# Biometrics, Sexual Dimorphism and Gender Determination of Griffon Vultures Gyps fulvus from Crete

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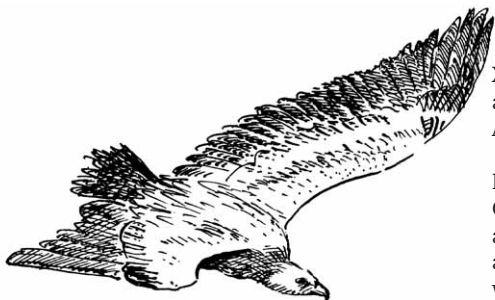
In the present study we describe the morphometrics of 97 live Eurasian Griffon Vultures *Gyps fulvus* collected on the island of Crete (Greece) and a sex determination model on the basis of various field techniques and multivariate data analysis. We determined the sex of 49 individuals with sex-specific DNA markers and polymerase chain reaction using DNA extracted from blood samples. Adult birds were generally larger than young ones with the wing chord being the only significantly different character. Male Griffons were smaller than females for most body characters and significantly larger (c. 3–5%) for head length, head width, bill length and bill length including the cere. A stepwise discriminant function analysis showed a high predictive power in gender determination by classifying 94.1% of the individuals of known sex that was not reduced by a jackknife cross-validation technique.

Key words: morphometrics, sexual dimorphism, molecular sexing, *Gyps fulvus*, Crete

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## INTRODUCTION

Biometrics and sex identification are of significance in avian biology. Data on body characteristics and gender differences have been used in a range of ecological or behavioural studies (Hughes 1998). Moreover, mating systems and demography have serious management implications in wild and captive populations, and sex identification is therefore important (Newton 1998, Dunn *et al.* 2001).

Eurasian Griffon Vultures *Gyps fulvus*, like all vultures of the hypergenus *Gyps*, lack plumage characteristics or external features from which sexes can be identified. Cloaca examination, laparoscopy, analysis of steroid hormones and DNA analysis, which have been used for sex determination in birds including vultures (Fry 1983, Richner 1989, Griffiths *et al.* 1998, Wink *et al.* 1998, Ito *et al.* 2003), require trained researchers and specialized equipment, and are expensive and time consuming (Palma *et al.* 2001). Therefore, being able to reliably distinguish sexes by measuring morphological traits in the field would be especially useful.

Gender determination is essential to assess sex-specific behavioural patterns (e.g. foraging strategy) or life-history traits (e.g. survival, dispersal). Although the species is widely distributed and still common among European vultures in the Mediterranean region, published accounts on its body

and sex morphology are scarce (Fernández & Fernández 1974, Cramp & Simmons 1980). The objectives of this paper were (1) to describe the morphometrics of the species and make comparisons of this island population to continental ones, (2) to test the existing molecular techniques for sex identification in vultures, and (3) to determine body measurements that best differentiate males and females.

## MATERIAL AND METHODS

### Study area and sample
Between 1998 and 2006 97 live vultures were collected from Crete (34°54'N 23°30'E – 35°41'N 26°19'E, 8261 km$^2$). All birds were found wounded, poisoned, emaciated or swept by strong northern winds into the sea. The majority came from central and eastern parts of the island, where population density is highest and most colonies occur (Xirouchakis & Mylonas 2004). Griffons were classified into three age groups according to the plumage characteristics provided by Forsman (1999): juvenile (0–1 yrs), immature (2–4 yrs) and adult (>5 yrs). After having been marked with metal rings from the Hellenic Ringing Scheme and with plastic engraved rings (Pro-Touch Engraving), birds were sent to wildlife rehabilitation centres. Normally the recovery period lasted from a few months to one year. Body measurements and blood samples were taken a few hours prior to the release in the wild.

### Molecular sexing
In 2002–2006 a small blood sample (0.5 ml) was taken from each vulture with an insulin syringe from the brachial vain (n = 55). The blood was transferred to capillary tubes with 95% ethanol and stored at –20°C. DNA was extracted from 200 $\mu$l of blood using the Macherey-Nagel Kit (Germany) following the manufacturer's instructions. Contamination with modern DNA or Polymerase chain reaction (PCR) products was ruled out by including two extraction blanks in every extraction round. For DNA sexing we used the method described by Griffiths et al. (1998) and Conway et al. (2004) based on two conserved CHD (chromo-helicase-DNA-binding) genes that are located on the avian sex chromosomes of all birds.

