Beak morphometrics and line analysis for accurate sex determination in juvenile Lovebird (*Agapornis fischeri*)

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Abstract. *Dhamayanti Y, Wahyunita ZH, Rantam FA, Mafruchati M, Eliyani H, Soeharsono, Hendarti GA, Palupi TDW, Kinasih KN, Rosyada ZNA, Zahro AN, Maslamama ST, Purnama MTE. 2025. Beak morphometrics and line analysis for accurate sex determination in juvenile Lovebird* (Agapornis fischeri). *Biodiversitas 26: 14-21.* Particular consideration must be dedicated to the welfare of birds as pets and trade animals in the context of biodiversity. Lovebirds (*Agapornis fischeri*(Reichenow, 1887)) are well-liked birds all around the world because of their lovely chirping sound and diverse ornaments. This study aimed to investigate the precision of differentiating between male and female juvenile Fischer lovebirds through beak morphometry and beak line analysis. A total of 54 Fischer's lovebirds, 27 males and 27 females aged 4, 5, and 6 months, were meticulously investigated in this study. The beak length, width, and depth were measured using a caliper with utmost care. The beak angle and beak line pattern were measured using the ImageJ application. Data was analyzed using MANOVA and crosstab in SPSS v.26. This study reported no significant difference in morphometric beak length, width, and depth in lovebirds aged 4, 5, and 6 months. In addition, this study reported differences in morphometry of the beak angle in Lovebirds aged 4, 5, and 6 months. The comparison of the interaction between sex and the line pattern to the eyes of Fischer lovebirds at the age of 4 and 6 months reported insignificant results, and at the age of 5 months, showed significant results. In conclusion, starting at 4 months of age, an alternative method for determining the sex of Fischer lovebirds is the morphometric technique of measuring the beak angle.

Keywords: *Agapornis fischeri*, beak morphometry, domesticated animals, genetic diversity, sex determination

INTRODUCTION

Lovebirds (*Agapornis fischeri* (Reichenow, 1887)) are prized for their vibrant plumage and impressive intellect (Guzmaliza and Puspita 2021). With their easily recognizable color patterns, lovebirds are often kept as pets. They are also popular as competition birds, especially because of their distinctive and melodious vocalizations, which are characteristic of female birds (Dewi et al. 2024). Bird breeding has become a profitable business due to the growing popularity of lovebirds, which may produce a range of chirps and ornaments (Argarini et al. 2020).

Lovebird breeding has been gaining traction in response to the growing demands of enthusiasts. The lovebird breeding industry aims to cater to these enthusiasts' needs. Accurately determining the sex of the parent birds is crucial for successful breeding among early breeders. Breeders often rely on various factors, such as observing body size, os-pubic filling, and cloaca inspection, when estimating the sex of the birds (Pratama et al. 2021).

A comprehensive examination of lovebird species reveals two distinct groups, for example, dimorphic and monomorphic. Dimorphic species exhibit noticeable differences between male and female individuals. In contrast, monomorphic species are less able to differentiate physical characteristics between the sexes (Akrom et al. 2020). In previous investigations, the Polymerase Chain Reaction (PCR) approach was used to visualize 400 bp and 350 bp for females and only 400 bp for males in feather samples from 4-month-old lovebirds (Dewi et al. 2024).

Various methods have been established for sexing, especially in monomorphic birds, with two general approaches being invasive and non-invasive. Invasive methods for sex identification in monomorphic birds include vent sexing, laparoscopic surgery, steroid sexing, and chromosome inspection (Purwaningrum et al. 2019). On the other hand, a traditional non-invasive method for sex determination in lovebirds involves measuring the cloacal region or the distance between the os pubis sinister and dexter by placing a finger between them. Female

lovebirds typically exhibit a wider cloacal distance compared to males. However, this method may be less reliable for juvenile birds due to the challenges of identifying the phallus. Alternatively, molecular techniques such as the Polymerase Chain Reaction (PCR) method have been employed for bird sexing (Dewi et al. 2024). However, PCR is also associated with several drawbacks. Its high susceptibility to contamination, dependence on prior sequence data for primer design, and limited capability to identify only known pathogens or genes are notable disadvantages. Additionally, non-specific annealing and the potential for incorrect nucleotides present further challenges, necessitating expert knowledge in the molecular examination for accurate results (Putra et al. 2020). Consequently, many breeders persist with traditional non-invasive methods for sex determination.

