Geometric morphometric methods show no shape differences between female and male kākāpō (*Strigops habroptilus*) bills and claws

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Abstract Kākāpō (*Strigops habroptilus*) are the only parrot species known to have a lek-based mating system. In competing for mating opportunities with females, males can fight intensely with one another, sometimes with fatal consequences. Males may have evolved more deeply hooked bills and raptorial claws than females if these confer advantage in conflicts with other males. We studied bill and claw shape in 28 museum specimens using geometric morphometrics and found no sex differences. While no claw shape sex differences were identified, we did find kākāpō lateral claws are significantly more hooked than their medial toe claws which are flatter. Claw shape in other parrot species has not yet been analysed via geometric morphometric methods, it is therefore unknown whether this claw-shape configuration is unique to kākāpō.

INRODUCTION

Sexual dimorphism occurs in many animal species where females and males have distinct life histories. This is especially true when the features of one sex are subject to either intra- or inter-sexual selection (West-Eberhard 1983; Andersson 1994). In birds, males often evolve exaggerated morphological traits when they are required to display to attract mates, or to fight each other over the territories they use to gain mates (Owens & Hartley 1998; Jones & Hunter 1999).

While many parrot species express some sexual dimorphism (Moorhouse *et al*. 1999; Berkunsky *et*

al. 2009; Barreira *et al*. 2012; de Araujo & Marcondes-Machado 2014), most parrots are monogamous and both sexes participate in nest preparation, egg incubation, and the care of dependent offspring (Juniper & Parr 1998; Masello *et al*. 2002; Ekstrom *et al*. 2007). In these species sexual selection is thought to be less intense than in polyandraous bird species (Payne 1984; Höglund 1989; Møller & Pomiankowski 1993).

Kākāpō (*Strigops habroptilus*) are the only parrots known to have a lek-based mating system, be flightless and nocturnal (Merton *et al*. 1984). Male kākāpō establish and maintain specialised mating arenas known as track and bowl systems, or "kākāpō gardens", for the purposes of display to females (Merton *et al*. 1984; Powlesland *et al*. 1992).

Male kākāpō vigorously defend their track and bowl territories from other males and fights between males resulting in the death of one combatant have been reported (Reischek 1884; Cockrem 1999). In the evenings, males call from their arenas to attract females, to whom the males also display (Merton *et al*. 1984). Females may visit and mate with multiple males per evening, and males in turn may receive multiple visits from different females (Eason *et al*. 2005). Following mating, females, who are less territorial than males (Whitehead et al. 2012), raise their chicks in nest burrows, conducting all incubation and chick provisioning without male assistance (Reischek 1884; Merton *et al*. 1984; Whitehead *et al*. 2012).

Given these behavioural differences, kākāpō may show some sexual dimorphism in the shape of their bills and claws. Some shapes may confer advantage in conflicts between rivals, for example, males may have evolved more re-curve bills and raptorial claws than females. We therefore tested the null hypothesis that male and female claw and bill shapes were the same using museum specimen kākāpō and geometric morphometric techniques.

MATERIALS AND METHODS
Specimens and photography
A total of 28 (*n* = 19 females, *n* = 9 males) kākāpō study-skins were analysed for this study. These were 10 female and 4 male specimens held by the Australian Museum, Sydney, and 9 female and 5 male specimens held by the University of Sydney Macleay Museum, Sydney. Specimen sex was determined through wing-feather patterning, which differs between female and male birds (Robertson *et al*. 2000), combined with a discriminate function analysis (DFA; see Gray *et al*. 2017 for details). The DFA predicted sex with 96.5% accuracy and was trained with "exposed culmen", "culmen", "bill gape", "tail", and "longest toe claw" length measures from 12 adult female and 17 adult male kākāpō of certain sex. For 8 museum specimens, wing patterning was not distinctively female or male, and the DFA prediction of sex was used.

Digital images of the lateral side of each parrot's bill, and of each of the 4 claws on both feet were taken with a IXUS 130 Canon digital camera. The body side from which the photographs were taken was also recorded. Six females and 2 males had only 3 of their claws included in the analysis due to the other claws being inaccessible. To ensure image parallax did not influence shape analyses (Mullin & Taylor 2002), specimens were supported by cushions so that the camera was always held directly perpendicular to the body part being imaged. For image scaling, all photographs included a ruler that was positioned level with the sagittal plane of the photographed bill or claw (see Fig. 1). Claw number was scored as follows: number 1 was on the medial anterior toe; number 2 was the lateral anterior toe; number 3 was on the lateral posterior toe; and number 4 was on the medial posterior toe (see Fig. 2).
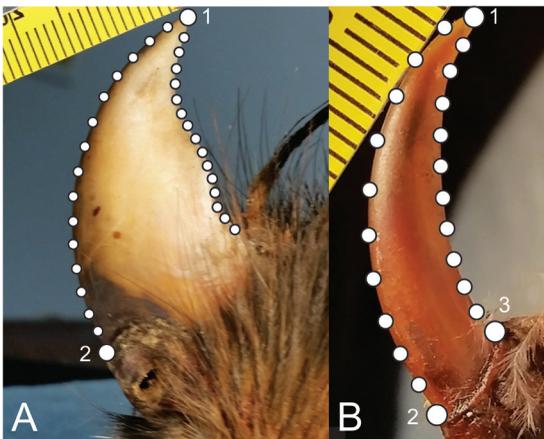

