

SHORT NOTE

A paler shade of brown kiwi: hatchling *Apteryx mantelli* plumage fairness is correlated with geography in the central North Island

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New Zealand's "brown" kiwi were formally regarded a single species, *Apteryx australis*, until the recent molecular genetics work of Baker *et al.* (1995) and Burbidge *et al.* (2003). This historical lumping of several species was due to the group's apparent morphological crypsis (Baker *et al.* 1995). Brown kiwi are now classified as 3 distinct species; North I brown kiwi (*A. mantelli*) and 2 South I species, rowi (*A. rowi*) and tokoeka (*A. australis*) (Burbidge *et al.* 2003). All species are threatened on the mainland, primarily due to mammalian predators (Robertson 2003).

Failure to recognise that different brown kiwi populations represented cryptic species led to neglect from conservationists, with the result that 2 of the newly recognised species declined in population size and genetic diversity (Shepherd & Lambert 2008). The small Westland population of rowi and subpopulations of tokoeka in Haast and Fiordland were given management priority after the publication of Baker *et al.* (1995) and the 1996 Kiwi Recovery Plan (Robertson 2003). The newly circumscribed South I populations (*A. australis* and *A. rowi*) are now intensively managed (Holzapfel *et al.* 2008), and experiencing population recovery. Understandably, separate management of sub-

populations of what was then considered a single species (i.e., *A. australis*) perhaps did not seem imperative given the relative abundance of "North Island" brown kiwi.

Evidence is now pointing toward brown kiwi populations in the North I also harbouring "cryptic" species or sub-species awaiting classification and management. Using mitochondrial DNA (mtDNA), Burbidge *et al.* (2003) resolved 3 clades of *A. mantelli* from the North I, each of which correlated with their geographical distribution (Northland, Bay of Plenty/Hawkes Bay and Taranaki). Shepherd and Lambert (2008) also identified genetic structuring correlated with geography, again using mtDNA. By comparing 45 cytochrome-*b* sequences from modern and sub-fossil *A. mantelli* material, they demonstrated that populations from Taranaki, Wanganui, Hawkes Bay and the Bay of Plenty shared no common haplotypes. Interestingly, while some haplotypes were shared among sampled populations (notably between Northland and Taranaki), none were shared across eastern and western regions of the North I (Shepherd & Lambert 2008).

While history may suggest otherwise, researchers looking to resolve the systematics of North I brown kiwi should reconsider morphological data, in addition to molecular, as potentially useful sources of taxonomic characters. Both tokoeka and rowi appear to show greater uniformity in plumage colour than observed across populations

Received 26 Feb 2011; accepted 8 Aug 2011

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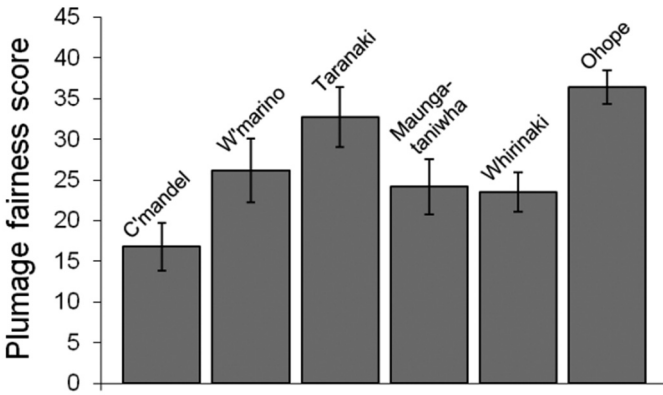


Fig. 1. Mean plumage fairness scores for 72 North I brown kiwi chicks from 6 regions of the central North I ($n = 12$ chicks per region). Chick plumage fairness was determined by comparing the average grey-scale values from digital photographs of individual 5 day old chicks. One way ANOVA and post-hoc Bonferroni comparisons showed Ohope and Taranaki chicks significantly differed from those from the Coromandel ($P < 0.010$). Error bars represent the standard error of the mean.

of North I brown kiwi. Tokoeka are noted as being either rufous, dark grey or brown, while rowi are described as only grey and white. North I brown kiwi, however, are noted as being variously black, brown, grey, and rufous (Baker *et al.* 1995) and, at least as juveniles, sometimes blonde (*pers. obs.*). I present evidence in this study which suggests that plumage colour, a heretofore neglected character, may have taxonomic utility in assessing the status of this otherwise cryptic species.

