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DNA Variation & Bioinformatics



Analysing DNA to identify who killed baby Tara iti

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A New Zealand Fairy Tern/Tara iti at Waipu River estuary
Tergiversation, CC BY-SA 4.0, via Wikimedia Commons

1. Introduction

Tara iti (*Sternula nereis davisae*) is probably New Zealand's most endangered indigenous breeding bird. It is estimated that there are fewer than 40 individuals left at present including approximately 9 breeding pairs. We can find Tara iti in the lower half of the Northland Peninsula. Breeding is limited to four regular sites: Waipū, Mangawhai, Pākiri and Papakānui on the South Kaipara Head. Te Ārai and Poutawa rivermouths are also intermittently used.

The most likely threats to Tara iti are:

- **Habitat depletion** – degradation of sand dunes caused by residential development, farming...
- **Predation** – predators such as rats, cats, hedgehogs and mustelids prey upon eggs and chicks.
- **Environmental events** – High tides, floods, and storms can destroy and wash away nests.
- **Death of embryos** – Nesting birds are eaten or chased away by predators, and the embryos die from exposure.
- **Recreational activities** – Beach activities such as drone use, dog walking, bonfires ... disturb the birds and scare them away from their nests.

Want to know more about Tara iti?

<https://www.doc.govt.nz/globalassets/documents/conservation/native-animals/birds/tara-iti-new-zealand-fairy-tern.pdf> (fact sheet)

<https://www.nzgeo.com/stories/fallen-from-grace/> (A New Zealand Geographic report on conservation efforts)

2. Task

Aim: To investigate WHO KILLED a Tara iti chick.

Method: You will play the role of a detective to solve a **fictional scenario**.

To have a better understanding of bioinformatics, an area of Genomics, you will analyse a DNA sequence obtained from a dead Tara iti chick to determine the identity of the predator, e.g. predatory bird or introduced pest who might be responsible for the death of the Tara iti chick.

A. Read the case report from the Department of Fauna.



Department of Fauna
Te Tari te Ao Kararehe

Case File Number: DOF-6060842

Officer assigned: Anthony McLeod

Date Filed: 13/1/2020

Incident Location: Waipu River Mouth Wildlife Refuge

Case Classification: Native species predation

Case status: Active investigation

Event Report:

At 9:54 AM on Saturday, January 11th 2020, DOF volunteer Susie Marsden discovered a dead fairy tern chick - Tara iti (*Sternula nereis davisae*) while conducting a nest survey at the Waipu River Mouth Wildlife Refuge. The chick was found approximately 260 meters south from the river mouth and 5 meters inland from the high-water mark. Marsden was unable to locate any nest site in the immediate vicinity (although shallow Tara iti nest scrapes in the sand are difficult to identify).

Autopsy of the chick revealed several small puncture wounds on the neck and back. The wounds, although not deep, are the likely cause of death and are consistent with either attack by a conspecific (same species) or predation by a small- to medium-sized predator. The puncture wounds suggest the latter scenario.

The stiffness and dehydrated condition of the body suggested death likely occurred within 48-72 hours prior. Despite a thorough search by Officer McLeod and a volunteer team, no informative animal tracks were found nearby. Strong easterly winds over the previous days could have filled over any predatory mammal tracks with sand.

Skin swabs and wound biopsies have been collected for forensic DNA sequence analysis. The DOF laboratory in Whangarei will file a MinION DNA sequence report within the next week. If sequence data indicate the involvement of an introduced pest, a predator trapping strategy will be rapidly implemented to protect the remaining Tara iti.

B. Track down the culprit

The Department of Fauna laboratory collected, processed and prepared DNA samples from skin swabs and biopsy tissues of the dead chick, then sequenced the prepared genomic DNA samples. The DNA sequences obtained were further screened through a computer program to remove sequence files closely matching the Tara iti's genome – this makes it possible to focus on foreign DNA sequences only for potential predator DNA sequences.

Results showed that one particular DNA sequence was the 'best guess' for a potential culprit. It is given as follows:

```
ATCAGGCTACATCCTGGAGCGCAAGAAGAAGAAGAGCTTCCGGTGGATGTGGC
TGAAC TTTGACCTGCTGCAGGAGCTGAGCCACGAGGCACGGCGCATGATTGAG
GGCGTGGTGTATGAGATGCGAGTCTACGCGGTCA
```

You are now going to analyse this sequence to properly identify the culprit.

Your aim is to answer two questions:

- Which species does this DNA sequence belong to?
- Does this DNA sequence indicate something special about the culprit?

FOLLOW THE STEPS BELOW.

- 1) Open the link to [NCBI BLAST](https://blast.ncbi.nlm.nih.gov/Blast.cgi). The BLAST tool of ncbi performs a sequence similarity search across the hundreds of millions of DNA sequences stored in the GenBank database.

- 2) Copy the following DNA sequence (including the title):

```
>Wound Biopsy Sequence
ATCAGGCTACATCCTGGAGCGCAAGAAGAAGAAGAGCTTCCGGTGGATGTGGC
TGAAC TTTGACCTGCTGCAGGAGCTGAGCCACGAGGCACGGCGCATGATTGAG
GGCGTGGTGTATGAGATGCGAGTCTACGCGGTCA
```

- 3) Paste the sequence into the "Enter Query Sequence Box" and make sure 'blastn' is selected (the tab appears blue).