Despite the widespread use of traditional sex determination methods, it is important to note that these methods can be invasive and cause stress to birds, potentially affecting their health and well-being. This underscores the urgent need to develop non-invasive, reliable, and efficient techniques to advance avian breeding practices (Purwaningrum et al. 2019; De Silva et al. 2023). Recent studies have showcased the potential of using morphometric and geometric techniques for sex determination in diverse bird species. For example, research on other avian species has revealed that specific morphometric measurements, such as beak dimensions and head shape, can be strong indicators of sex. Morphometric variables derived from hard body structures like beaks and bones (e.g., upper beak depth or forearm) are preferred as stable predictors. The existence of small but significant differences in morphometrics between sexes allows for the application of statistical techniques that enhance classification accuracy (Baehaqi et al. 2018; Pohlen et al. 2021). These findings suggest that similar approaches could be effectively adapted for lovebirds.

Furthermore, enhancing the accuracy of sex prediction methods could be achieved by integrating line pattern analysis with morphometric measurements. In certain bird species, the arrangement and distribution of distinct beak markings, known as line patterns, have been observed to differ between sexes (Lequitte-Charransol et al. 2022); beak corrugations were also used to predict the age and the sex of the Central American hummingbirds (*Trochilus* sp.) (Carnes and Ash 2023). Through the combination of morphometric data and line pattern analysis, there is

potential to develop a comprehensive and dependable method for determining the sex of juvenile lovebirds.

In light of the limited investigation on this topic, this study aimed to investigate the implementation of beak morphometrics and line pattern analysis for accurate sex prediction in juvenile lovebirds. By providing a detailed analysis of these variables, we hope to establish a non-invasive, efficient, and reliable method for early sex determination. Once developed, this method could significantly benefit breeders, reducing the time and resources required for sexing birds and ultimately enhancing the profitability of breeding.

## MATERIALS AND METHODS

Ethical approval
The Research Ethics Commission has approved this study, Animal Care and Use Committee, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia, with approval No.1.KEH.133.10.2022. An ethical declaration was completed to prevent harm to animals and undue stress during the investigation. This study was conducted at the Veterinary Anatomy Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga, from September to November 2022.

Animals
The sample size was determined using Federer's formula: $t(n-1) \geq 15$ with a combination of age and gender for six groups. This study adopted a 2×3 factorial design and was quasi-experimental in the natural environment, in which no variables were treated. There were nine replications in each group, meaning a total of 54 juvenile lovebirds of different sexes (27 males and 27 females) were investigated, which, according to birth certificate records, represented the ages of 4, 5, and 6 months and the male and female sexes, respectively. These lovebirds were reared in a fenced enclosure measuring $75 \times 45 \times 110$ cm in a 12-h night/day cycle, fed millet seeds and fresh water ad libitum.

