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Apteryx spp. (Kiwi) possess an uropygial gland: Anatomy and pathology

Sian M. Reynolds[1,2], Isabel Castro1, Maurice R. Alley[3], Susan J. Cunningham[4]

[1]P.O. Box 11222, Institute of Agriculture and Environment, Massey University, Palmerston North, New Zealand, [2]New Zealand Department of Conservation, Haast Awarua Field Centre. P.O. Box 50, Haast 7844, New Zealand, [3]P.O. Box 11222, Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand, [4]Percy FitzPatrick Institute, DST/NRF Centre of Excellence, University of Cape Town, Private Bag X3, Rondebosch 7701, South Africa

SUMMARY

The uropygial gland is a prominent feature of the avian anatomy but there is limited information on its structure and function. The gland is of current interest because it provides a source for volatile chemicals that can be used by birds in communication. We examined the anatomy of uropygial glands in *Apteryx* for the first time. The gland was located immediately caudal to the cloaca and surrounding the coccygeal bone rather than rostral to the coccygeal bone and above the posterior free caudal vertebrae as in other birds. This may explain why it has not been recognised until relatively recently. Like most uropygial glands *Apteryx*'s were bilobar but possessed eight primary sinuses, each opening through its own orifice in the gland's papilla.

Primary ducts were compact and branches of connective tissue extending from the capsule internally formed interfollicular septae that were thicker in some areas, grouping follicles into discrete lobules. Striated muscle was present in the capsule, a characteristic so far unique to *Apteryx* that may be used in controlling the expulsion of secretion. There were significant differences in the architecture of the follicles between species and sexes that suggest differences in the production, storage and availability of uropygial gland secretion. This was supported by variations in live bird's gland volume between two years of sampling. Atrophy of the uropygial gland was seen in two birds in poor condition suggesting that health impacts the functioning of the gland. This finding suggests an adaptive significance for the gland and offers a possible way for birds to communicate their health status through the production or composition of the secretion. More research is needed to fully understand the relationship between the anatomy of the gland in *Apteryx* and its function, but we propose that it plays roles in both feather maintenance and sociality.

Key words: Holocrine – Oil gland – Rump gland – Preen gland – Integumentary gland – Apterygidae

INTRODUCTION

The uropygial gland, also known as the oil gland, rump gland or preen gland (Jacob and Ziswiler, 1982; Sadoon, 2011), is one of only three integumentary glands found in birds. These skin glands are very few when compared to the array of integumentary glands of reptiles and mammals (Quay, 1972). The evolutionary origins of the uropygial gland are unknown. Homology between the uropygial gland and reptilian glands is uncertain (Elder, 1954), although there seem to be phylogenetic relationships between uropygial gland secretions and secretions produced by some reptilian

Corresponding author: Isabel Castro. Institute of Agriculture and Environment, Massey University, P.O. Box 11222, Palmerston North, New Zealand. Phone: +64 6 3569099 ext. 84830; mobile: +64 274491261.

E-mail: i.c.castro@massey.ac.nz

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integumentary glands (Quay, 1972). Histologically, the avian uropygial gland is most similar to the mammalian sebaceous gland because of its holocrine structure (Wagner and Brood, 1975; King and McLelland, 1985; Sara et al., 2006).

Externally, the uropygial gland is visible as an eminence generally located dorsomedially in the synsachrocaudal area, generally rostral to the pygostyle and above the posterior free caudal vertebrae (Jacob and Ziswiler, 1982). The organ is generally bilobate, with ducts leading from the secretory tissue to the papilla and reaching the surface of the body through two or more orifices (Jacob and Ziswiler, 1982). The size, shape, number of circlet feathers, and proportions of the gland cells can vary greatly (Jacob and Ziswiler, 1982). The parenchyma is composed of small follicles with intertwining ducts leading to primary sinuses. Secondary sinuses drain secretory product from follicles to primary sinuses for storage (Lucas and Stettenheim, 1972). In species in which the gland's histology has been studied it has four layers of cells within each follicle (germinative, intermediate, secretory and degenerative) (Jacob and Ziswiler, 1982; Chandrasekar et al., 1990; El-Bargeesy et al., 1995; Kale et al., 1999; Sunanda et al., 2001; Sawad, 2006; Onal et al., 2013; Shafiian and Mobini, 2014; Chiale et al., 2014; Chiale et al., 2016; Harem et al., 2010; Kozlu, 2011). Many features of this gland remain a mystery, for example its innervation, mode of secretion, and the expulsion of the secretion from the papilla (Jacob and Ziswiler, 1982).

Because of the oily nature of the secretion, waterproofing and maintenance of feathers were its first postulated roles. However, research has shown that the role of the uropygial gland and its secretion may vary greatly between species. Experiments by Elder (1954) supported a feather maintenance role (Stettenheim, 2000): removing the uropygial gland in ducklings of two species, he showed that the plumage of experimental birds deteriorated with age, and survival decreased compared to control birds, because the damage to the plumage exposed them to injury and disease. Salibian and Montalti (2009) did not find any difference in the size, degree of development of the gland and the bird's relationship with water, suggesting that it may not have a role in waterproofing. Giraudeau et al. (2010) experimentally showed that when access to the uropygial gland was prevented for three months in a group of mallards (*Anas platyrhynchos*), their plumage showed lower water-repellence compared to control birds. Arguably, this may have resulted from deterioration of the plumage with disruption to the microstructure of the feathers, which is the most commonly proposed strategy for waterproofing.

In some birds the secretion may be involved in social cues; Kolattukudy and colleagues in 1987 showed that the female mallard's uropygial gland secretion contained a chemical which actively attracted males during the mating season, and thus the composition of the secretion exhibited seasonality. Likewise Hirao et al. (2009) found that the uropygial gland of female chickens (*Gallus gallus domesticus*) acts as a source of social odour cues. The uropygial gland secretion of blue petrels (*Halobaena caerulea*) has an individual signature and is used in conspecific recognition (Mardon et al., 2011). Starlings (*Sturnus vulgaris*) can identify the sex of conspecifics by using volatiles in their uropygial gland secretion (Amo et al., 2012). The uropygial gland secretion can be used in cosmetic colouration (*Phoenicopterus roseus*; Amat et al., 2011) or incorporate toxic, unpalatable, and foul smelling chemicals that the bird applies to its plumage as a defence mechanism against predators (Martín-Vivaldi et al., 2009; Dumbacher et al., 1992; Rajchard, 2010) or microbial parasites (Shawkey et al., 2003; Moyer et al., 2003; Chiale et al., 2014, 2015).

To fully understand how the uropygial gland works and the possible effect of phylogeny on its structure and function, it is necessary that we examine the uropygial gland of different bird groups histologically and through cytochemistry. Recent studies for example are analysing information regarding the gland's structure and chemical production with the function (Chiale et al., 2014; Chiale et al., 2015), habitat (Chiale et al., 2016), and phylogeny of the study birds (Chiale et al., 2014). Of particular interest is the gland of Paleognathous birds as this group is basal to the entire Aves. Amongst the ratites, only adult Apterygidae (five species) and Tinamidae (c. 40 species) are known to have uropygial glands (Johnston, 1988; Jacob and Ziswiler, 1982). Interestingly, early authors did not realize that *Apteryx* had an uropygial gland until Beddard (1899) examined the gland in three specimens, one of each *Apteryx australis*, *A. haasti* and *A. Mantelli.* Beddard (1899) described the gland as *enormous* but did not consider it to be bilobar. He found that the papilla had two inconspicuous nipples and that these had two orifices each. No measurements were taken or other descriptions made although the author provides an illustration of a ventral and lateral view of the uropygial gland close to the cloaca. In this paper we provide the first detailed description of the anatomy, morphology, and histology of the uropygial gland of *Apteryx*, and provide descriptions of some pathological changes that were observed in the uropygial gland of individuals of three *Apteryx* species. We discuss our findings in terms of the possible function of the gland.

MATERIALS AND METHODS

External morphometry

We measured and photographed twice (in February/March 2008 and March 2009) the uropygial

gland of a set of 29 (15 male and 14 female) wild brown kiwi (*Apteryx mantelli;* all species follow Burbidge et al. 2002) from Ponui Island, in the Hauraki Gulf, New Zealand (1770 ha; 36 55', 175 11'E). Nine additional birds (six females and three males) were sampled once in 2008. We recorded the following measurements: uropygial gland base length (rostral to caudal part of kiwi body) and width (side to side of *Apteryx* body); uropygial gland height from the point where the uropygial gland met the body to the tip of the papilla (Fig. 1A). To determine an index of volume, the cone volume formula was used: $v= 1/3*\pi*r^2*h$; where r = {[(maximum length + maximum width)/2]/2)} and h = uropygial gland height. We also collected a sample of uropygial gland secretion (~35-70 µL) for another study by gently massaging the gland and collecting the secretion in a capillary tube. We recorded the colour of the secretion as it appeared to the naked eye and whether it had a noticeable odour.