### Morphological measurements
The following biometric data were taken, using standard measurements as described for vultures (Mendelssohn et al. 1989, Mundy et al. 1992): 1) wing chord (WC), from the carpal joint of the bent wing to the tip of the longest straightened primary; 2) wing span (WS), the distance between the extended wing tips by keeping the bird on its back with the wings fully outstretched; 3) tail length (TL), from the insertion of the central rectrices to their tips; 4) tarsus length (TL), the distance from the tarsometatarsal joint to the articulation of the middle toe; 5) middle toe (MTO), from the first scale of the extended toe to the base of its claw; 6) middle talon (MTA), the distance between the base of the claw to its tip; 7) head length (HL), from the supraoccipital to the tip of the bill; 8) head width (HW), distance between the widest points in the auricular patches behind the eyes; 9) bill length (BL), from the tip of the culmen to its junction to the cere; 10) bill plus cere length (BCL), from the bill tip to the edge of implantation of feathers; 11) bill width (BW), from the junction of the tomium of the upper jaw to the cere at both sides of the culmen; 12) bill depth (BD), dorso-ventral distance at the nostrils, and 13) body mass (W).

Wing span was measured with a tape meter (accuracy 0.5 cm). Wing chord and tail length were measured with a steel stopped ruler to the nearest millimetre. The remaining measurements were taken to the nearest 0.1 mm by using a vernier caliper. Body mass was determined with a pesola 15-kg spring balance to the nearest 0.1 kg. Vultures without physical injuries were weighed before being dispatched to rehabilitation centres, recovered birds again after having fasted for 2–3 days prior to release.

Two aerodynamic statistics were estimated (Pennycuick 1972), namely wing loading (weight/ wing area) expressed as kg m$^{-2}$ and aspect ratio (wingspan$^2$/ wing area). Wing area was calculated

as the average wingspan divided by average wing width (Mendelsohn *et al.* 1989, Mundy *et al.* 1992). The latter character was measured from a sample of 14 alive (8 males and 6 females) and 5 dead specimens that were all immature. All measurements were made by the same person to minimize variability, and are presented as means and standard deviations.

## Statistical analysis

Data were tested for normality by using the Shapiro-Wilk's statistic, and homogeneity of variances with the Levene's test. A univariate analysis (ANOVA) was applied for differences in body measurements between age groups and sexes, and Tukey tests and *t*-tests on a post-hoc analysis, respectively. When the assumption of normality was violated, comparisons were made with Kruskal-Wallis and Mann-Whitney tests (Zar 1996). A multiple analysis of variance (MANOVA) was performed on both age and sex in order to investigate their main effect and interaction on body characters (Hair *et al.* 1998). For sex determination, frequency distributions, although overlapping, indicated that some characters might discriminate birds. The percent of sexual dimorphism in each measurement was taken as $\bar{x}_m - \bar{x}_f / \bar{x}_m$ where $\bar{x}_m$ and $\bar{x}_f$ were the mean values of males and females respectively. Gender determination was pursued by applying Discriminant Function Analysis (DFA; Klecka 1980) with sex being the categorical variable. For selection of the dependent variable (body measurements) we used Wilks' lambda statistic. The main criteria established for the application of the method were: a) normality of the data, b) linearity of inter-variable relationships (inspection of scatterplot matrix), c) lack of multicollinearity (inter-variable correlation coefficients $r < 0.6$) and d) homoscedasticity (equality of the variance/ covariance matrices, Box's M statistic). Prior probabilities in the groups formed in the classification were determined by the observed sample sizes.

The DFA with all variables combined detected the most informative ones and predicted membership into two mutually exclusive groups. It created a regression equation (discriminant function) that provided individual functions (D scores) where a cut-point between male-female mean scores (the weighted average of group centroids) distinguished birds by gender. The effectiveness of the method was assessed by the proportion of birds of known sex that were correctly classified using all individuals in the analysis. A jackknife procedure (Sokal & Rohlf 1995) was also performed where each bird was singly removed from the analysis and classified using a separate function derived from the remainder of the data. This cross-validation technique was considered a good indicator of the DFA accuracy since it works better for small sample sizes. All analyses were made at a 0.05 level of significance by using SPSS 14.0 software (Norušis 1989).