Fig. 1. Representative digital photographs of a kākāpō specimen bill (A) and claw (B) showing how specimens were orientated for photography. Numbered white points indicate the position of landmarks. Non-numbered white points indicate sliding semi-landmarks.
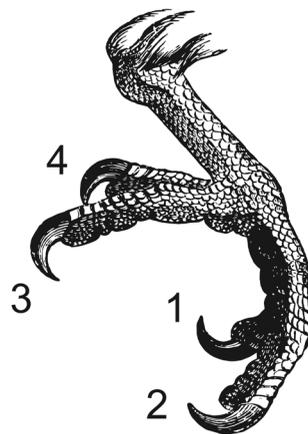

Fig. 2. Parrot foot indicating the number of each kākāpō claw. Lateral side is shown outer-most. Re-drawn from Cooper (1887).

Table 1. Bill Principal Component Analysis results showing the proportion of bill shape variation accounted for by the first 10 principal components (PC).

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Standard deviation	0.0352	0.0305	0.0162	0.0010	0.0080	0.0063	0.0061	0.0047	0.0037	0.0035
Proportion of variance	0.4505	0.3375	0.0950	0.0356	0.0230	0.0143	0.0136	0.0080	0.0051	0.0045
Cumulative proportion	0.4505	0.7880	0.8830	0.9189	0.9416	0.9559	0.9695	0.9775	0.9826	0.9871

Geometric morphometric analyses
Landmarks
Landmark-based geometric morphometric methods quantify shape through homologous morphological landmarks (Patterson 1982). We used tpsDig2 software (ver. 2.16 [Rohlf 2010]) to digitise 2 landmarks on each bill photograph, and 3 landmarks on each claw photograph. For the bills, the first landmark was the bill tip, and the second was the point where the upper-culmen meet the cere (see Fig. 1a). Fifteen sliding semi-landmarks were then placed equidistant between landmarks 1 and 2 along the dorsal surface of upper-culmen. Fifteen sliding semi-landmarks were also placed equidistant along the ventral edge of the upper-culmen. These extended backwards from landmark 1 to where the culmen met the lores feathers (Fig. 1a). For the claws, 3 landmarks were placed as follows: landmark 1 on the claw tip, landmark 2 at the junction where the dorsal surface of the claw met the toe skin, and landmark 3 at the junction of the ventral surface of the claw and the toe (Fig. 1b). Dorsally between landmarks 1 and 2, and ventrally between landmarks 1 and 3, 10 sliding semi-landmarks were placed equidistant along the claw edges. Sliding semi-landmarks are used to describe the shape of curves, and differ from true landmarks as rather than remaining fixed in a fixed position in shape-space during analyses, they shift laterally toward and away from one another to provide a comprehensive estimate of shape curvature (see Adams *et al*. 2004).

Statistical analyses
We used Geomorph (ver. 3.0.1) in R (ver. 3.2.4) for all analyses (Adams & Otarola-Castillo 2013) and analysed the bill and claw landmark sets separately. First, landmark sets were aligned using Generalised orthogonal Procrustes superimposition analysis (GPA). This removes inter-specimen size, position and orientation variation, and calculates a series of *x,y* Procrustes coordinates which define the shape of each specimen in the set within Procrustes shape-space. GPA removes inter-specimen differences in

size by scaling each specimen to unit centroid size (Rohlf & Slice 1990; Klingenberg & McIntyre 1998; Adams *et al*. 2004). Scaling is achieved by taking the square root of the sum of squared distances from each specimen's set of landmarks to their centroid (Klingenberg & McIntyre 1998). We then extracted the landmark set's partial warp scores, i.e. the "shape variables" (Adams *et al*. 2004) of each specimen. Principal components analysis (PCA) was then conducted on the partial warp scores, providing a quantitative description of the main way the specimens varied in shape from one another. Next, we plotted the bill and claw PCA results showing the proportion of variation in shape among the specimens due to principal component (PC) 1 and 2 in a PC space called tangent-space (Adams & Otarola-Castillo 2013). On these plots we also included transformation grids representing how specimen shape varied along the PC1 axis (Adams & Otarola-Castillo 2013).