Histology and pathology

Uropygial glands of eight brown kiwi (two adult males, four adult females and two juvenile males; two captive and six wild), five wild great spotted kiwi (*Apteryx haastii*; one adult male, two sub-adult females and two juvenile females) and three wild Haast tokoeka (*Apteryx australis* var. Haast) (two juvenile males and one juvenile female) that died from a variety of causes unrelated to this study were sourced from birds submitted in fresh condition to Massey University's Wildbase (New Zealand's central wildlife hospital and wildlife pathology centre) between March 2011 and June 2012. Uropygial glands were dissected from carcasses with a tissue margin sufficient to include the surrounding fat and connective tissue. This sometimes involved the inclusion of part of the coccygeal bone. The glands were fixed in 10% neutral buffered formalin. Each gland was cut in half sagittally between the two papillae (Fig. 1B). One half was used to produce three pieces: a midline trimming and two lateral trimmings (Fig. 1B) with the midline and outermost trimming used for data collection. The other half we used for measuring length and depth of the gland's lobes. We measured the maximum length and the maximum depth of the lobes (usually about 2/3rds craniodorsally from the papilla) including the capsule.

Each trimmed section was embedded in paraffin, processed routinely and cut at 4µm on a rotary microtome. Blocks containing bone were decalcified before being cut to avoid tearing the glandular region of tissue. Decalcification was undertaken in Osteomoll® (Merck) overnight prior to processing. Slides were routinely stained with Haematoxylin and Eosin (HandE) and examined under a light microscope. Measurements of the glandular tissue were made with an Olympus ColourView - 5 Mega-Pixel colour digital camera and an AnalySIS 5 software-image analysis system. Masson's trichrome and Van Gieson stains were used to identify connective tissue and muscle, Sudan Black to identify lipid and Gram stain to identify bacteria. Techniques for each stain followed procedures in Bancroft and Gamble (2008).

The uropygial gland of seven brown kiwi and three great spotted kiwi were used to describe the normal uropygial gland histology. The uropygial gland of four birds (one brown kiwi, two great spotted kiwi and one Haast tokoeka) showed pathological changes and were described separately. The remaining two tokoeka were not used for histological measurements, but the length and depth of their gland's lobes were taken.

Histomorphometrics

A longitudinal transect representing the maximum glandular diameter was made at 1.25x mag-

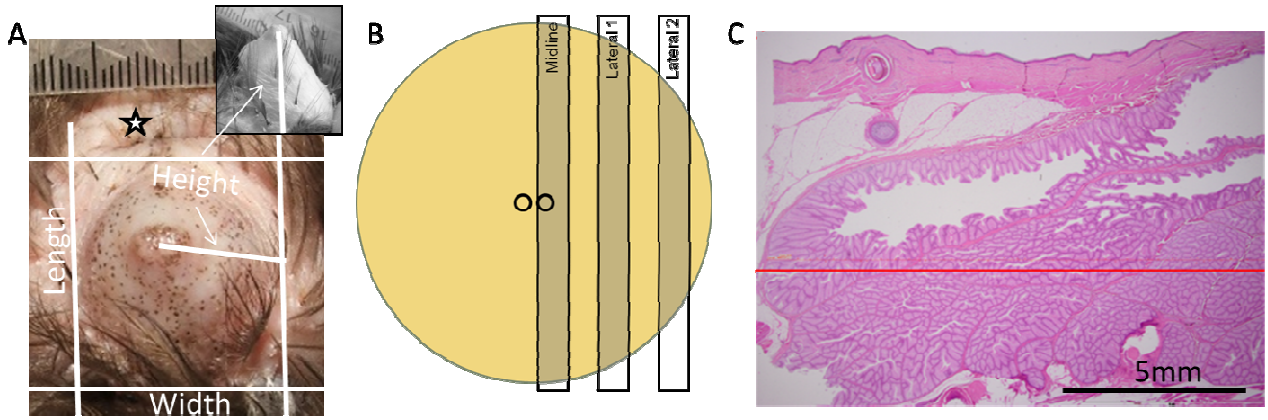

Fig 1. A) Brown kiwi uropygial gland (UG) from above showing how width and length were measured externally. Inset: view of UG from the side, height was measured from the top of the papillae to the point where the UG meets the body of the bird. Cloaca = ★. **B**) Model illustrating histological sections produced from each UG - the two small circles in the middle represent papillae. **C**) H&E photomicrograph of the UG of a brown kiwi showing the longitudinal transect of maximum glandular diameter as a black line approximately in the middle of the picture.

nification (Fig. 1C). Measurements at 20x magnification were taken of follicles in the secretory tissue which this transect intersected. The mean follicular diameter and mean luminal diameter of each follicle were calculated. The number of cells contributing to the follicle cell layer along the line and their degree of vacuolation were recorded. Degree of vacuolation was determined by the amount of secretory products present in a cell: germinative cell = no secretory products present, intermediate cell = small vacuoles of secretion present, and secretory cell = secretory products filled entire cytoplasm displacing nucleus to the side of the cell.

Statistical analysis

Live birds: A Mixed Model Repeated Measures Analysis (MMRMA) was carried out to investigate the effect of age (juvenile, adult) and sex (male, female) on uropygial gland volume for the sample of 29 Ponui Island brown kiwi that were measured twice. A model was built where bird identity was the repeated measure, and volume (year 1 vs. year 2) the dependent variable; age and sex were between subjects factors and tarsus measurement was used as a covariate to control for differences in size between the sexes (male brown kiwi are generally smaller than the females; Cunningham and Castro 2011). Uropygial gland volume data were not normally distributed (Shapiro-Wilkinson test [S-WT] = 0.87; P = 4.8E-6) so the data were log10 transformed to conform to normality (post-transformation S-WT = 0.99; P = 0.90).

We looked at the effect of age (chick, juvenile, adult), species (great spotted kiwi, brown kiwi, tokoeka), and sex (male, female) on the length and depth of the lobes using a multivariate general linear model with bird identification as WLS weight variable to account for variance in diameters attributable to the different individual birds sampled. We used the same type of test to look at the effect of sex, age and species on the length and depth of the uropygial gland and on the proportions of each cell type in follicles.

Data for lumen and follicle diameter were log transformed to make them conform to normality. We then fitted multivariate General Linear Models with log follicle and log lumen diameters as the dependent variables; section, age, and sex as factors and bird identification as WLS weight variable.

To examine the predictive value of section, species, age and sex on follicle architecture we carried out individual negative binomial models with log link with total number of cells, number of germinative, number of intermediate and number of secretory cells as dependent variables.

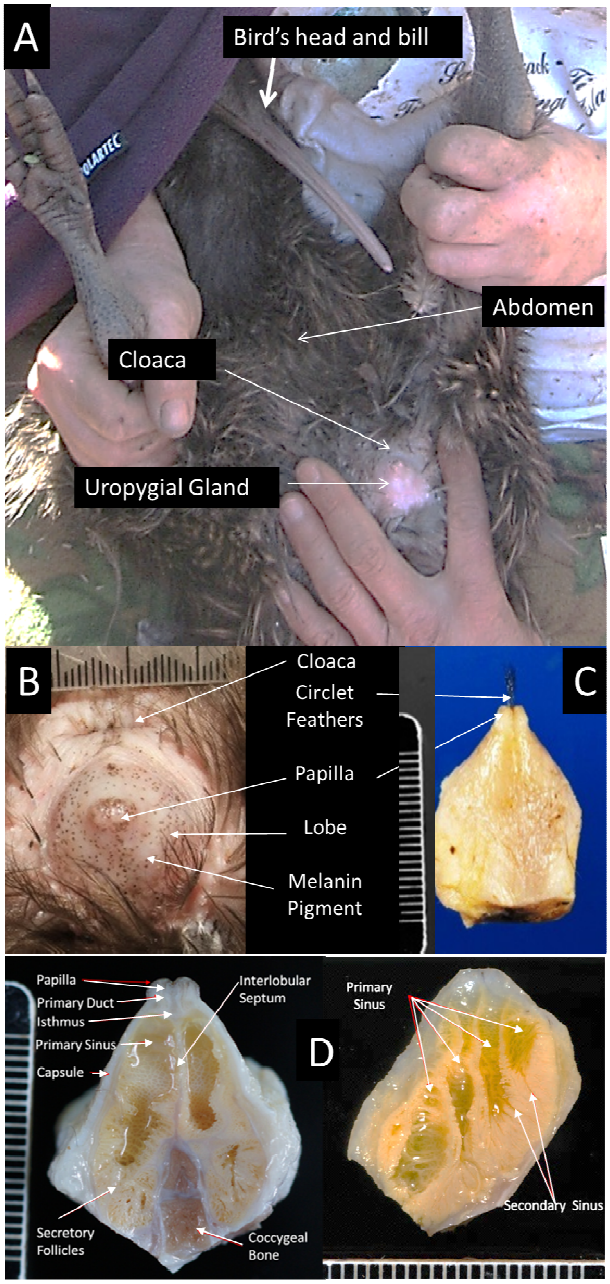

Fig 2. Brown kiwi uropygial gland (UG) anatomical location and organisation. **A**) Position of the bird for measurements and photographs. **B**) UG showing dark melanin pigmentation spots on its surface. **C**) Ventral view of UG showing two lobes, a papilla, and uropygial circlet feathers. **D**) Inside of a lobe of a brown kiwi UG. Mid transverse section left and lateral sagittal section right. Four primary sinuses and some secondary sinuses are clearly visible. Scale = millimetres.