## RESULTS

Descriptive statistics for all birds sampled are presented in Table 1. All variables were normally distributed (Shapiro-Wilk's statistic $P > 0.05$), with the exception of body mass which was discarded from the discriminant function analysis. The weight of Griffons differed between age groups (Kruskal-Wallis $\chi^2 = 8.6$, $P = 0.014$), but not between the sexes (Mann-Whitney $U = 294$, $P = 0.634$) although females were slightly heavier than males. Extreme values below 6 kg (with a minimum of 4 kg), apparently the threshold for a Griffon to become grounded, were excluded from the comparisons.

Regarding age, 41 vultures were classified as juveniles, 46 as immatures and 10 as adults. Means for body measurements did not differ between age groups apart from wing chord that was significantly larger in adult than in juvenile and immature birds (Tukey test $P = 0.003$). However, non-adult birds (pooled data) were smaller than adults for eight out of 12 morphometrics variables examined (Table 1). Although the small sample size might have produced some bias, adult birds were on average smaller in tail, middle talon, bill width and bill depth. By examining the coefficient of variation we concluded that

**Table 1.** Descriptive statistics of body measures of Griffon Vultures in Crete. Units in mm unless otherwise mentioned. Variable that differs among age groups (*F*-test) in bold.

| Variable | $n$ | Pooled $\bar{x} \pm$ SD | Range | Juvenile $\bar{x} \pm$ SD ($n$) | Immature $\bar{x} \pm$ SD ($n$) | Adult $\bar{x} \pm$ SD ($n$) | *F*-value | *P* |
|---|---|---|---|---|---|---|---|---|
| **Wing chord**, cm | 97 | 69.9 ±2.65 | 64–75.3 | 70 ±2.56 (41) | 69.1 ±2.47 (46) | 72.9 ±1.58 (10) | 9.74 | < 0.001 |
| Wingspan, cm | 97 | 252.5 ±9.13 | 230–272 | 250.3 ±8.55 (41) | 253.6 ±8.57 (46) | 256.2 ±12.35 (10) | 2.45 | 0.09 |
| Tarsus | 97 | 118.3 ±7.93 | 99.5–135.04 | 117.9 ±7.99 (41) | 118.4 ±7.09 (46) | 119.2 ±11.57 (10) | 0.13 | 0.88 |
| Tail, cm | 97 | 30.4 ±2.80 | 24–38 | 30.3 ±2.63 (41) | 30.7 ±2.88 (46) | 29.3 ±3.12 (10) | 1.08 | 0.34 |
| Middle toe | 97 | 107.9 ±3.83 | 100–116.8 | 107.7 ±3.10 (41) | 107.7 ±4.27 (46) | 110.6 ±3.86 (10) | 2.63 | 0.07 |
| Middle talon | 97 | 35.5 ±2.73 | 27.8–43 | 35.6 ±2.42 (41) | 35.6 ±3.01 (46) | 35 ±2.78 (10) | 0.20 | 0.82 |
| Head length | 89 | 142.8 ±5.07 | 133.3–153.8 | 142.8 ±5.33 (35) | 142.5 ±5.10 (44) | 144 ±4.24 (10) | 0.36 | 0.70 |
| Head width | 59 | 61.1 ±2.54 | 56.9–66.8 | 60.6 ±2.83 (16) | 61.2 ±2.45 (37) | 62.2 ±2.30 (6) | 0.97 | 0.38 |
| Bill length | 91 | 52.6 ±2.58 | 45.9–61.1 | 52.1 ±2.44 (35) | 52.6 ±2.74 (46) | 54 ±1.84 (10) | 2.12 | 0.13 |
| Bill-cere length | 74 | 73.5 ±2.5 | 67–78.6 | 73.7 ±2.78 (25) | 73.4 ±2.37 (40) | 73.9 ±0.53 (9) | 0.20 | 0.82 |
| Bill width | 75 | 24.8 ±1.84 | 21.4–29 | 24.9 ±1.89 (27) | 24.8 ±1.82 (39) | 24.4 ±1.95 (9) | 0.25 | 0.78 |
| Bill depth | 74 | 35.5 ±1.94 | 30.8–40 | 35 ±1.96 (26) | 35.9 ±1.70 (39) | 35.4 ±2.66 (9) | 1.79 | 0.17 |
| Weight, kg | 92 | 7.44 ±0.72 | 6–9 | 7.16 ±0.68 (37) | 7.66 ±0.55 (45) | 7.47±1.13 (10) | | |
| Wing area, m² | 19 | | 0.88–0.89 | | 0.88 ±0.01 | | | |
| Wing loading, kg m⁻² | 19 | | 8–9.5 | | 8.69 ±0.38 | | | |
| Aspect ratio | 19 | | 7.2–7.3 | | 7.23 ±0.04 | | | |