Beak morphometry evaluation
For this investigation, beak morphometry was measured using a Sony L59 type camera equipped with a 90 mm macro lens and NRT-PRO brand calipers with an accuracy of 0.05 mm. All beak morphometric measurements were illustrated in landmarks (Figure 1).
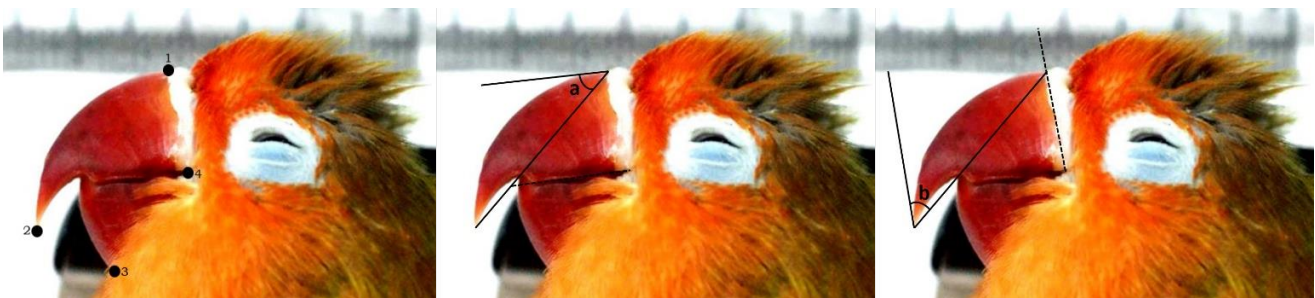
Figure 1. Beak morphometry landmarks in lovebird. 1-2. Beak length; 1-3. Beak depth; 4-4. Beak width; a. Beak axis angle; b. Beak tip angle

The beak length was assessed from the beak tip to the base of the beak or the edge of the cere (Baldwin et al. 1931; Eck et al. 2011). The beak was measured between the most dorsal base of the maxilla and the most ventral base of the mandible. The beak width was determined from both edges of the beak, perpendicular to the dorsal beak (Eck et al. 2011). The beak length, width, and depth were measured using a caliper. The beak angle was ascertained through macro photography. Subsequently, the curvature of the beak axis angle and the beak tip angle was analyzed through the application of the angle tool in ImageJ version 1.34e software (NIH & LOCI, University of Wisconsin). The beak line to the eyes was ascertained from the tip of the upper beak through the gap between the upper and lower beak towards the back of the head (Akrom et al. 2020). The beak line to the eyes was also observed using the line feature in the ImageJ application.

Data analysis

At the time of data collection, age and gender were the main classification factors. Levene's test and the Shapiro-Wilk statistic were used to determine the homogeneity of variances and the normality of the data, respectively. All collected data for beak length, depth, width, and beak angle were tabulated and analyzed using Multivariate Analysis of Variance (MANOVA) followed by Duncan's multiple comparison test, then presented in the form of mean and Standard Deviation (SD). The crosstab comparison test was used to compare the beak line to the eyes and breeder predictions. Statistical analysis was conducted using the statistical product and service solution (SPSS, IBM®, USA) v.26 computer statistics program.

RESULTS AND DISCUSSION

Evaluation of lovebird beak morphometry

This study reported no significant differences in the parameters of beak length (p = 0.123), depth (p = 0.904), and width (p = 0.803) in males and females aged 4, 5, and 6 months respectively (Table 1). On the other hand, the evaluation of beak angle morphometry included two variables, i.e., the beak axis angle and the beak tip angle. The results of the MANOVA analysis demonstrated a significant interaction between sex and age on both the beak axis angle (p = 0.001) and beak tip angle (p = 0.001), indicating that the beak angles in male and female Fischer's lovebirds at the ages of 4, 5, and 6 months are significantly different (Table 1). The findings of this study highlighted that only the beak angle parameter, out of the five beak morphometric characteristics typically employed for comparing bird age, may be utilized by breeders to identify sex in lovebirds aged 4, 5, and 6 months old.

Meanwhile, this study also reported a positive correlation between beak axis angle on beak length (y = 0.0088x + 1.228; y = 0.0009x + 1.6532) and depth (y = 0.0011x + 1.5108; y = 0.0015x + 1.562) in both males and females (Figure 2). Positive correlations were also reported between beak tip angle on beak length (y = 0.0068x + 1.3156) and width (y = 0.0035x + 0.8855) in males and beak tip angle on beak depth (y = 0.0004x + 1.613) in females, respectively (Figure 3). The present findings emphasized that male Fischer's lovebirds have a wider average curved beak tip size compared to females at 4 and 5 months of age (Figure 4).