I assessed the plumage of newly hatched kiwi chicks at Kiwi Encounter in Rotorua, an incubation and chick rearing facility for Bank of New Zealand Operation Nest Egg (ONE) in the central North I. In ONE, kiwi eggs are removed from the wild and hatched in captivity. Chicks are raised to ~1 kg (large enough to fend off mustelid predators) and then returned to the wild. Fourteen ONE organisations incubate ~250 North I brown kiwi eggs each breeding season at Kiwi Encounter. Variation in plumage colour in chicks from different regions is well known to staff at the centre. Staff noticed that a majority of chicks from Ohope were blonde while those from Coromandel were black. Given these observations, and the demonstration of genetic structuring among *A. mantelli* populations, I hypothesised that chick plumage colour varied according to geographic origin because of each sub-population's genetic distinctiveness. I therefore predicted that plumage colour would show significantly more variation between regions of origin than within. As a component of record keeping at Kiwi Encounter, digital colour photographs of every chick are taken 5 days post-hatch. I used digital image analyses of these photographs to quantify and compare the observed plumage differences across regions.

Plumage "fairness" differences between kiwi chicks were compared across 6 regions: Ohope (37.968807S, 177.032716E), Wairarapa (39.446353S, 175.146703E), Whirinaki (38.698996S, 176.687657E), Maungataniwha (38.960784S, 176.843794E), Taranaki (39.098618S, 174.133346E), and Kuaotuna Peninsula (hereafter Coromandel; 36.735110S, 175.750800E).

Twelve chicks from each region were used in the analysis and photographs were selected haphazardly from the 2007/2008 and 2008/2009 breeding seasons. Seventy-two chicks in total were used and none were siblings. Photographs were taken in the same corner of a fluorescently lit room with an Olympus μ Tough Zm/6.6 ft 12 megapixel camera set to macro using no flash. Chicks were placed ~1 m from the camera lens when photographed. All photographs showed chicks standing lateral to the camera. Photographs were rejected if a chick was in an atypical posture. Canon Digital Photo Professional (v. 2.2) software was used to overlay the area of the image comprising the chick's main body (head excluded) with a series of 4900 pixel-sized grid-squares. Typically, 10 squares fitted over the body area. Random coordinates were used to sample an individual grid-square from each photograph for analysis.

Each chick's 4900 pixel-square was converted to 16 bit greyscale, and Apple Digital Color Meter software (v. 1.3) was then used to measure the average greyscale value for each 4900 pixel-square. This provided a measure for "fairness" ranging from 100 to 0, with 0 representing a completely black chick. The entire "main-body" portion of the photograph was not evaluated, as the method for obtaining fairness values from individual pixels is time consuming. Plumage colour is reasonably uniform within individual kiwi chicks; therefore I considered 4900 pixel-squares representative of total body fairness. Between and within region fairness scores were compared using 1-way ANOVA, accompanied by Levene's test for homogeneity of variance and Bonferroni post-hoc multiple comparisons. Analyses were performed in PASW Statistics (v. 18).

ANOVA revealed that plumage fairness of kiwi chicks differed significantly across regions ($F_{5,66} = 4.94$, $P = 0.001$; Fig. 1), and Levene's test showed homogeneous variance ($F_{5,66} = 1.32$, $P = 0.27$). Bonferroni comparisons of the 6 regions showed that Ohope chicks (mean = 36.37, 95% CI [30.09, 42.65],) were significantly fairer than those from the