Table 1- Sex and age of specimens used for measuring follicle and lumen diameters and number/type of cells in follicles. BK = Brown kiwi; GSK = Great Spotted kiwi.

Species	Female				Male			
	Total	Chick	Juvenile	Adult	Total	Chick	Juvenile	Adult
BK	4	0	2	2	4	0	2	2
GSK	2	1	1	0	1	0	0	1

In all tests we examined main effects only because relatively few individuals of each species were used and therefore interactions were in most cases not present or a single individual was available (Table 1). We used a P value of 0.006 to correct for multiple comparisons (nine) (Dunn 1961). All tests were carried out in IBM SPSS statistics 23.

RESULTS

Morphology of the Apteryx uropygial gland

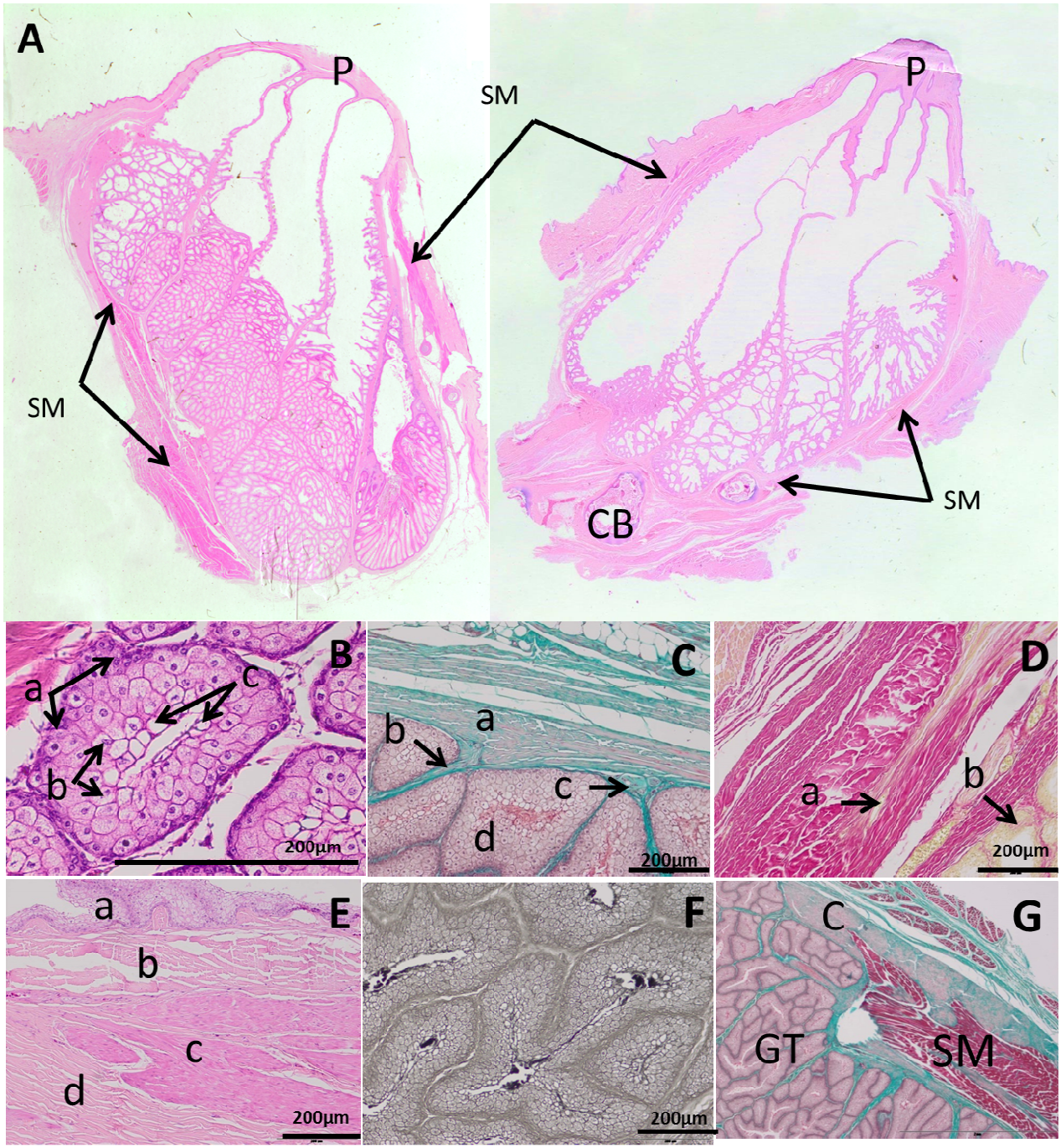

Fig. 3.- *Apteryx* uropygial gland (UG) histology. **A**) H&E staining of a Tokoeka (left) and a great spotted kiwi (right) showing the histology of entire UG. SM = striated muscle; P = papilla; CB = coccygeal bone. **B**) H&E staining of brown kiwi UG Midline section. Follicle cell types: a) germinative cells; b) intermediate cells; c) secretory cells. **C**) Masson's trichrome staining of brown kiwi UG: a) capsule; b) branching of interfollicular septae into glandular area c) neural tissue; d) follicle. **D**) Van Gieson staining of great spotted kiwi UG: a) striated muscle within the capsule of UG; b) secretory area. **E**) H&E staining of brown kiwi UG: a) glandular area; b) capsule c) smooth muscle; d) dermal collagen. **F**) Sudan Black staining of brown kiwi UG lateral section showing lipids in the secretion staining black. **G**) Masson's trichrome staining of a brown kiwi UG showing extensive striated muscle bundles (SM) within the capsule (C). GT = glandular tissue.

The gland was located immediately caudal to the cloaca (Figs. 2A and B) and surrounding the coccygeal bone (Fig. 2D). The uropygial gland was bilobar (Figs. 2C and D) with individual variation in shape and size of the gland. The external measurements were (Average ± SD): height: 16.27 ± 4.18mm; length: 18.77 ± 5.69 mm; width: 15.93 ± 2.94 mm. Uropygial gland volume ranged from 0.35 to 4.77 cm^3 in 2008 (Median = 1.57); and from 0.27 to 2.97 cm^3 in 2009 (M = 0.94). Mean uropygial gland volume was significantly higher in 2008 than 2009 (paired t-test $t = 5.8$; $df = 28$; $P < 0.0001$). Age (MMRMA, $t = 0.102$; $df = 25.16$; $P = 0.92$) and sex (MMRMA, $t = 1.15$; $df = 27.54$; $P = 0.26$) were not good predictors of uropygial gland volume.

Each lobe of the gland had a papilla protruding from it and the transition from lobe to papilla was gradual with no clear division being visible (Figs. 2C and D). Up to two small uropygial circlet feathers were present at the top of the gland (Fig. 2C). Pigmentation of the uropygial gland consisted of freckling of the skin and was very prominent in some birds (e.g. Fig. 2B) but absent in others (Fig. 2C). The exact freckle pattern was consistent year to year in individuals that possessed it. Pigmentation was significantly more common in females (10 of 18) than males (5 out of 17; Chi square = 10.0; $df = 2$; $P < 0.05$). In addition, some birds had pigmented papillae but lacked pigmentation on the lobes of the uropygial gland; this was also more common in females (5/9) than males (1/12). Some glands presented a prominent forward extension we called cartilaginous protuberance (18/19 males and 14/18 females).

The uropygial gland secretion had four different colorations easily discerned by eye: colourless, pale gold, gold, and ivory. Secretion colouration was not always consistent between years within the same individual; half of the birds sampled showed differences in secretion colour between 2008 and 2009. The most common colour in both years was pale gold (14/30 and 18/34, in 2008 and 2009 respectively), followed by gold (12/30 and 7/34). The secretion was usually translucent but in two females (one adult, one juvenile) in 2008 it was turbid at one of the sampling periods (that looked ivory in colouration).

There were no differences in the length and depth of the gland once dissected between species, sexes or ages (Appendix 1) and the overall mean measurement was 21.73±2.67 for length and 13.32±2.35 for depth (mean ± SE).