**Table 2.** Sexual dimorphism, univariate comparisons and stepwise discriminant function analysis of morphometric measurements of male and female Griffon Vultures in Crete. See Table 1 for units. Variables that differ between sexes (by Wilks' lambda) in bold.

| Variable | Male ($n = 29$) $\bar{x} \pm$ SD (range) | Female ($n = 22$) $\bar{x} \pm$ SD (range) | % dimorphism | Wilks' lambda | *F*-value | *P* |
|---|---|---|---|---|---|---|
| Wing chord | 69.5 ±2.72 (64–75) | 70.3 ±2.84 (65–75) | –1.3 | 0.976 | 1.23 | 0.27 |
| Wingspan | 253.4 ±7.80 (238–270) | 253.9 ±10.58 (230–272) | –0.2 | 0.999 | 0.03 | 0.87 |
| Tarsus | 120.5 ±9.67 (99.5–135.04) | 117.2 ±8.42 (101–134) | 2.8 | 0.967 | 1.69 | 0.20 |
| Tail | 30 ±3.02 (24–35) | 31 ±2.79 (27.2–38) | –3.4 | 0.969 | 1.55 | 0.22 |
| Middle toe | 108.2 ±4.53 (101.3–116.8) | 107.6 ±3.41 (100.4–113.8) | 0.6 | 0.994 | 0.27 | 0.60 |
| Middle talon | 35.2 ±2.24 (30.2–39.1) | 36.5 ±3.74 (27.8–43) | –3.7 | 0.952 | 2.46 | 0.12 |
| **Head length** | 146.1 ±3.92 (139.5–153.8) | 138.9 ±2.50 (134.3–143.2) | 5 | 0.461 | 57.35 | <0.001 |
| **Head width** | 62.1 ±2.14 (58.4–66.1) | 59.8 ±2.51 (57.3–66.8) | 3.7 | 0.799 | 12.29 | 0.001 |
| **Bill length** | 54.1 ±2.18 (49.9–61.1) | 51.4 ±1.81 (45.9–55) | 5 | 0.687 | 22.35 | <0.001 |
| **Bill-cere length** | 74.7 ±1.93 (71.5–78.6) | 72.6 ±1.77 (69.1–76) | 2.8 | 0.754 | 16.01 | <0.001 |
| Bill width | 24.8 ±1.98 (21.4–29) | 25.3 ±1.86 (21.8–29) | –1.8 | 0.986 | 0.70 | 0.40 |
| Bill depth | 35.4 ±1.64 (30.8–38.6) | 36 ±1.90 (32.5–40) | –1.8 | 0.969 | 1.56 | 0.22 |
| Body mass | 7.648 ±0.69 (6.5–9) | 7.741 ±0.42 (6.2–9) | –0.012 | | | |
| Wing area | 0.89 ±0.004 (0.8–0.89, $n = 8$) | 0.89 ±0.01 (0.8–0.89, $n = 6$) | 0 | | | |
| Wing loading | 8.7 ±0.21 (8.5–9.1, $n = 8$) | 9.04 ±0.28 (8.8–9.5, $n = 6$) | –3.9 | | | |
| Aspect ratio | 7.24 ±0.03 (7.2–7.3, $n = 8$) | 7.26 ±0.04 (7.2–7.3, $n = 6$) | –0.3 | | | |

relative variability was marginally significant (homogeneity Levene's test, $P = 0.053$), increasing with age for wingspan, tarsus length, tail length and bill depth, and decreasing for wing chord and bill length.

Molecular techniques (i.e. P2 and P8 sexing methods) proved successful in gender identification. We determined the sex of 49 individuals (27 males, 22 females) out of a total of 55. Males showed a single band at 379bp (CHD-Z) on the polyacrylamide gel, while the females exhibited two bands (379bp, CHD-Z) and (388bp, CHD-W), both of them readily discernible. The identifying fragments of CHD gene were sequenced to confirm their size. GenBank accession numbers to the nucleotide sequence of these regions are EU430640 (CHD-W) and EU430641 (CHD-Z). Two additional birds were sexed as males by gonad inspection after dissection.