Interpretation of the beak line to the eyes

The male and female Fischer lovebirds typically have a line pattern above the eye in over 50% of cases. However, observations of Fischer lovebirds aged 4, 5, and 6 months showed that the line pattern parallel to the eye was present in less than 50% of cases, while the line pattern above the eye was present in more than 50% of cases. The results of the crosstab comparison of the interaction between sex and the line pattern against the eyes of Fischer lovebirds at the age of 4 months showed no significant results, with a p-value of 0.235 (p > 0.05). At the age of 5 months, the results were not applicable, and at the age of 6 months, the results showed no significance, with a p-value of 0.765 (p > 0.05) (Table 2). These findings suggested that the line pattern is not a reliable indicator for sex determination in lovebirds.

Table 1. Beak morphometry evaluation of lovebird among sexes and ages

Parameters	Sex	Ages			Multivariate interactions p-value
		4 months old	5 months old	6 months old	
Beak length (cm)	Male (n = 9)	1.60 ± 0.07^a	1.67 ± 0.03^a	1.67 ± 0.03^a	0.123
	Female (n = 9)	1.66 ± 0.03^a	1.73 ± 0.02^a	1.68 ± 0.03^a	
Beak depth (cm)	Male (n = 9)	1.55 ± 0.03^a	1.56 ± 0.03^a	1.56 ± 0.05^a	0.904
	Female (n = 9)	1.60 ± 0.04^a	1.62 ± 0.05^a	1.63 ± 0.02^a	
Beak width (cm)	Male (n = 9)	1.03 ± 0.05^a	1.06 ± 0.03^a	1.07 ± 0.03^a	0.803
	Female (n = 9)	1.06 ± 0.03^a	1.07 ± 0.04^a	1.08 ± 0.04^a	
Beak axis angle (°)	Male (n = 9)	46.92 ± 3.44^b	48.62 ± 0.99^{ab}	49.21 ± 2.24^a	0.001**
	Female (n = 9)	42.07 ± 3.20^d	44.63 ± 2.00^c	50.24 ± 1.39^a	
Beak tip angle (°)	Male (n = 9)	48.17 ± 1.79^c	50.16 ± 2.09^b	50.63 ± 2.66^b	0.001**
	Female (n = 9)	45.30 ± 1.63^d	47.89 ± 2.61^{cd}	53.37 ± 1.86^a	

Note: [a,b,c,d] Different superscripts in the same column for each parameter indicate significant differences (p < 0.05) between males and females. Interaction between ages and sex is represented by a significance at: * p < 0.05; ** p < 0.01; *** p < 0.001

Accuracy of breeder predictions in determining sex

According to breeder predictions, as reported in Table 3, male birds demonstrate a higher accuracy in sex prediction compared to female birds at the ages of 4 and 6 months. The breeders' prediction method yields an accuracy rate exceeding 50% for male Fischer birds at 4 ($p = 0.009$), 5 ($p = 0.018$), and 6 ($p = 0.003$) months, respectively. Breeders used standard methods, such as cloacal differentiation and feather color brightness, to estimate the sex of the lovebirds in this study.

**Table 2.** Interpretation of the beak line pattern to the eyes

Ages	Sex	Eyes position to the beak line		p-value
		Parallel (%)	Top side (%)	
4 months old	Male (n = 9)	2 (22.2)	7 (77.8)	0.235
	Female (n = 9)	0 (0)	9 (100)	
5 months old	Male (n = 9)	0 (0)	9 (100)	N/A
	Female (n = 9)	0 (0)	9 (100)	
6 months old	Male (n = 9)	1 (11.1)	8 (88.9)	0.765
	Female (n = 9)	1 (11.1)	8 (88.9)	

Interaction between ages, sex, and eye position is represented by a significance at: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 3. Breeders' accuracy in differential sexing