Histology of the Apteryx uropygial gland

The glandular elements of the *Apteryx* uropygial gland were bound together by a connective tissue capsule. This capsule consisted of thick, branched, and linked collagen fibres visible with a Masson's trichrome stain (Fig. 3). Bands of collagen and elastic fibres formed the interfollicular septae, penetrating into the glandular area (Fig. 3C). Within the uropygial gland papilla was a connective tissue isthmus through which the primary ducts for secretion of the glands contents ran (Fig. 3A). These ducts were of a compact type where connective tissue surrounding the ducts was dense causing them to be narrow (Jacob and Ziswiler, 1982). Small nerves penetrated both the capsule and interfollicular septae (Fig. 3C) and could be related to expulsion of the secretion from the gland. There was smooth muscle both around (Fig. 3E) and within the capsule but not always extending into the interlobular septae as only thin strips of collagen separated the follicles. Large bundles of striated muscle were present within the capsule on the dorsal aspect of the gland (where it meets the body) surrounding both lobes (Figs. 3A, D and G).

Each of the two uropygial gland lobes possessed four distinct primary sinuses. Each of these sinuses was associated with its own primary duct (Fig. 3A). There were no cross-channel connections between primary ducts therefore the secretion has to be expelled through individual orifices. In total therefore, there were eight orifices (duct openings) in the papillae; four for each lobe of the gland. Each lobe was elongated and sat ventral and lateral to the coccygeal bones which curve cranioventrally beneath the body (Fig. 2D). The parenchyma predominated at the base of its associated sinus (Fig. 2D). Sudan Black staining revealed the presence of dense sudanophilic lipids within the lumen of many follicles (Fig. 3F).

Within follicles, three separate cell types were identified; 'germinative', 'intermediate', and 'secretory/degenerative' (Fig. 3B). The germinative layer was between 1-3 cells thick, and consisted of cells with dense basophilic nuclei and a uniform, eosinophilic cytoplasm indicating that very little secretion had accumulated. These cells were predominantly flattened, cuboidal, or a transition between the two (Fig. 3B-a). The intermediate layer was more variable and thickness ranged from 3-8 cells. Similar to descriptions by Jenik et al. (1987) for other species, intermediate cells were most commonly polyhedral and had small vacuoles of secretion within their cytoplasm. They could be distinguished from the secretory cells by their centrally located nuclei (Fig. 3B-b). The secretory layer ranged from 1-3 cells thick. The secretory cells were clearly vacuolated and all the cytoplasm was filled with secretory products (Figs. 3B-c and 3F). In these cells nuclei were displaced peripherally. These cells corresponded to those of the secretory and degenerative areas described by Jacob and Ziswiler (1982), although the degenerative cells were much flatter, smaller and more disrupted than the underlying secretory cells as described by Jenik et al. (1987) for other species. In this study we did not separate between secretory and degenerative layers because cells in the degenerative layer were rarely seen as once apoptosis had oc-

curred cellular debris was lost to the lumen (Fig. 3F).

Histomorphometrics of healthy uropygial glands

There was no effect of section in the diameter of follicles and lumen (Table 2). The single sampled chick had significantly smaller average lumen and follicular diameters than the juveniles and juveniles had significantly smaller diameters than adults (Table 2). Great spotted kiwi had significantly larger lumen average diameter than brown kiwi (Table 2). Males had larger follicular diameter than females and females had larger lumen diameter than males (Table 2).

Age was a good predictor of the number of cells in each follicle with the chick having significantly less cells in follicles than juveniles and juveniles less than adults (Tables 3 and 4). When each layer was looked at individually, the chick had 50% less germinative and intermediate cells than adults and juveniles. Interestingly, there was no significant age difference in the secretory layer, but species (great spotted kiwi had more) and sex (males had more) were both significant predictors of cell numbers in this layer (Tables 3 and 4). When we examined the proportion of each cell type within the follicles we found no effect of section therefore we rerun the model excluding this factor. There were significant effects of species, sex, and age on proportions of cell types (Table 5). Males had a greater proportion of cells in the germinative and intermediate layers and lesser in the secretory layer than females. Brown kiwi had proportionally more germinative and intermediate cells than great spotted kiwi and GSK had proportionally more secretory cells. The single chick sampled had proportionally more intermediate cells than adults and juveniles. Juveniles had proportionally more germinative and less intermediate and secretory cells than adults (Table 6).

Pathological findings in Apteryx uropygial glands

Cases one and two: Atrophy of the uropygial gland

Two cases of uropygial gland atrophy were observed, both occurring in sub-adult female great spotted kiwi. The first female was brought to the Wildbase hospital from the wild in an emaciated state. During her short time in captivity she progressively lost weight and at necropsy weighed 780 g (healthy weight for a sub-adult female great spotted kiwi ranges between approximately 800 g and 1200 g; Robertson et al., 2003). Starvation was diagnosed as the cause of death in this bird. She exhibited minimal epicardial fat reserves and had very little slightly reddened subcutaneous fat. The gastrointestinal tract was empty but helminths or coccidia were not found, nor were any pathogenic bacteria cultured from intestinal contents. The uropygial gland showed severe generalised atrophy of epithelial elements (Fig. 4A-a).

The second sub-adult female had been translocated from Riccarton Bush to North Canterbury, but died nine days after release. She had no epicardial or subcutaneous fat reserves and had severe generalised muscle atrophy. The lungs were very dark red in colour and contained several 3-5 mm yellow areas of necrosis. On histopathology, the lungs showed a severe mycotic bronchopneumonia. Macrophages, lymphoid cells and multinucleate giant cells filled airways and air capillaries

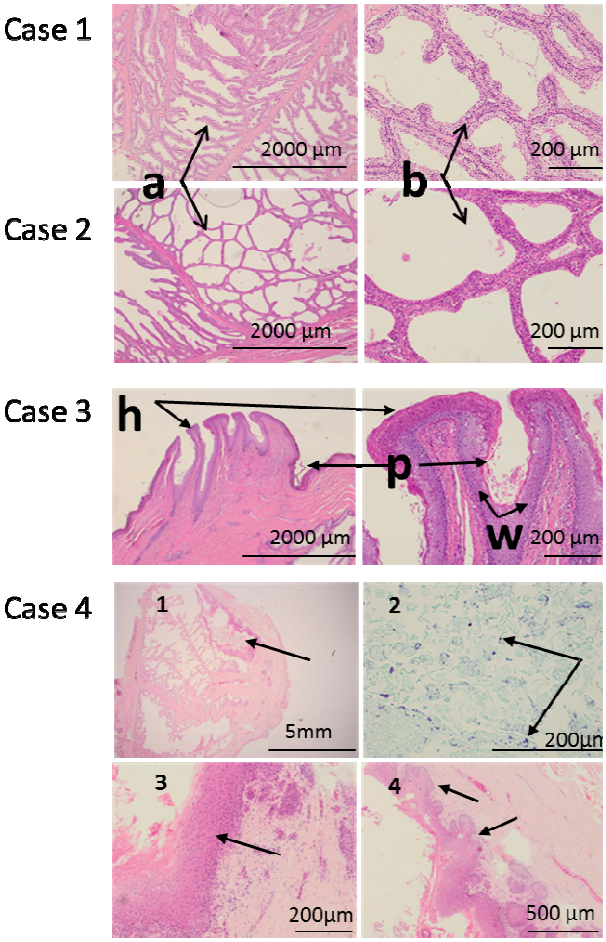

Fig 4. Pathology in the Uropygial Gland (UG) of *Apteryx*. **Cases 1 and 2**: H&E staining showing atrophy of the UG in great spotted kiwi; a) Follicle numbers and epithelial thickness reduction; b) Follicles presenting severely reduced epithelial thickness and expanded lumina. **Case 3**: H&E staining of the uropygial papilla in a female brown kiwi showing hyperkeratosis (h) and pustule (p) formation (w) heterophil infiltration of the dermis. **Case 4**: H&E staining showing localized adenitis of a Haast Tokoeka UG 1) ventral UG sinus filled with necrotic keratinaceous debris (arrow); 2) Gram stain of ventral UG sinus filled with necrotic keratinaceous debris and clumps/pairs/singles of Gram-positive cocci (arrows); 3) H&E staining showing severe diffuse hyperkeratosis, squamous metaplasia, and hyperplasia of affected glandular epithelium (arrow); 4) H&E staining showing numerous epithelial pegs extending from the base of the epithelium into the surrounding connective tissue (arrow).

and often surrounded necrotic foci containing large numbers of branching septate fungal hyphae typical of *Aspergillus* sp. The uropygial gland showed moderate generalised atrophy; the follicles throughout the gland were reduced in number and their lumina were diffusely expanded (Fig. 4A-b). The follicular epithelium was markedly attenuated with no secretory cells present. Chronic mycotic bronchopneumonia due to *Aspergillus* sp. was diagnosed as the most likely cause of death.

Case three: Pustular dermatitis involving the uropygial gland

An adult female brown kiwi was found injured close to a road. She was taken into care and underwent surgery to fix broken toes. Following this she was placed in a small outside run where she appeared to be doing well until she was found dead a week later.

Gross examination revealed that the bird was in good body condition with adequate internal fat reserves. There was a small amount of free blood in the abdomen and left thoracic cavity and a large clot of blood covered the ventral surface of the proventriculus and gizzard. The left lung was firm in texture and both lungs presented with hyperaemia. In addition the adrenal glands were enlarged bilaterally.