Males were smaller than females for most body measurements, including wing loading and aspect ratio, though the degree of sexual dimorphism differed significantly only between head and bill dimensions ($F$-tests, all $P < 0.05$, Table 2). In particular females were larger for all physical characters, apart from tarsus length and middle toe length, but male birds were significantly larger than females (c. 3–5%) for length and width of the head as well as length of the bill up to the cere or to the edge of the feathering (Table 2). The effect of age on sexual dimorphism (MANOVA, Wilks' Lambda = 0.399, $F = 1.65$, $P = 0.055$, interaction: Wilks' Lambda = 0.421, $F = 1.53$, $P = 0.087$) was not significant, therefore we pooled data of all age classes and performed a discriminant function analysis with sex as independent variable. A linear discriminant function analysis was used since all relevant assumptions were met, i.e. linear relationship between the variables, no inter-variable correlations ($r < 0.5$) and homoscedasticity (Box's M statistic = 15.1, $F = 1.38$, $P = 0.18$). The Wilks' Lambda criterion indicated that head length, head width, bill length and bill-cere length were the best physical characters in discriminating gender (Table 2). The application of DFA where all four variables were entered simultaneously, exhibited a success rate of 96.6% and 95.5% in correctly predicting males and females respectively (eigenvalue = 2.92, $\chi^2 = 0.255$, $df = 4$, $P < 0.001$). On the whole sex was accurately predicted for 96.1% of the sample, although it dropped to 94.1% when the jackknife cross-validation technique was performed. However, a stepwise procedure showed that sex discrimination was possible by using head length, head width and bill length (eigenvalue = 2.89, $\chi^2 = 0.257$, $df = 3$, $P < 0.001$) with an overall accuracy of 94.1% (Table 3).

Both unstandardized discriminant functions were similarly effective, as shown by the cross-validated values (Table 3). However, we considered the discriminant formula with the fewer variables to be the most useful (Fig. 1), because the cross-validation technique did not reduce its predictive ability even if prior probabilities in the DFA were taken of equal values (Table 3, correctly classified cases, $D_2$: 96.6% of males, 90.9% of females, 94.1% overall vs. $D_1$: 89.7% of males, 90.0% of females, 90.2% overall). For the simpler discriminant function the group centroids were 1.45 for males and –1.912 for females, thus the cutting score 0.31 could be used as a gender index (males: $D$ scores > 0.31, females: $D$ scores < 0.31).

**Table 3.** Discriminant functions (four and three variables) for gender determination of Griffon Vultures in Crete. HL = head length, HW = head width, BL = bill length, BCL = bill-cere length.

| Discriminant function | Group centroids | | Selftest (%) | Cross-validated (%) |
|---|---|---|---|---|
| | Males | Females | | |
| D1 = 0.26 HL+0.17 HW+0.34 BL+0.06 BCL–71.4 | 1.459 | –1.924 | 96.1 | 94.1 |
| D2 = 0.27 HL+0.19 HW+0.37 BL–69.12 | 1.450 | –1.912 | 94.1 | 94.1 |

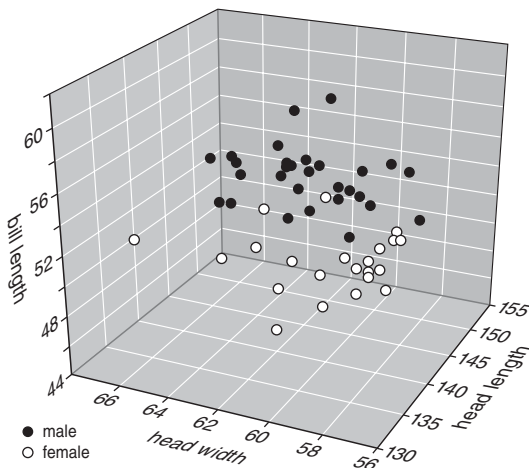**Figure 1.** Head length, head width and bill length (all in mm) are effective measurements for gender determination of Griffon Vultures in Crete.