To test for differences in bill and claw shape due to sex we performed Procrustes ANOVA on specimen Procrustes-aligned coordinates (Adams & Otarola-Castillo 2013).

RESULTS
Bill shape
The proportion of bill-shape variation explained by the first principal component (PC1) was 0.4505, and 0.338 for PC2 (Table 1). The shape deformation described by PC1 represented a shift from an elongate, more deeply re-curve, hooked bill to a stouter, less hooked bill. However, there was no clustering or separation of male and female bills along the first or second principal components (Fig. 3). This was confirmed by Procrustes ANOVA which showed that there was no significant difference in bill shape due to sex ($F_{(1,26)}$ = 0.4938, P = 0.735, Table 2).

Claw shape
PCA on claw specimen partial warp scores found the proportion of claw shape variation explained
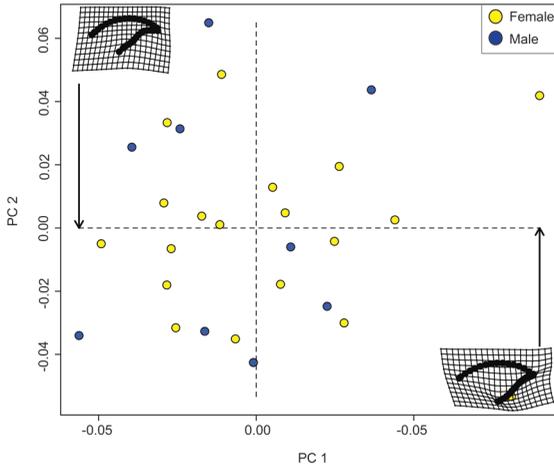
Fig. 3. The position of Procrustes-aligned female (yellow points) and male (blue points) specimens within tangent-space plot showing the major axes of kākāpō bill shape variation, PC1 and PC2. Deformation grids show the shape of kākāpō bills at either end of PC1.

Table 2. Results from the Procrustes ANOVA comparing female and male bill shape.

	df	SS	MS	Rsq	F	Z	P
Sex	1	0.00138	0.00138	0.0186	0.4938	0.3914	0.735
Residuals	26	0.07283	0.00280				
Total	27	0.07421					

Table 3. Kākāpō claw Principal Component Analysis values, indicating the proportion of claw shape variation explained by the first 10 principal components (PC).

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Standard deviation	0.0304	0.0290	0.0159	0.0118	0.0071	0.0063	0.0050	0.0043	0.0041	0.0029
Proportion of variance	0.3905	0.3555	0.1064	0.0595	0.2118	0.0166	0.0106	0.0078	0.0069	0.0037
Cumulative proportion	0.3905	0.7460	0.8525	0.9119	0.9331	0.9498	0.9604	0.9681	0.9750	0.9787

by PC1 was 0.391, and 0.355 by PC2 (Table 3). The claw PCA plot showed that PC1, the main axis of claw shape variation, described a transition from a thick, deeply re-curved claw to a narrower, flatter claw. However, colour-coding specimens according to sex indicated that claw shape variation was not associated with sex (Fig. 4a), and Procrustes ANOVA confirmed there was no significant difference in claw shape between females and males ($F_{(1,152)}$ = 2.2455, P = 0.106, Table 4). Procrustes ANOVA did however show claw shape varied significantly with claw number ($F_{(1,152)}$ = 12.3216, P = 0.001, Table 4). Colour

coding the claw specimens according to their claw number within the PCA plot and examining their relative position in tangent space indicated that this is due to the lateral toe claws, number 2 and 3, being more hooked in shaped than the medial toe claws, number 1 and 4 (Fig. 4b). Unfortunately, we were unable to conduct *post-hoc* multiple comparisons to identify formally which claws differed from one another statistically, as appropriate tests have not yet been developed (Adams & Otarola-Castillo 2013). Procrustes ANOVA also showed there were no differences in claw shape due to body side, or
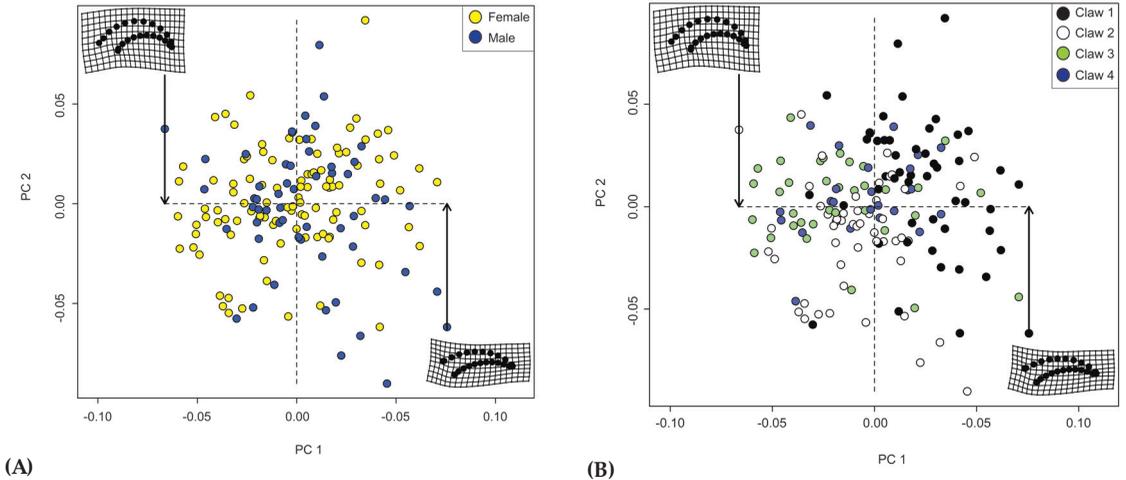
Fig. 4. Tangent-space showing the position of Procrustes-aligned kākāpō specimen claws along PC1 and PC2. Deformation grid plots at either end of PC1 indicate the main way kākāpō claws differed from one-another in their shape. Panel A shows the position of female (yellow) and male (blue) specimen claws in tangent-space. Panel B shows the same specimens with points coloured according to claw number 1 (black), number 2 (white), number 3 (green) and claw number 4 (blue).