Histopathology showed a severe diffuse bacterial ventriculitis which was thought to be the primary cause of death. The gizzard had largely lost the koilin layer and the underlying glands were distended with eosinophilic pyknotic debris, degenerate heterophils, and large numbers of bacteria streaming into the lumen. The lamina propria, submucosa, muscle layers, and serosa all contained moderate numbers of infiltrating heterophils. Bacteria present in the deep glands were Gram-negative, short rods, while bacteria within the superficial layers of remaining koilin were a mixture of Gram-negative, short rods and Gram-positive, medium sized rods. The lung showed an acute bronchopneumonia associated with aspirated koil-

Table 2. Results of a multivariate general linear model examining the effects of section (midline, lateral), age (Ch = chick, J = juvenile, A = adult) species (BK = Brown kiwi; GSK = Great Spotted kiwi), and sex (male, female) on mean follicle (FD) and lumen (LD) diameter. In bold = significant effect after Bonferroni correction (p £ 0.006).

Source	Dependent Variable (log)	Type III Sum of Squares	df	Mean Square	F	P-value	Average (µm)	Standard error
Corrected Model	FD	160145.32b	5	32029.06	34.31	0.000		
	LD	309649.55c	5	61929.91	12.21	0.000		
Intercept	FD	149762806.85	1	149762806.85	160444.33	0.000		
	LD	77707991.17	1	77707991.17	15323.56	0.000		
Section	FD	5.18	1	5.18	0.01	0.941	201.37 201.37	1.02 1.02
	LD	6750.62	1	6750.62	1.33	0.249	46.99 44.36	1.04 1.04
Species (BK/GSK)	FD	1532.17	1	1532.17	1.64	0.200	239.90 203.71	3.28 5.15
	LD	113089.36	1	113089.36	22.30	0.000	62.15 71.25	2.09 3.41
Sex (F/M)	FD	10695.95	1	10695.95	11.46	0.001	208.98 251.24	3.43 4.27
	LD	91106.76	1	91106.76	17.97	0.000	68.17 61.08	2.48 2.56
Age	FD	60365.49	2	30182.75	32.34	0.000	165.01 220.06 246.98	5.56 5.00 3.64
	LD	234078.46	2	117039.23	23.08	0.000	52.98 63.51 67.44	3.70 3.39 2.39
Error	FD	880220.13	943	933.43				
	LD	4782088.07	943	5071.14				
Total	FD	242915039.66	949					
	LD	130406004.41	949					
Corrected Total	FD	1040365.45	948					
	LD	5091737.62	948					

in and bacteria. Occasional small parabronchial and pleural granulomas were also present.

An acute superficial dermatitis was present in the skin of the papilla of the uropygial gland. Superficial epithelial cells showed multifocal ballooning degeneration and heterophil infiltration of the stratum spinosum and the upper dermis was prominent. Between the stratum corneum pustule formation occurred in several areas extending into the stratum spinosum of the papilla (Fig. 4B-c and d). In addition to the pustule formation, there was extensive hyperkeratosis of the papilla (Fig. 4B-c and d).

Case four: Uropygial gland adenitis

A sub-adult male Haast tokoeka was found dead under thick vegetation in a reserve in the West Coast region of New Zealand's South Island. The carcass was in moderate to poor body condition with gelatinous reddening of the remaining subcutaneous fat reserves. On gross examination the coelomic cavity contained a large amount of dark yellow, turbid and flocculent fluid. Numerous thick plaques of yellow fibrinous material was adherent to most of the ventral body wall, the heart, left thoracic air sac, left liver lobe, the ventral aspect of the gizzard and proventriculus, and the serosal surfaces of the small and large intestines. The lungs were diffusely deep red, glistening and slightly swollen. The cause of death was diagnosed as a severe fibrinous coelomitis due to bacterial infection, possibly from an ingested foreign body which penetrated the wall of the gizzard where the infection was localised.

Histopathology revealed a localised adenitis (inflammation) in the uropygial gland ventral lobes. The ventral uropygial gland sinus was filled with necrotic keratinaceous debris (Fig. 4C) which contained numerous and widely distributed Gram-positive cocci. These bacteria were predominantly arranged in clumps although a few were scattered individually or in pairs (Fig. 4C-f). The surrounding glandular epithelium showed severe diffuse hyperkeratosis, squamous metaplasia, and hyperplasia (Figs. 4C-g and h). Some areas of the gland showed numerous epithelial pegs extending from the base of the uropygial gland epithelium into the surrounding connective tissue (Fig. 4C-h). At the base of the epithelium moderate numbers of infiltrating lymphocytes were present. Intraepithelial migrating granulocytes were also seen as well as a hyperplastic nodule of lymphocytes surrounding a capillary in the adjacent connective tissue.

DISCUSSION

This investigation has revealed that the uropygial gland of the *Apteryx* species studied differs markedly from that of all other birds examined to date, but it is similar between *Apteryx* species. Unlike

Table 3. Parameter estimates for each of the factors tested in a Negative Binomial Model (Table 4). The mean is the average number of total cells, germinative cells, intermediate cells and secretory cells in a transect line across the follicles of Uropygial glands in this study. In bold are factors found significant.

Factors		Mean Number	Standard Error	95% Wald CI		Factors		Mean Number	Standard Error	95% Wald CI	
				Lower	Upper					Lower	Upper
Section	Middle	6.37	.345	5.73	7.09	Section	Middle	2.14	.135	1.90	2.43
	Lateral	6.42	.379	5.72	7.21		Lateral	2.14	.144	1.88	2.45
Species	BK	6.17	.378	5.47	6.95	Species	BK	2.40	.168	2.09	2.75
	GSK	6.64	.456	5.80	7.60		GSK	1.91	.151	1.64	2.23
Sex	Female	5.82	.306	5.25	6.45	Sex	Female	2.08	.126	1.85	2.35
	Male	7.03	.456	6.19	7.99		Male	2.21	.162	1.91	2.55
	Chick	5.86	.705	4.63	7.42		Chick	1.41	.205	1.06	1.87
Age	Juvenile	6.59	.398	5.85	7.41	Age	Juvenile	2.47	.165	2.16	2.81
	Adult	6.79	.430	6.00	7.69		Adult	2.84	.195	2.48	3.24
Germinative						Secretory					
Section	Middle	.86	.068	.74	1.01	Section	Middle	2.64	.174	2.32	3.01
	Lateral	.72	.062	.61	.85		Lateral	3.96	.287	3.43	4.56
Species	BK GSK	0.77	0.067	0.65	0.92	Species	BK GSK	2.54	.147	2.27	2.85
		0.8	0.078	0.67	0.97			4.12	.288	3.59	4.72
Sex	Female	.82	.062	.71	.95	Sex	Female	2.54	.147	2.27	2.85
	Male	.76	.070	.63	.91		Male	4.12	.288	3.59	4.72
	Chick	.46	.084	.32	.66		Chick	4.01	.506	3.13	5.14
Age	Juvenile	.87	.069	.75	1.02	Age	Juvenile	3.32	.219	2.92	3.78
	Adult	1.23	.102	1.04	1.44		Adult	2.54	.177	2.21	2.91

most birds, the *Apteryx* uropygial gland was located at the end of the coccygeal bone and surrounding it, with the papillae pointing ventrally towards the cloaca. The gland was bilobar with eight primary sinuses, four per lobe of the gland. Each primary sinus was clearly associated with its own orifice, making eight openings in the papillae. In all *Apteryx* studied, the uropygial gland had little smooth muscle within the interlobular septae or interfollicular septae. Unlike in other birds (Jacob and Ziswiler, 1982) we found striated muscle within the capsule around the caudoventral part of the gland. The *Apteryx* uropygial gland's absolute size is similar to that of their relative, the Red-winged tinamou (*Rhyncotus rufescens*), and species in the orders Anseriformes, Phoenicopteriformes, Falconiformes and Galliformes (when comparing measurements of entire lobes, as measured by Jacob and Ziswiler, 1982).

The uropygial glands of approximately 16% of avian species representing all orders have been examined so far and in most of them the uropygial gland has two lobes, each with its own primary sinus and associated primary duct. Exceptions include the hoopoe (*Upupa epops*) which has three lobes which empty into a papilla that has one broad orifice (Jacob and Ziswiler, 1982; Martín-Vivaldi et al., 2009) and the European nightjar (*Caprimulgus europaeus*) with only one lobe (Jacob and Ziswiler, 1982). Jacob and Ziswiler (1982) described bird species that have more than two orifices within the papillae of the uropygial gland. In these species they found that the secondary sinuses led to the base of the papilla (no primary sinuses existed) where associated ducts continued through the papilla, opening at individual orifices. In the *Apteryx*, however, primary sinuses were apparent and these areas were large and had many secondary sinuses running into the parenchyma. Having many openings may allow the birds to obtain a larger amount of secretion from the gland while preening. A higher flow rate, however, would indicate a need for an increased amount of secretion synthesis and raises the possibility that birds with more than two orifices in the uropygial gland may have differences in the cellular composition of the glands. The size and number of the storage sinuses in *Apteryx* uropygial gland suggests that large amounts of secretion may sometimes be available at one time and this is supported by changes in the volume we found between years in the same individual birds. These differences in volume may be associated with costs of producing the secretion being higher some years and/or higher use of secretion in some years or seasons or under particular environmental or hormonal conditions. It is also interesting to note that most bird species with more than two orifices in the uropygial gland are associated with aquatic habitats (members of the Procellariidae, Phalacrocoracidae, Sulidae, Laridae, Sternidae, Pelecanidae, Phoenicopteridae, Ciconiidae, Gruidae, Alcidae), with exception of *Geronticus eremita* a member of the family Threskiornithidae that inhabits alpine, semi-desert or rocky habitats (Kumerloeve, 1984).