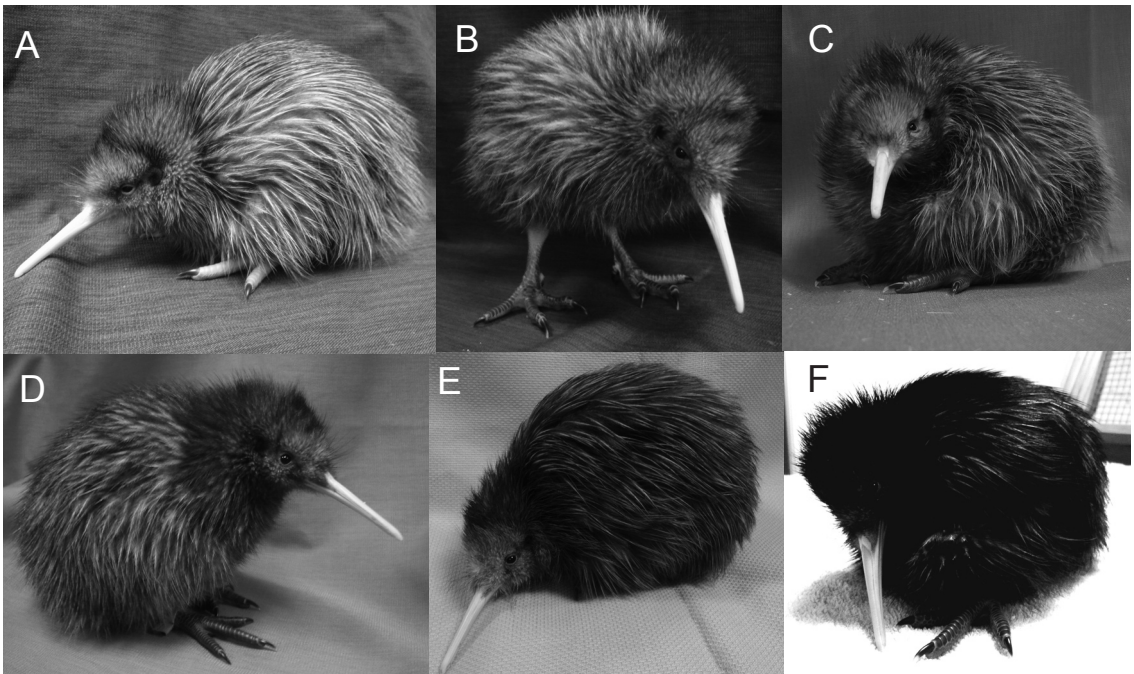


Fig. 2. Digital photographs of 5 day old North I brown kiwi chicks (*Apteryx mantelli*), demonstrating regional differences in plumage fairness. Regions are: (A) Taranaki, (B) Ohope, (C) Whirinaki, (D) Maungataniwha, (E) Waimarino, and (F) Coromandel. All photographs © Kiwi Encounter, Rotorua.

Coromandel (mean = 16.80, 95% CI [10.52, 23.08]; $P = 0.001$), and that Coromandel chicks were also significantly darker than those from Taranaki (mean = 32.71, 95% CI [26.42, 38.10]; $P = 0.010$). Taranaki and Ohope chicks did not differ significantly in plumage fairness, nor did any other regions differ significantly with remaining comparisons having $P > 0.05$ (Fig. 1).

Chicks from Ohope displayed the lowest variance in plumage fairness, which is consistent with previous observations of their distinctive “blondeness” (fairness scores ranged from 23.0 to 44.05). While Taranaki chicks did not differ significantly from Ohope, they displayed greater variance in plumage fairness, ranging from 17.55 to a high 61.5. Strikingly, Coromandel chicks were much darker than those from other areas (with mean Coromandel fairness = 19.2), though only significantly darker than Ohope and Taranaki chicks.

The results show a strong relationship between chick plumage fairness and the geographical location of each population. While the hypothesis that plumage fairness varies more between regions than within regions is only in-part supported, the results do not reject outright that a population’s plumage differs significantly according to their provenance. Indeed, if these plumage variations reflect genetic variability, then these results are potentially relevant to taxonomists and conservationists alike.

It appears that for central North I *A. mantelli*, gene flow may be restricted from east to west, landward of the Bay of Plenty. This is indicated by Ohope and Coromandel chicks displaying the greatest difference in fairness among all studied regions. According to the results, Ohope and Coromandel represent 2 extremes of a plumage fairness spectrum, with the populations of other regions displaying evidence of greater and perhaps even graded gene exchange. The “v” shaped Taupo Volcanic Zone (TVZ), an area of extensive Pliocene and Quaternary volcanic activity (Wilson *et al.* 1995) in fact presents itself as a potential geographical determinate of plumage fairness variation. The TVZ may provide a geographical structure for maintaining clinal variation in plumage fairness, or could even be contributing to vicariant speciation in *A. mantelli*.

