New submission from ARC Award Final Report

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Name of Researcher

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Department/Faculty

Biology/Math and Science

Position in Department/Faculty

Instructor

Project Title

And DNA Barcoding

Term of Project

Sumer 2019 - Summer 2023

Please introduce yourself – include pertinent background information relating to the topic of your research project.

I have been a faculty member in the Langara College Biology Dept. since 1994, and I love telling people things that I have learned about the natural world. My research interests have focused on the ecology of social insects, notably honey bees and ants.

Please discuss your educational background and your work experience that led you to taking on this research project. If possible, include a quote that helps define your interest in this project.

I have published over 20 reviewed papers on insect ecology, including an overview of the distribution of ant species in British Columbia. Ants are fascinating but most species are small and difficult to tell apart and so I have spent enough frustrating hours working through dichotomous keys of ant species to know that such keys are often incomplete and subjective. DNA barcoding offers an objective way to confirm species identifications - if the process can be carried out reliably and relatively quickly.

Please summarize your project in plain language that others not in your field could understand.

All animal species carry versions of certain shared genes. The information in these genes is carried in long sequences of four types of nitrogen bases, like a language consisting of four letters, and the particular sequences of nitrogenous bases can be reliably used to identify individual species. If each of the four nitrogenous bases in a sequence is represented by a different coloured line, then the unique sequence for each species can be presented as a sort of four-coloured bar code, not unlike the bar codes that identify every unique product in store.

In this study, we collected the DNA from several dozen different ant specimens, from over a dozen presumed species, made millions of copies of the segment of DNA that we were interested in, cleaned and purified it, determined the sequence of nitrogenous bases in each sample, and then compared the sequences (bar codes) to those already published in a database. Each step required some experimentation and practice.

We found that we could reliably use this method to generate bar code sequences that allowed us to confirm species identifications or even suggest identifications when traditional keys based on anatomical features had left us unsure.

Identify the project goals and objectives. Explain how the results may be used to solve a problem or inform further research in the field.

The goal of this project was to determine if DNA bar coding could be carried out in the department, as a potential technique to include in lab exercises, as a tool for future research projects, or even as a commercial service. Another objective was to give some of our current students an opportunity to gain practical experience in molecular biology.

Briefly explain the steps taken (methods used) to conduct the research, and describe the key findings.

A general (and more detailed) flowchart of the process is shown below:

1. Individual worker ants collected
(From various sites in southern British Columbia)

2. Storage of the ants
(Individual ants preserved in vials of 70% ethanol)

3. DNA Extraction
(Preserved ants were removed from ethanol and air dried in an 1.5 ml Eppendorf tube)
(A 2 mm metal ball added to each tube)
(each tube received extraction buffer consisting of 30µl of boiling 5% Chelex (which protects from degradative enzymes and potential contaminants that might inhibit downstream analyses) in TE (a buffer).
(Each tube was then placed into a boiling water bath for 15 min, with periodic vigorous handshaking to crush the samples)
(Samples were centrifuged at 13,000 rpm for 10 min.)
(20µl of the supernatant was removed from each sample, and put into storage at -20 C for later use as template DNA in PCR reactions)

4. Multiple copies of a specific section of the mitochondrial DNA were made
(Custom synthesized forward and reverse primers were used to amplify a 702 base pair section of the mitochondrial COXI gene)
(The forward primer LCOI1490: GGT CAA CAA ATC ATA AAG ATA TTG G)
(The reverse primer HCO2198: TAA ACT TCA GGG TGA CCA AAA AAT CA)

(These sections of the COXI gene were amplified numerous times using polymerase chain reaction (PCR) using 2µl of PCR buffer, 1 µl of 1µM of primer solution, 1µl of Taq polymerase (Amplitaq, from Life Technologies), 1 µl of a 2 mM dNTP, and 1 µl ant DNA, in a total volume of 20µl. PCR was run on a Techne
Techgene thermal cycler. The program settings were: initial denaturation at 95 C for 2 minutes; 30 cycles of the following: 30 seconds at 94 °C, 45 seconds at 50 °C, 2 minutes at 72 °C, and a final extension for 5 minutes at 72 °C)

5. Confirmation of successful amplification
(Successful amplification of single 710 bp DNA from all ants was confirmed by agarose gel electrophoresis)

6. Purification of the amplified DNA
(The amplified DNA samples were purified using a QIAquick PCR Purification Kit)

7. Quantitation of the DNA
(The amount of amplified DNA quantitated using a NanoDrop Microvolume spectrophotometer)

8. Storage of the DNA
(Samples were frozen at -20°C until further use)

9. Determination of the nucleotide sequence of each sample's DNA
(Individual samples were custom sequenced by Genome Quebec) and the data presented as FASTA files)

10. Comparison of the sequences to those in existing DNA databases, including BLAST
(All of the sequences could be matched to existing sequences in the database, confirming their identifications based upon anatomy-focused dichotomous keys)

In summary, we developed a relatively reliable, rapid protocol for the DNA barcoding of animals. This could be used for research projects or even to test, for example, if fish samples being sold in shops or restaurants are the species being claimed.

Who was involved in this project (eg. faculty, students, community partners)? How did their involvement contribute to the project's success? Were there any challenges to overcome?

Like most modern scientific projects, this one was a collaboration of many individuals. A key colleague in this work was Dr. Mario Moniz de Sa, from the Langara Biology Dept. He provided the molecular expertise and did much of the hands on work. Dr. Ji Yang, also from the Biol Dept. facilitated the sequencing of the copied DNA samples. Several students contributed skilled lab work including Avneet Kaur, who cleaned and purified DNA samples, Himmeshwar Singh, who did some DNA extractions and optimized PCR conditions, and Livia Vieira Lopes de Silva, who did much of the DNA extraction from the ants. Dr. Rob Higgins from Thompson Rivers University supplied some of the ant specimens.

Please share any personal stories that made this research experience memorable/valuable.

The most memorable feature of this project was the series of roadblocks that kept delaying its completion! As this project was about to begin, back in 2019, a fire and the associated water damage blocked access to the labs in the T building for the entire non-teaching term. Then Covid arrived, which effectively put the project on the hold over the next two summers. By the time it was reawakened, all of the original student participants had moved on and the we waffled between contracting out the sequencing of the DNA samples, and training people to do it in house. In the end, it was less expensive and simpler to send them to a sequencing service.

What are the next steps for this project and for you as a researcher?

The techniques learned and practiced in this project can potentially be used within the Biology Dept. to create laboratory exercises in molecular biology courses. DNA barcoding of any animal species could also be carried out as part of future research projects or even as contract work but he ant-themed research that I have been carrying out since 2013 is winding down and I am unlikely to follow up on this work directly.

Please upload any images that will help to showcase your project.

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Langara Institutional Repository Consent

By submitting, I consent to uploading my ARC Fund final report to the Langara Institutional Repository (The LaIR).