However, *Apteryx* species are not aquatic but, like *Geronticus ermita*, live in a wide range of terrestrial habitats including temperate rain forests, alpine and coastal areas, as well as (but not predominantly) swampy habitats. Based on our findings and these comparisons, we suggest that the large uropygial gland in *Apteryx* species. as well as the differences in volume may be related to maintenance of feathers associated with *Apteryx's* niche and the very wet conditions found in New Zealand's rain forests where these birds evolved. *Apteryx* feathers are hair-like; lacking the barbules that maintain stiff structure of feathers in other avian taxa. Maintaining insulating feathers in a damp environment, both directly above and below the ground in roosting and nesting burrows, may require much oiling. This proposition is supported by

Table 4. Results of Negative binomial with log link models. Dependent variables are numbers of cells in each cell type category. Factors examined: section (midline, lateral), age (chick, juvenile, adult) species (GSK, BK), and sex (male, female). In bold = significant effect after Bonferroni correction ($p \leq 0.006$).

Cell type	Source	Wald Chi-Square	df	Significance
All cells	(Intercept)	1767.66	1	0.000
	Section	0.01	1	0.911
	Species	0.60	1	0.440
	Sex	5.83	1	0.016
	Age	0.98	2	0.613
Germinative	(Intercept)	12.752	1	0.000
	Section	3.538	1	0.060
	Species	.100	1	0.751
	Sex	.564	1	0.453
	Age	24.483	2	0.000
Intermediate	(Intercept)	212.775	1	0.000
	Section	.000	1	0.995
	Species	4.549	1	0.033
	Sex	.469	1	0.494
	Age	16.266	2	0.000
	(Intercept)	212.775	1	0.000
Secretory	(Intercept)	639.854	1	0.000
	Section	2.191	1	0.139
	Species	15.326	1	0.000
	Sex	29.604	1	0.000
	Age	13.047	2	0.001

our finding that the great spotted kiwi, a species that lives in the wet cold mountains of the South Island, has a significantly larger follicle lumen diameter and more secretory cells than brown kiwi, a species living in comparatively drier conditions in the North Island. Another non-mutually exclusive possibility is that *Apteryx* use the secretion of this gland for social purposes as suggested by Castro et al. (2010). Great spotted kiwi, a cooperative breeder, may require larger amounts of secretion for their social interactions than brown kiwi a predominately socially monogamous species. It was interesting that the only kiwi chick, a female great spotted kiwi, despite having less cells overall when compared with older birds, had similar numbers of secretory cells and proportionally a large number of cells in the intermediate layer. Maintenance of feathers for warmth is crucial for the chicks of precocial species, and we suggest that the *Apteryx* uropygial gland may have adaptations to ensure enough oil is produced at all life stages.

The presence of smooth and striated muscle in the capsule of the *Apteryx* uropygial gland may be of some benefit in expelling secretion from the gland, especially the striated muscle which could be contracted voluntarily. Studies of the internal and external musculature surrounding the uropygial gland of birds are scarce. Lucas and Stettenheim (1972) and Jacob and Ziswiler (1982) provide brief descriptions, but these are very general. Lucas and Stettenheim (1972) discovered the presence of smooth muscle within the papilla region of the uropygial gland in the chicken and stated that an important function of these transverse muscles is expansion of duct lumen. Thus, muscular contraction in the chicken causes the opening of the primary ducts, aiding in expulsion of secretion from the gland.

Table 5. Results of a multivariate general linear model examining the effects of age (Ch = chick, J = juvenile, A = adult) species (BK = Brown kiwi; GSK = Great Spotted kiwi), and sex (female, male) on the proportion of each cell (Germinative, Intermediate and Secretory) category. In bold = significant effect after Bonferroni correction ($p \leq 0.006$).

Tests of Between-Subjects Effects[a]

	Dependent Variable Proportion of cells	Type III Sum of Squares	df	Mean Square	F	Sig.
	Germinative	9.390[b]	4	2.348	18.185	**0.0000**
Corrected Model	Intermediate	46.941[c]	4	11.735	54.479	**0.0000**
	Secretory	77.923[d]	4	19.481	76.557	**0.0000**
	Germinative	104.369	1	104.369	808.481	**0.0000**
Intercept	Intermediate	928.244	1	928.244	4309.205	**0.0000**
	Secretory	867.996	1	867.996	3411.153	**0.0000**
	Germinative	1.119	1	1.119	8.671	0.0030
Species	Intermediate	9.29	1	9.29	43.127	**0.0000**
	Secretory	16.974	1	16.974	66.706	**0.0000**
	Germinative	6.35	1	6.35	49.188	**0.0000**
Sex	Intermediate	11.194	1	11.194	51.964	**0.0000**
	Secretory	34.26	1	34.26	134.64	**0.0000**
	Germinative	3.328	2	1.664	12.89	**0.0000**
Age	Intermediate	40.07	2	20.035	93.008	**0.0000**
	Secretory	53.602	2	26.801	105.326	**0.0000**
	Germinative	121.864	944	0.129		
Error	Intermediate	203.347	944	0.215		
	Secretory	240.209	944	0.254		
	Germinative	246.183	949			
Total	Intermediate	1175.67	949			
	Secretory	1570.635	949			
	Germinative	131.254	948			
Corrected Total	Intermediate	250.288	948			
	Secretory	318.131	948			

[a] Weighted Least Squares Regression - Weighted by bird ID; [b] R Squared = .072 (Adjusted R Squared = .068); [c] R Squared = .188 (Adjusted R Squared = .184); [d] R Squared = .245 (Adjusted R Squared = .242)

Jacob and Ziswiler (1982) suggested that the striated muscle of the tail region can have both direct and indirect effects on expulsion of secretion from the uropygial gland. In some species, for example the common loon (*Gavia immer*), 80% of the *levator caudae* muscle fibres attach to the capsule of the uropygial gland and just 20% to the bones of the vertebral column. Contractions of this muscle cause lateral and lift movements of the tail, and longitudinal extension of the uropygial gland (Jacob and Ziswiler, 1982). In the *Apteryx* the presence of striated muscle, both around the coccygeal region and in the caudoventral capsule surrounding the gland, may relate to these observations. Video recordings of brown kiwi on our study site (Cunningham and Castro, 2011) show that they are able to control the spread of their rump feathers when defecating and preening, and we suggest that they may be able to use these movements to expel uropygial gland secretion.

Histological characteristics of the follicle cell layers in *Apteryx* were similar to other species in which follicles have been described, including the osprey (*Pandion haliaetus*) (Harem et al., 2010), moorhen (*Gallinula chloropus*) (Sawad, 2006), European starling (Sadoon, 2011), and chicken (Lucas and Stettenheim, 1972). Male and female *Apteryx* had similar number of cells contributing to

Table 6. Basic statistics and pairwise comparisons for the proportion of the types of cells composing the follicle tissue according to age (Ch = chick, J = juvenile, A = adult) species (BK = Brown kiwi; GSK = Great Spotted kiwi), and sex (female, male). In bold significant comparisons after Bonferroni correction ($p \leq 0.006$).