Ages	Breeders' prediction	Laparotomy confirmation		Breeders' accuracy (%)	p-value
		Male (n)	Female (n)		
4 months old	Male (n = 9)	9	0	100	0.009**
	Female (n = 9)	4	5	55.6	
5 months old	Male (n = 9)	7	2	77.8	0.018*
	Female (n = 9)	2	7	77.8	
6 months old	Male (n = 9)	9	0	100	0.003**
	Female (n = 9)	3	6	66.7	

Interaction between ages, sex, and breeders' accuracy is represented by a significance at: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$
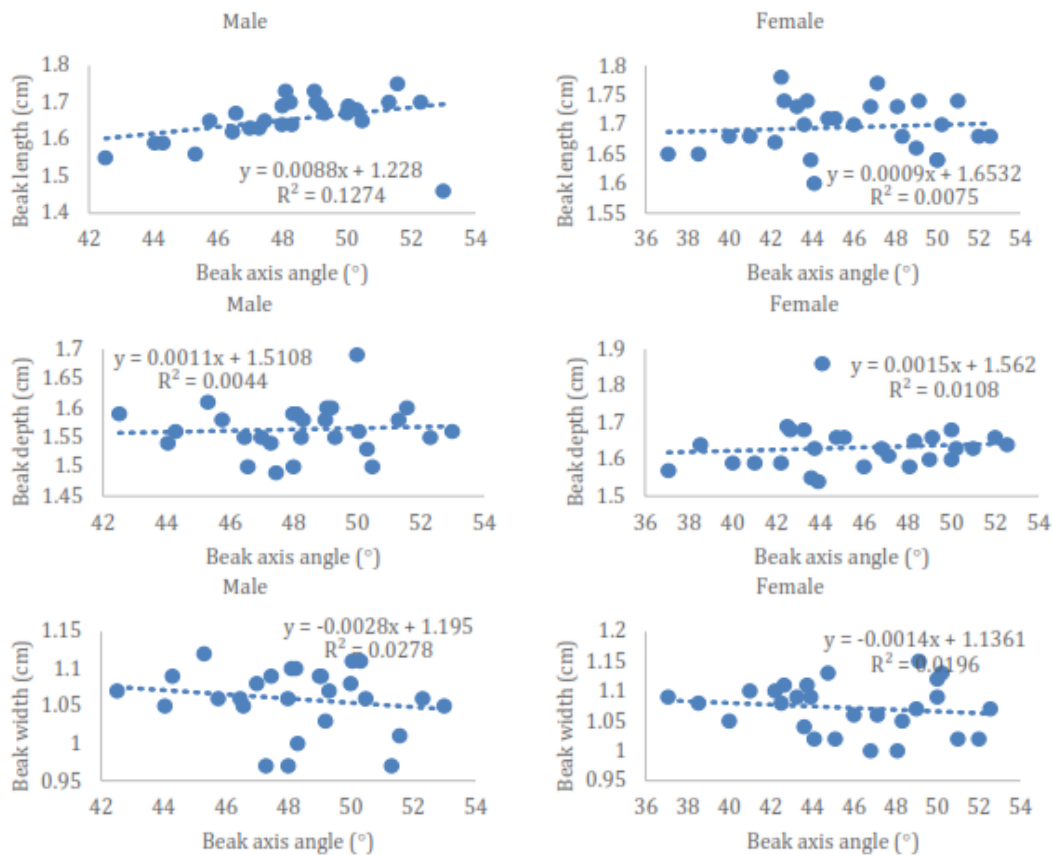
Figure 2. Correlation between beak length, depth, and width to the overall beak axis angle in males and females, respectively
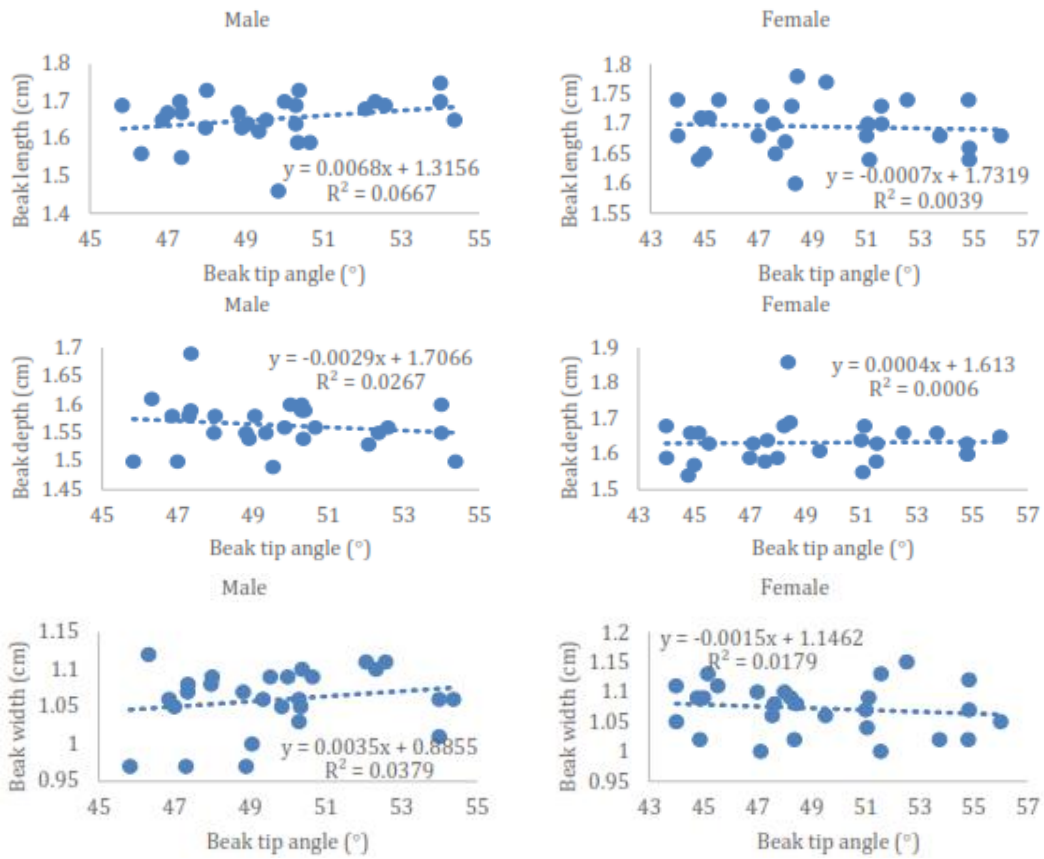
Figure 3. Correlation between beak length, depth, and width to the overall beak tip angle in males and females, respectively
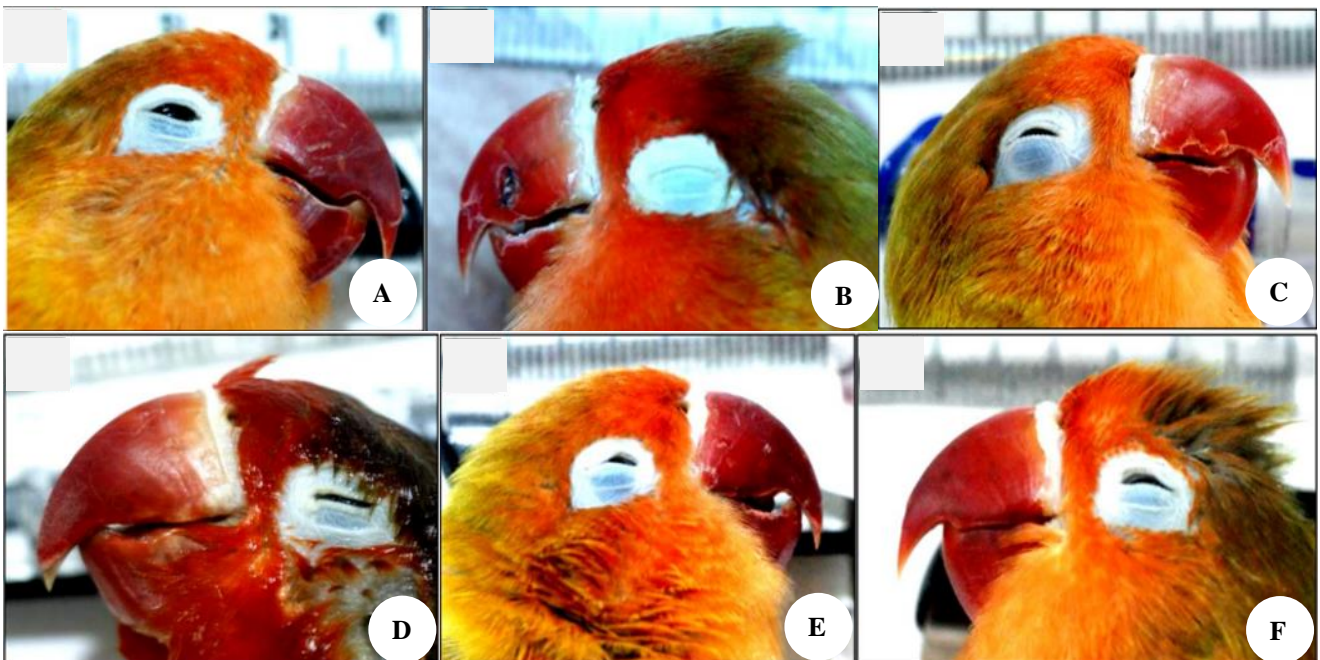