The screenshot shows the NCBI BLAST web interface. At the top, there are tabs for 'blastn', 'blastp', 'blastx', 'tblastn', and 'tblastx'. The 'blastn' tab is selected and highlighted in blue. Below the tabs, the text 'Standard Nucleotide BLAST' is visible. The main area is titled 'Enter Query Sequence'. It contains a large text input box for the query sequence, which is highlighted with a red arrow. To the right of the input box are fields for 'Query subrange' with 'From' and 'To' sub-fields. Below the input box, there is a section for 'Or, upload file' with a 'Choose File' button and a 'No file chosen' status. At the bottom, there is a 'Job Title' field with a placeholder text 'Enter a descriptive title for your BLAST search'.

- 4) Scroll to the bottom of the webpage and click the blue BLAST button.
- 5) Wait for a few minutes.
- 6) Scroll down the report page. The "descriptions" tab will be highlighted in Blue. You get 'hits' of sequences stored in NCBI that are most closely related to your query sequence.

Descriptions

Graphic Summary

Alignments

Taxonomy

Sequences producing significant alignments

Download

Select columns

Show

100

☒

select all

100 sequences selected

GenBank

Graphics

Distance tree of results

MSA Viewer

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<input checked="" type="checkbox"/>	PREDICTED: Panthera tigris myosin binding protein C3 (MYBPC3), mRNA	Panthera tigris	246	246	97%	4e-61	99.26%	5005	XM_042958215.1
<input checked="" type="checkbox"/>	PREDICTED: Panthera uncia myosin binding protein C3 (MYBPC3), mRNA	Panthera uncia	246	246	97%	4e-61	99.26%	4988	XM_049647407.1

7) Write down the names of the top 5 “hits” the DNA sequence matches with.

8) Reviewing the 5 top ‘hits’, can you infer who would be the most likely culprit?
Hint: you will probably need to google what ‘Panthera and Felis catus’ mean to guide your guess.

9) Deselect the ‘Select all’ check box

Descriptions

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Sequences producing significant alignments

☒ select all 0 sequences selected

10) Click on the Felis catus myosin binding protein C3 (MYBPC3), transcript variant X2, mRNA. You will get something like as below:

PREDICTED: Felis catus myosin binding protein C3 (MYBPC3), transcript variant X2, mRNA

Sequence ID: [XM_019812397.2](#) Length: 4282 Number of Matches: 1

Range 1: 2528 to 2663 [GenBank](#) [Graphics](#)

[Next Match](#) [Previous Match](#)

Score	Expect	Identities	Gaps	Strand
246 bits(133)	1e-60	135/136(99%)	0/136(0%)	Plus/Plus
Query 5	GGCTACATCCTGGAGCGCAAGAAGAAGAAGAGCTTCCGGTGGATGTGGCTGAACCTTTGAC	64		
Sbjct 2528	GGCTACATCCTGGAGCGCAAGAAGAAGAAGAGCTTCCGGTGGATGCGGCTGAACCTTTGAC	2587		
Query 65	CTGCTGCAGGAGCTGAGCCACGAGGCACGGCGCATGATTGAGGGCGTGGTGTATGAGATG	124		
Sbjct 2588	CTGCTGCAGGAGCTGAGCCACGAGGCACGGCGCATGATTGAGGGCGTGGTGTATGAGATG	2647		
Query 125	CGAGTCTACGCGGTCA	140		
Sbjct 2648	CGAGTCTACGCGGTCA	2663		

- 11) Identify the common and different base(s) between your query (the sequence that you copied and pasted in steps 2 and 3) and the GenBank database. What is/are the difference(s) if any?
Note: matching bases are linked by a line.

- 12) This single base change is actually known to change the MYBPC3 protein in cats.

- 13) Click on the 'CDS feature' check box to change the DNA sequence into a protein sequence made of amino acids. Can you spot any difference(s) in the series of letters?

Descriptions

Graphic Summary

Alignments

Taxonomy

Alignment view

Pairwise

☒ CDS feature

Restore defaults

Record what you see. Refer to the table of codons at the link below to identify the names of the amino acids that changed <https://pixabay.com/vectors/dna-amino-acids-biology-code-152135/>

- 14) Open the National Library of Medicines [PubMed search engine](#)

- 15) Type 'MYBPC3 in cats' in the search box and learn about MYBPC3 variants by reading the first article.

- 16) The following table shows different cat breeds with MYBPC3 variants.

Occurrence of MYBPC3 variants in different cat breeds

Pathogenic Variant	Maine Coon	Scottish Fold	British Longhair	Munchkin	Ragdoll
91G>C [A31P]	✓	✓	✓	✓	✓
2453C>T [R818W]	●	●	●	●	✓

- 17) Note that for technical reasons, the DNA position 2453 in the above table corresponds to position 2573 in *Felis catus* MYBPC3 transcript variant X2 (the first 120 nucleotides of the X2 variant mRNA are untranslated).

Based on your sequence data analysis, can you identify which type of cat killed baby Tara iti?

- 18) Watch these videos to find out about the consequences of MYBPC3 mutation in humans and cats:

<https://www.youtube.com/watch?v=oF6h6LNDBiU> (in cats)

<https://www.youtube.com/watch?v=ZIB9BLtE0-A> (in humans)

<https://www.youtube.com/watch?v=wQTmaRCeDE> (in humans)