An analysis of pair stability in Brown Kiwi using eggshell DNA extraction

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1. **Brwon kiwi egg inside a nest. Ponui Island, 2018.**

My research focuses on finding an ecological explanation to the unique traits of Brown Kiwi (*Apteryx mantelli*); in this case, the mating system and pair stability of the Brown Kiwi at the high density population at Mangataniwha (-38.8249, 176.7865)

Pair stability in Brawn Kiwi has been addressed in the past, and it is the common belief that Brown Kiwi is a monogamous species, but current research in many allegedly monogamous species has found a great deal of extra pair copulations, and more intricate and plastic mating systems. It is possible that monogamy was observed to be the mating system of kiwi because of the low density of the populations, which could make pairs to remain together, more likely due to a reduced chance to encounter new potential partners than because is an adaptive strategy.

Brown kiwi, like other ratites have been shown to have a variable mating system with some degree of extra-pair paternity. However, the extent of these behaviours is unknown. The brown kiwi egg is incubated by the male instead of the female, as it is the case for most birds; in this scenario it could be expected that the female once free from incubation could find other potential partners increasing her individual fitness by literally putting her eggs in more than one basket.

This unusual incubating system of Brown Kiwi provides a unique opportunity to test the pair stability. The Operation Nest Egg program tracks males using telemetry during the year and when eggs are laid, the males are found and the egg retrieved from their nests. This process indicates the paternity of the egg as the adult is used to find the egg, but the maternity of the egg is unknown. By extracting DNA from the eggshell, which belongs entirely to the female we can track the number of times a particular Kiwi female has laid eggs in the nest of a particular nest, comparing different years we can establish the potential number of partners of a particular male.

As the embryo develops inside the egg an extremely vascularised corio-allantoic membrane grows on top of the egg innermost membrane. This corio-allantoic membrane contains the chick's DNA. In this project, we want to extract DNA from the eggshells and membranes of hatched eggs from Operation Nest Egg to assess parentage, the degree of relatedness between eggs in several clutches of the same male, and the individuals in a clutch. In this way we will

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explore the extra pair mating that can occur from both the male perspective (Chick's not related to him) and the female perspective (different females in a male's nest)

We have obtained sufficient DNA form Kiwi eggshells and membranes; this is the first time this has been done for this species. We developed a cost-effective and fast method to obtain and amplify DNA from these materials giving us the opportunity to quantify and inform on Brown kiwi pair stability and not rely on personal observations in a reclusive species where mating rituals are difficult to observe. This method could be used with other species in the wild, which means that mating systems can be studied in endangered species with minimum disturbance.

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