due to the interaction of any of sex, body size, or claw number (Table 4).

DISCUSSION

Despite marked behavioural differences between female and male kākāpō, our results indicate no sexual dimorphism in the shape of kākāpō bills or claws. All Strigoipedean parrots are however sexual dimorphic in size, with male kākāpō, kea and kaka possessing larger bills and bodies than females (Moorhouse *et al*. 1999; Gray *et al*. 2017). Among these species, sexual difference in body size is most pronounced in kākāpō (Merton *et al*. 1984; Livezey 1992). Given all parrot species have raptorial claws and hooked bills, it is possible these exaptations have proved sufficient weapons for fighting male kākāpō, and body size has been a more important target of intra-sexual selective pressure for kākāpō relative to other family members. Larger male kākāpō may be are able to wield their weapons to greater effect than smaller males.

The 28 museum specimen kākāpō did differ from one another in their bill shape (Fig. 3), however this was not sex based. While not explicitly quantified, younger kākāpō are reported to have narrower, more pointed bills than older birds (Powlesland *et al*. 2006), and possible age differences among the museum specimens at their time of death may account for the inter-individual

differences. To verify these museum specimen-based findings, geometric morphometric analyses on the bills of kākāpō of known age and sex should be conducted. Several specimens from our dataset had their sex determined using DFA only (Gray *et al*. 2017), therefore it is appropriate to regard the current findings as preliminarily.

We identified statistically significant differences in claw shape across the 28 specimens related to claw position on the foot. Our PCA analysis indicated that the 2 lateral claws on each kākāpō foot were more raptorial than the medial toes (Fig. 4). These differences in claw shape could be due to an increased need for grip strength on the lateral side of the kākāpō body during terrestrial locomotion. Kākāpō have several distinctive morphological adaptions associated with their terrestriality. Compared to more arboreal parrots, kākāpō have longer femur bones and shorter tarso-metatarus (Livezey 1992) and phalanx bones (Hopson 2001). It is possible the claw shape of kākāpō is similarly unique, however we unable to investigate this further as to date no other geometric morphometric studies of parrot claw shape have been conducted.

Future comparative studies would also show whether the opposite possibility is true – that all parrots share the kākāpō claw configuration of flatter medial claws and more raptorial lateral claws. In addition to their ground based-activities, kākāpō do have arboreal habits. They spend a large

Table 4. Procrustes ANOVA results from comparison of claw shape variation due to kākāpō sex, body size and claw number.

	df	SS	MS	Rsq	F	Z	P
Sex	1	0.0049	0.0049	0.0130	2.251	1.6014	0.106
Claw number	1	0.0270	0.0270	0.0717	12.353	9.0365	0.001
Body side	1	0.0031	0.0031	0.0082	1.4195	1.1347	0.219
Sex*claw number	1	0.0015	0.0015	0.0041	0.7053	0.5746	0.548
Sex*body side	1	0.0013	0.0013	0.0035	0.5959	0.4790	0.645
Claw number*body size	1	0.0042	0.0041	0.0111	1.9118	1.5320	0.113
Residuals	153	0.3346	0.0022				
Total	159	0.3766					

portion of their day roosting in low canopy trees and foraging in trees during the evening. These activities require kākāpō to climb, hang and grasp branches (Ksepka & Clarke 2012; Carril *et al*. 2014). Potentially all parrots require extra lateral grip facilitated by deeply hooked lateral claws.

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