencing reagents + sequencing facilities <sup>(3)</sup>										
- Laboratory Technical Assistance = £ 20/day x 30 days	600	<b>3738</b>				3738		600		
<b>Education and Outreach activities</b>										
-Brochures and educational materials	1000	<b>6230</b>				6230		1000		currently in preparation
<b>Total</b>	<b>5769</b>	<b>35940.87</b>	<b>5719</b>	<b>8153.58</b>	<b>31682.21</b>	<b>31682.21</b>		<b>5085.43</b>	<b>683.57</b>	<b>difference between budgeted and approved amount plus difference due to currency exchange applied</b>





*Bombus ruderatus*



*Bombus terrestris*

Site of release of *B. ruderatus* and probable release of *B. terrestris* in Chile, and sites of first report of each species in Argentina (Aizen & Roig Alsina 1996, Torreta et al. 2006 respectively). In green, sites surveyed during 2011.