The incidence of both dark and fair Taranaki individuals suggests that Taranaki *A. mantelli* may have been (or are still) in genetic exchange with populations from both Ohope and Coromandel. Fair Ohope genes can transgress southwest and dark Coromandel genes southeast as far as Taranaki, but neither penetrate directly east to west. The comparative similarity in plumage between Waimarino, Whirinaki and Maungataniwha populations may represent a sustained north-east, south-west population exchange along the perimeter of the TVZ. While the adaptive significance of chick

plumage is open to speculation, the greater number of fair chicks in Ohope and Taranaki could reflect local selection for fair over dark plumage. Kiwi chicks are sometimes diurnally active (*pers. obs.*). Possibly predation from extinct diurnal predators such as the Haast eagle (*Harpagornis moorei*) could have selected for individuals better camouflaged against the local environment.

Excitingly, these findings support Shepherd and Lambert's (2008) hypothesis that the TVZ has been a geographic impediment to *A. mantelli* gene exchange over the last 2 million years. Their finding that no mtDNA haplotypes were shared across eastern and western regions of the North I is a pattern *A. mantelli* has in common with several other divergent taxa (Shepherd & Lambert 2008). These results support the current separate management of geographically distinct populations of *A. mantelli* (Holzapfel *et al.* 2008) and the push for further taxonomic scrutiny of *A. mantelli* (Burbidge *et al.* 2003; Shepherd & Lambert 2008; Holzapfel *et al.* 2008).

Despite evidence for a genetic basis to geographic variation in plumage, there exists a non-genetic, or a potential gene by environment interaction explanation for the observed differences. It is possible that plumage difference may be due to carotenoid availability in the maternal environment. Females allocate carotenoids to yolk, and yolk carotenoid levels have a weak influence on hatchling feather chroma (Ikaksson *et al.* 2006). If carotenoid availability varies geographically, regional differences in hatchling plumage could arise. Nonetheless, this explanation fails to account for the heavy melanisation of Coromandel chicks, as the expression of melanin is under strong genetic control and therefore largely insensitive to the environment (Roulin & Dijkstra 2003). Further, while reared in captivity on a common artificial diet, Ohope and Coromandel chicks retain their distinctive darkness and fairness following their first moult (*pers. obs.*).

The quantification of variable morphological characters in this study demonstrates their potential utility for *A. mantelli* systematists. Full spectrum colour analyses of plumage itself (rather than from digital photographs) may prove informative. Chicks from Taranaki in particular show a greater incidence of rufous plumage, and Ohope and Coromandel chicks appear distinctive in their respective plumage "blondeness" and "blackness" (*pers. obs.*). In fact, chicks from Ohope and Coromandel are perhaps the most distinctive overall with regards to other potentially informative traits. Compared to other regions, Ohope chicks tend to have broader, "fluffier" feathers, with more widely spaced barbs, while Coromandel birds have silkier, sleeker plumage, with more heavily melanised bills, scales and claws (*pers. obs.*). As yet, the unique Coromandel kiwi have

not been included in any analysis of population genetics.

North I brown kiwi remain management dependant, and despite extensive recovery efforts, the species is still in decline (Holzapfel *et al.* 2008). Given that up to 41% of genetic diversity within kiwi species is known to occur between allopatric sub-populations (Shepherd & Lambert 2008), it is imperative to accurately characterise sub-populations. This would enable a strategic approach to conservation management where distinctive populations can be prioritised appropriately. Considering the ease and economy of acquisition, the use of morphological characters could assist in achieving these aims for North I Brown Kiwi.

ACKNOWLEDGEMENTS

Many thanks to Claire Travers, Emma Bean, Carmel Richardson and Beverley Wilkinson of Kiwi Encounter, and Bridget Evans of the Whakatane Kiwi Trust for their observations of kiwi chicks and suggestions for the study. Thanks also to the National Kiwi Trust, Kiwi Encounter at Rainbow Springs for the use of chick photographs. Thank you to Matt Renner, Ingrid Stirnemann, and 2 anonymous reviewers for their helpful feedback on the manuscript.

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Keywords North Island brown kiwi, *Apteryx mantelli*, plumage colour, taxonomy, conservation