Figure 4. A-B. External morphology of the beak of a lovebird at 4 months old; C-D. External morphology of the beak of a lovebird at 5 months old; E-F. External morphology of the beak of a lovebird at 6 months old; A, C, E. The beak of the female lovebird looks flat and wide; B, D, F. The beak of the male lovebird looks sharp

Discussion

The present study found no significant differences in the beak length, width, and depth between male and female lovebirds. This suggests that these particular beak characteristics are not reliable indicators for determining the sex of lovebirds. Additionally, the study noted that the shape of a lovebird's beak can impact the positioning of the beak line pattern near the eye. Since Fischer lovebirds exhibit distinct variations in the shape, size, and curvature of their beaks compared to other birds, it follows that the beak line pattern cannot conclusively distinguish between male and female Fischer lovebirds. This study analyzed the differences in shape between the sexes and the dorsal, caudal, and ventral views of the landmarks where these differences were identified. The male and female samples' form variances were found to be relatively close to one another during the investigation. Male and female birds differ significantly on the dorsal side, which includes the maxillary tomium and upper beak curvature. However, there are no discernible differences in the beak's angle or labial commissure (Kurniawati et al. 2024). That being stated, it can be claimed that sex determination within the same species can benefit from geometric form analysis, offering a promising avenue for future research. Differences between males and females were noted in longitudinal measures using standard morphometric techniques in various skulls (Szara et al. 2022).

Identifying the sex of monomorphic bird species presents a substantial challenge due to the striking similarity in the physical traits of males and females within these species. Notably, sexual characteristics in birds often become discernible only after they reach sexual maturity. For example, birdsong's involvement in female reproductive activity serves as a prominent indicator of sexual characteristics in certain avian species (Akrom et al. 2020). Body shape (the ratio of body and tail length to tarsus and culmen length) and overall body size were found to be the two main components of variation in body morphology. In line with previous studies on the morphology of Darwin's finches' beaks, beak shape variation was concentrated along the long/slender/pointed axis compared to the short/robust/blunt axis (Shao et al. 2016). However, in terms of beak shape variation, most species overlap. It seems that the long/slender/pointed vs. short/robust/blunt axis is where the beak form varies most. Compared to other adaptive radiations that support a higher range of beak morphology, such as Darwin's finches (Grant and Grant 1993), Malagasy vangas (Jønsson et al. 2012), and Hawaiian honeycreepers (Lerner et al. 2011), Paridae species have a rather conservative beak shape.