## DISCUSSION

Regarding age-related biometrics, relevant conclusions should be drawn with caution as the sample size of adult birds was small. Nevertheless, excluding characters of significant sexual dimorphism, adult griffons were larger than immatures in all morphological measurements apart from tail length, probably a feature that serves manoeuvrability of flight-inexperienced birds. The length of the middle talon was found to be smaller in adult birds but this difference could have been caused by some degree of wear in a few individuals. The negative trend in the variation of measurements with regard to age should be attributed to the variable body condition of fledglings and first-year birds. However, an increase in wing chord and a decrease of tail length with age seems rather common among medium-sized raptors and eagles (Mundy 1982, Bortolotti 1984, Ferrer & de le Court 1992, Donohue & Dufty 2006).

In general raptors exhibit reverse sexual dimorphism where females are generally larger than males (Newton 1979). In our case the differences

observed, with female vultures being marginally larger than males for most linear measurements as well as weight, were in line with the existing literature (Cramp & Simmons 1980, Donázar 1993). More specifically, our results on sex-specific discrepancies in head and bill dimension corresponded with Fernández & Fernández (1974), who observed morphological differences in head width, bill length and width of the lower mandible of male and female Griffons in a sample of 16 pairs. The fact that head and bill dimensions were not influenced by age indicates that inter-sexual differences between these characters build up quite early, probably already during the nestling stage.

Head and bill length have a strong discriminant power to distinguish males from females. These 'skeletal' measurements have the advantage not to vary in relation to factors such as food availability, season, phase of the breeding cycle, moult or wear. Moreover, they are consistent and routinely measured in morphometrical studies in contrast to other body characters that are effective in adult gender discrimination but not traditionally collected in the field e.g. footpad length, forearm length or tarsus length (Edwards & Kochert 1986, Ferrer & de le Court 1992).

The present field method for gender determination of Griffon Vultures is rapid and simple, and cheaper than molecular techniques. Its applicability corresponds to live birds and possibly to study skins. Geographic variation may affect the sex discriminant coefficients of the function we developed. However, given the long-distance movements of the species within its distribution range (Bernis 1983, Berthold *et al.* 1991, Sušic 2000, Bahat *et al.* 2001), this is unlikely.

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## SAMENVATTING

Door middel van inspectie van de cloaca, laparoscopie en een analyse van steroïde hormonen en DNA is het mogelijk de sekse van Vale Gieren *Gyps fulvus* te bepalen. Deze technieken vergen echter veel ervaring, kennis en gespecialiseerde apparatuur. Ze zijn bovendien niet goedkoop. De onderhavige studie probeert morfologische maten te identificeren waarmee de geslachten van Vale Gieren uit elkaar kunnen worden gehouden. Onderscheid naar geslacht is wenselijk, omdat veel biologische parameters – zoals dispersie, overleving en gedrag – verschillen naar sekse. Om inzicht in de populatiedynamiek van een lokale populatie te krijgen, zoals in dit geval bij Vale Gieren op Cyprus, is seksebepaling van gevangen en gevonden vogels een eerste vereiste. Dat klinkt eenvoudiger dan het is, omdat Vale Gieren – in tegenstelling tot andere roofvogelsoorten – slechts een geringe seksuele dimorfie kennen. Verder is onbekend of de eilandpopulatie op Cyprus afwijkt van die op het vasteland van Europa. Het onderzoek werd verricht aan 97 gieren die tussen 1998 en 2006 in vogelasiels terecht waren gekomen, voornamelijk als juveniele (41) en onvolwassen (46) vogels. Slechts 10 vogels werden als adult aangemerkt. Van alle vogels werden twaalf lengtematen bepaald, verdeeld over vleugel, staart, poot en kop, daarnaast gewicht en afgeleiden als vleugelbelasting en vleugelslankheid. Een deel van de vogels werd moleculair op geslacht gebracht. Voor de meeste lichaamsmaten waren mannen kleiner dan vrouwen. Significante verschillen tussen de seksen werden gevonden in koplengte, kopbreedte en snavellengte (zowel met als zonder washuid), waarbij mannen 3–5% groter waren dan vrouwen. Kopmaten zijn 'hard' (immers betrekking hebbend op bot), vertonen geen seizoenvariatie, zijn niet onderhevig aan leeftijdsafhankelijke variaties, en zijn beter reproduceerbaar dan 'zachte' maten als vleugel, staart en tarsus. Door gebruik te maken van snavellengte, koplengte en kopbreedte kon bijna 95% van de Vale Gieren foutloos op geslacht worden gebracht. Daarmee is het een goed en goedkoop alternatief voor meer geavanceerde methoden.

(RGB)