Estimates [a]						Pairwise comparisons [a]					
Dependent Variable	Species	Mean	Std. Error	95% CI		Species	Mean Difference	Std. Error	Significance	95% CI for Difference	
Proportion of cells				Lower Bound	Upper Bound					Lower Bound	Upper Bound
Germinative	BK	0.16	0.01	0.15	0.18	vs GSK	.034*	0.01	0.003	0.01	0.06
Intermediate	BK	0.48	0.01	0.46	0.51		.099*	0.02	0.000	0.07	0.13
Secretory	BK	0.35	0.01	0.33	0.38		-.134*	0.02	0.000	-0.17	-0.10
Germinative	GSK	0.13	0.01	0.11	0.14	vs BK	-.034*	0.01	0.003	-0.06	-0.01
Intermediate	GSK	0.39	0.01	0.37	0.40		-.099*	0.02	0.000	-0.13	-0.07
Secretory	GSK	0.49	0.01	0.47	0.51		.134*	0.02	0.000	0.10	0.17
Germinative	Female	0.10	0.01	0.09	0.12	vs Male	-.083*	0.01	0.000	-0.11	-0.06
Intermediate	Female	0.38	0.01	0.36	0.40		-.110*	0.02	0.000	-0.14	-0.08
Secretory	Female	0.52	0.01	0.50	0.54		.193*	0.02	0.000	0.16	0.23
Germinative	Male	0.19	0.01	0.17	0.20	vs Female	.083*	0.01	0.000	0.06	0.11
Intermediate	Male	0.49	0.01	0.47	0.51		.110*	0.02	0.000	0.08	0.14
Secretory	Male	0.32	0.01	0.30	0.35		-.193*	0.02	0.000	-0.23	-0.16
Germinative	Chick	0.19	0.01	0.16	0.21	vs Juvenile	.036*	0.02	0.056	0.01	0.07
						vs Adult	.083*	0.02	0.000	0.05	0.12
Intermediate	Chick	0.60	0.02	0.56	0.63	vs Juvenile	.271*	0.02	0.000	0.23	0.31
						vs Adult	.215*	0.02	0.000	0.17	0.26
Secretory	Chick	0.22	0.02	0.18	0.25	vs Juvenile	-.307*	0.02	0.000	-0.35	-0.27
						vs Adult	-.299*	0.02	0.000	-0.35	-0.25
Germinative	Juvenile	0.15	0.01	0.14	0.16	vs Chick	-.036*	0.02	0.056	-0.07	-0.01
						vs Adult	.047*	0.01	0.000	0.02	0.07
Intermediate	Juvenile	0.33	0.01	0.31	0.35	vs Chick	-.271*	0.02	0.000	-0.31	-0.23
						vs Adult	-.055*	0.02	0.001	-0.09	-0.03
Secretory	Juvenile	0.53	0.01	0.51	0.55	vs Chick	.307*	0.02	0.000	0.27	0.35
						vs Adult	0.008	0.02	1.000	-0.03	0.04
Germinative	Adult	0.10	0.01	0.08	0.12	vs Chick	-.083*	0.02	0.000	-0.12	-0.05
						vs Juvenile	-.047*	0.01	0.000	-0.07	-0.02
Intermediate	Adult	0.38	0.01	0.36	0.41	vs Chick	-.215*	0.02	0.000	-0.26	-0.17
						vs Juvenile	.055*	0.02	0.000	0.03	0.09
Secretory	Adult	0.52	0.01	0.49	0.54	vs Chick	.299*	0,02	0.000	0.25	0.35
						vs Juvenile	-0.008	0.02	1.000	-0.04	0.03

* The mean difference is significant at the .05 level. [a] Weighted Least Squares Regression - Weighted by bird ID

their follicular epithelium. However, there were significant differences in the diameter of follicles and lumen as well as the proportions of cells within the follicles. This could have implications for the amount and/or composition of the secretion produced as more cells are likely to result in increased secretion, and larger lumen may represent more storage or production of secretion. Sex-specific differences in follicular epithelia may also suggest a social role for the uropygial gland secretion that is more strongly selected in one of the sexes and requires further research.

Pathological changes were found in the uropygial glands of a high proportion of birds examined histologically in this study. This can probably be explained by the fact that samples were obtained from a veterinary centre to which *Apteryx* carcasses were sent by conservation authorities for necropsy. Many of these individuals will have died from disease-related causes, and therefore the high proportion of uropygial glands showing pathological changes is unlikely to represent the situation in the *Apteryx* population as a whole. In all cases, cause of death was not specifically related to pathology of the uropygial gland. Rather, systemic illnesses seem to affect the uropygial gland in *Apteryx* and this in turn has implications on the overall condition of the bird – both physically and ecologically. For example, birds with atrophied glands may lose feather condition and be more prone to skin infections, cold, or ectoparasites. A decrease in gland function may affect social interactions through reduced secretion production or secretion composition and *Apteryx* may use this to assess the health status of conspecifics. Avian pox has recently been reported in brown kiwi (Ha et al., 2011); and our discovery of pustular dermatitis of the uropygial gland in an adult female brown kiwi may be an early indication of this disease.

Pustular formation has been associated with avian pox virus in a variety of birds (Yoshikkawa and Alam, 2002).

Both the innervation and mechanism for the expulsion of secretion and the control of secretion synthesis require further investigation in *Apteryx*. Few studies have examined the role of hormones in the control of secretion production in any bird species. In the pigeon, treatment with the hormone estriol resulted in inhibition of uropygial gland activity (Manna et al., 1983) while in mallards, estradiol increased the number of peroxisomes (associated with fat biosynthesis; Sara et al., 2006) within uropygial gland secretory cells and consequently enhanced fatty acid diester biosynthesis (Bohnet et al., 1991). Furthermore, hormones control the synthesis of secretion in sebaceous glands of mammals (Schneider and Paus, 2010) and knowledge of the hormonal status of birds could reveal key information regarding the gland's function.

Appendix 1. Results of a multivariate general linear model (including basic statistics) examining the effects of age (Ch = chick, J = juvenile, A = adult) species (BK = Brown kiwi; GSK = Great Spotted kiwi), and sex (female, male) on the length and depth of the uropygial gland.

Source	Dependent Variable	Type III Sum of Squares	df	Square		
Corrected Model	Length	500.235b	5	100.05	1.65	0.221
	Depth	253.529c	5	50.71	1.08	0.419
Intercept	Length	4001.59	1	4001.59	66.04	0.000
	Depth	1502.57	1	1502.57	32.01	0.000
Species	Length	195.08	2	97.54	1.61	0.240
	Depth	61.20	2	30.60	0.65	0.539
Sex	Length	2.82	1	2.82	0.05	0.833
	Depth	3.13	1	3.13	0.07	0.801
Age	Length	209.56	2	104.78	1.73	0.219
	Depth	141.22	2	70.61	1.50	0.261
Error	Length	727.12	12	60.59		
	Depth	563.33	12	46.94		
Total	Length	107797.57	18			
	Depth	36304.22	18			
Corrected Total	Length	1227.35	17			
	Depth	816.85	17			

Dependent Variable	Factors	Mean	Std. Error	95% CI Lower Bound	95% CI Upp Bound
Length	GSK	23.99	2.81	17.86	30.12
	BK	20.97	2.75	14.99	26.95
	Tokoeka	20.22	3.25	13.14	27.31
Depth	GSK	14.39	2.48	9.00	19.78
	BK	12.63	2.42	7.37	17.90
	Tokoeka	12.92	2.86	6.69	19.16
Length	Female	21.59	2.59	15.74	27.44
	Male	21.87	2.82	15.72	28.02
Depth	Female	13.16	2.36	8.02	18.31
	Male	13.47	2.49	8.05	18.88
Length	Chick	14.48	7.95	-2.84	31.80
	Juvenile	24.53	1.01	22.32	26.74
	Adult	26.18	1.07	23.85	28.50
Depth	Chick	10.58	7.00	-4.67	25.82
	Juvenile	13.70	0.89	11.76	15.65
	Adult	15.66	0.94	13.62	17.71

Uropygial glands appear to be absent in paleognaths other than *Apteryx* and tinamous. Future studies should therefore aim to investigate the histological organisation and structure of the uropygial gland of Tinamiformes, as this would complete knowledge of uropygial glands of the Palaeognathae lineage. The apparent lack of the uropygial gland in adults of the other ratite species, which are much larger and all but one group (the cassowaries *Causarius* spp.) live in open areas, suggests that the uropygial gland in *Apteryx* and tinamous may provide benefits related to their forest ground dwelling existence. Histological comparison of the uropygial gland in these two groups as well as studies of the embryogenesis of *Apteryx*, tinamous and ratites lacking uropygial glands will aid in answering questions regarding development of the uropygial gland and reasons for its absence and will also assist in closing the knowledge gap on this still enigmatic gland.

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REFERENCES

AMAT JA, RENDÓN MA, GARRIDO-FERNÁNDEZ J, GARRIDO A, RENDÓN-MARTOS M, PÉREZ-GÁLVEZ A (2011) Greater flamingos *Phoenicopterus roseus* use uropygial secretions as make-up. *Behav Ecol Sociobiol*, 65: 665-673.

AMO L, LOPEZ-RULL I, PAGAN I, MACIAS-GARCIA C (2012) Male quality and conspecific scent preferences in the house finch, *Carpodacus mexicanus. Anim Behav*, 84: 1483-1489.

BANCROFT JD, GAMBLE M (2008) Theory and practice of histological techniques. Sixth edition. China: Churchill Livingstone Elsevier.

BEDDARD FE (1899) Notes on the anatomy of the genus *Apteryx. Novit Zool,* 6: 361-402.

BOHNET S, ROGERS L, SASAKI G, KOLATTIKUDI PE (1991) Estradiol induces proliferation of peroxisome-like microbodies and the production of 3-hydroxy fatty acid diesters, the female pheromones, in the uropygial glands of male and female mallards. *J Biol Chem*, 266*:* 9795-9804.

BURBIDGE ML, COLBOURNE RM, ROBERTSON HA, BAKER AJ (2003) Molecular and other biological evidence supports the recognition of at least three species of brown kiwi. *Conserv Genet*, 4: 167-177.

CASTRO I, CUNNINGHAM SJ, GSELL AC, JAFFE K, CABRERA A, LIENDO C (2010) Olfaction in birds: a closer look at the kiwi (Apterygidae). *J Avian Biol*, 41: 213-218.

CHIALE MC, FERNÁNDEZ PE, GIMENO EJ, BARBEITO CG, MONTALTI D (2014) Morphology and histology of the uropygial gland in Antarctic birds: relationship with their contact with the aquatic environment? *Australian J Zool*, 62: 157-165.

CHIALE MC, MONTALTI D, FLAMINI MA , FERNÁNDEZ PE, GIMENO EJ, BARBEITO CG (2015) Histological and histochemical study of the uropygial gland of chimango caracara (*Milvago chimango*, Vieillot 1816). *Biotech and Histochem*, 91(1): 30-37.

CHIALE MC, MONTALTI D, FLAMINI MA, BARBEITO CG (2016) The uropygial gland of the Southern Caracara (*Caracara plancus*; Falconidae: Falconinae): histological and histochemical aspects. *Acta Zoologica* (Stockholm), doi: 10.1111/azo.12171.

CHANDRASEKAR V, KUMAR NS, LALITHA PS (1990) Histology and histochemistry of the uropygial gland of the Japanese quail *Coturnix coturnix japonica*. *J Vet Ani Sci*, 21(1): 77-82.

CUNNINGHAM SJ, CASTRO I (2011) The secret life of wild brown kiwi: studying behaviour of a cryptic species by direct observation. *N Z J Ecol*, 35: 209-219.

DUMBACHER JP, BEEHLER BM, SPANDE TF, GARRAFFO HM, DALY JW (1992) Homobatrachotoxin in the genus *Pitohui*: Chemical defense in birds? *Science,* 258: 799-501.

DUNN OJ (1961) Multiple comparisons among means. *J Amer Stat Assoc*, 56 (293): 52-64.

EL-BARGEESY GA, EL-SHAMMAA MA, EL-GHARBAWY SM (1995) Histological and some histochemical studies on the uropygial gland of young and mature pigeons. *Vet Med J Giza*, 43(4): 429-448.

ELDER WH (1954) The oil gland of birds. *Wilson Bull,* 66*:* 6-31.

GIRAUDEAU M, DUVAL C, GUILLON N, BRETAGNOLLE V, GUTIERREZ C, HEEB P (2010) Effects of access to preen gland secretions on mallard plumage. *Naturwissenschaften*, 97: 577-581.

HA HJ, HOWE L, ALLEY M, GARTRELL B (2011) The

phylogenetic analysis of avipoxvirus in New Zealand. *Vet Microbiol.* 150: 80-87.

HAREM IS, KOCAK-HAREM M, TURAN-KOZLU T, AKAYDIN-BOZKURT Y, KARADAG-SARI E, AL-TUNAY H (2010) Histologic structure of the uropygial gland of the osprey (*Pandion haliaetus*). *J Zoo Wildl Med*, 41: 148-151.

HIRAO A, AOYAMA M, SUGITA S (2009) The role of uropygial gland on sexual behavior in domestic chicken *Gallus gallus domesticus*. *Behav Processes*, 80(2): 115-120.

JACOB J, ZISWILER V (1982) The uropygial gland. In: Farner DS, King JR, Parkes KC (eds). *Avian biology: Volume VI*. America: Academic Press, New York, pp 199-314.

JENIK RA, FISCH JE, GOODRIDGE AG (1987) Terminal differentiation in the avian uropygial gland. Accumulation of fatty acid synthase and malic enzyme in non-dividing cells. *Cell Tissue Res,* 250: 315-321.

JOHNSTON DW (1988) A morphological atlas of the avian uropygial gland. *Bull Br Mus Nat Hist Zool,* 54: 199-259.

KALE MA, BHAMBURKAR VR, DALVI RS, BANUBAKODE SB, PATIL VA (1999) Histological and histochemical study of preen gland in fowl (*Gallus gallus domesticus*). *Indian J Vet Anat*, 11(2): 176-177.

KING AS, MCLELLAND J (1985) *Form and function in birds.* Great Britain: Academic Press, London, pp 1-52.

KOLATTUKUDY PE, BOHNET S, ROGERS L (1987) Diesters of 3-hydroxy fatty acids produced by the uropygial glands of female mallards uniquely during the mating season. *J Lipid Res,* 28: 582-588.

KUMERLOEVE H (1984) The waldrapp, *Geronticus eremita* (Linnaeus, 1758): Historical review, taxonomic history, and present status. *Biological Conservation*, 30: 363-373.

LUCAS AM, STETTENHEIM PR (1972) *Avian anatomy: Integument.* Agriculture Handbook 362, U.S. Department of Agriculture, Washington, D.C.

MANNA CK, DEADHIKARI H, BHATTACHARYYA SP (1983) Action of estriol on the uropygial gland activity in pigeon. *Z Mikrosk Anat Forsch,* 97*:* 386-392.

MARDON J, SAUNDERS SM, BONADONNA F (2011) From preen secretions to plumage: the chemical trajectory of blue petrels' *Halobaena caerulea* social scent. *J Avian Biol,* 42: 29-38.

MARTÍN-VIVALDI M, RUIZ-RODRÍGUEZ M, JOSÉ SOLER J, PERALTA-SÁNCHEZ JM, MÉNDEZ M, VALDIVIA E, MARTÍN-PLATERO AM, MARTÍNEZ-BUENO M (2009) Seasonal, sexual and developmental differences in hoopoe *Upupa epops* preen gland morphology and secretions: evidence for a role of bacteria. *J Avian Biol,* 40: 191-205.

MOYER BR, ROCK AN, CLAYTON DH (2003) Experimental test of the importance of preen oil in rock doves (*Columba livia*). *The Auk,* 120: 490-496.

ONAL O, TURK S, CINAR K (2013) The histological and histochemical characteristics on the uropygial gland of female and male partridge (*Alectoris chukar*). *Saglik Bilimleri Veteriner Dergisi, Firat Universitesi*, 27(1): 7-12.

QUAY WB (1972) Integument and the environment: Glandular composition, function, and evolution. *Am Zool*, 12: 95-108.

RAJCHARD J (2010) Biologically active substances of bird skin: a review. *Vet Med,* 55: 413-421.

ROBERSTON H, COLBOURNE R, CASTRO I, MILLER C, CRESSWELL M, MCLENNAN J, DEW L, MILES J, PIERCE R. OLSEN D, FLUX II, POTTER M (2003) Kiwi (*Apteryx* spp.) Best Practice Manual. Department of Conservation, Wellington, New Zealand.

SADOON AH (2011) Histological study of European starling uropygial gland (*Sturnus vulgaris*). *Int J Poult Sci,* 10: 662-664.

SALIBIAN A, MONTALTI D (2009) Physiological and biochemical aspects of the avian uropygial gland. *Braz J Biol,* 69: 437-446.

SARA Q, MALENTACCHI C, DELFINO C, BRUNASSO AMG, DELFINO G (2006) Adaptive evolution of secretory cell lines in vertebrate skin. *Caryologia,* 59: 187-206.

SAWAD AA (2006) Morphological and histological study of the uropygial gland in the moorhen (*Gallinula chloropus*). *Int J Poult Sci*, 50: 938-941.

SCHNEIDER MR, PAUS R (2010) Sebocytes, multifaceted epithelial cells: Lipid production and holocrine secretion. *Int J Biochem Cell Biol,* 42: 181-185.

SHAFIIAN AH, MOBINI B (2014) Histological and histochemical study on the uropygial gland of the goose (*Anser anser*). *Bulgarian J Vet Med*, 17(1): 1-8.

SHAWKEY MD, PILLAI SR, HILL GE (2003) Chemical welfare? Effects of uropygial oil on feather-degrading bacteria. *J Avian Biol,* 34: 345-349.

STETTENHEIM PR (2000) The integumentary morphology of modern birds – an overview. *Am Zool*, 40: 461-470.

SUNANDA K, RAO TSCS, NAGAMALLESWARI Y, LAKSHMI MS, RAMAYYA PJ, PURUSHOTHAM G (2001) Histological studies on the uropygial gland of the domestic duck (*Anas boschas domesticus*). *Indian J Vet Anat*, 13(2): 123-129.

KOZLU T, BOZKURT YA, ATES S (2011) A macroanatomical and histological study of the uropygial gland in the white stork (*Ciconia cicionia*). *Int J Morphol*, 29(3): 723-726.

WAGNER RC, BROOD RL (1975) Cytological differentiation in the Uropygial Gland. *J Morphol,* 146: 395-414.

YOSHIKKAWA MGT, ALAM J (2002) Histopathological studies of fowl pox in bantams. *Int J Poult Sci,* 1: 197-199.