Another study found that two important elements underlying the structure of the skull in psittaciform birds are the integration between the shape variation of the braincase and the beak and that these factors combined predict over half of the shape of the skull and beak (Pecsics et al. 2020). Despite the fact that phylogenetic similarity was considered throughout the whole analysis process, phylogenetic inertia seems to be primarily responsible for the remaining 50.5% of the shape variation. This is likely due to the fact that cockatoos differ greatly from real parrots in terms of their skull and beak shapes (Habl and Auersperg 2017). In other earlier research on raptors, 80% of the shape of the skull was predicted by allometry and integration (Klingenberg 2013).

Anatomical factors additionally contribute to the challenging comparison of studies across vertebrate classes. For example, the premaxilla of birds is remarkably variable compared to mammals due to the wide variation in the beak, and the quadrate bone, a prominent component of the articulation of the jaws and jugal bar in birds, is homologous to the incus, one of the middle ear ossicles, in mammals. Due to these variations in skull anatomy, it might be hard to locate landmarks in a particular category that are homologs of those in another, and even if homologous landmarks are found, patterns of integration and modularity might not be comparable if variations in skull organization significantly change the anatomical and functional context (Klingenberg and Marugán-Lobón 2013).

The success of the ring-necked parakeet as an invasive species may be attributed to high levels of variation in the palate and rostrum. The beaks of ring-necked parakeets in non-native areas are typically larger and stronger, according to a prior study examining the morphology of populations in their native range and the invasive populations that inhabit Europe (Le Gros et al. 2016). Due to the direct involvement of the palate and rostrum in feeding, the significant variety in these two modules facilitated the species' easier adaptation to a new environment and food supply. Therefore, the complex and intriguing relationship between nutrition and skull shape is complex, coupled with significant levels of rostrum and palate variation within the species, may have aided in the selection of particular beak morphologies (Wang et al. 2020).

A previous study reported that high modularity can promote wide ecological tolerances and, hence, quick encroachment in new areas. Nonetheless, *Psittacula krameri* (Scopoli, 1769) integration and modularity patterns are comparable to those of all birds. To investigate how cranial modularity might significantly affect ecological flexibility and niche conservatism in this lineage, a direct comparison between this cosmopolitan taxon and comparable parrot species that are susceptible to habitat loss or climate change is required (Mitchell et al. 2021). It is evident from a prior study on quail that males have wider skulls when viewed from the caudal perspective. In the same way as turkeys, female quail have larger occipital heights than male quail. In males, the roof of the cranium was wider than in females. The male quail's skulls were broader in the ventral view, just like those of the turkeys. The main components of integration and modularity patterns are comparable to all birds. The turkey skull was shown to account for 39.61% of the variation in dorsal appearance, 51.9% in tail appearance, and 34.32%; these percentages were 33.05%, 41.21%, and 34.5% in quail, which must be compared with this study for a comprehensive comparison. In both experiments, the biggest shape variance was described by the principal component caudal appearance. The foramen magnum showed the greatest gender difference in caudal appearance in those two investigations. Morphometric research on humans mentioned sexual

dimorphism of the foramen magnum; conversely, additional geometric morphometry and morphometric investigations may investigate whether the foramen magnum in chickens exhibits sexual dimorphism (Szara et al. 2022).

Sexual immaturity complicates the differentiation between male and female pubis sinister et dexter distance in Fischer lovebirds, impacting breeders' ability to determine the sex of the birds before sexual maturity (Turcu et al. 2020). Sexual maturation generally involves cycles of reproductive activity and inactivity, with individuals transitioning from juveniles to reproductively active adults (Ball and Wade 2013). During this process, the birds' gonads also develop, and gonadal hormones play a significant role in sexual differentiation, affecting not only physical attributes but also neurological and behavioral traits (Arnold and Itoh 2011). Thyroid hormones impact growth and metabolism in a variety of mechanisms. Thyroid are crucial for maintaining a high and steady body temperature in homoeothermic animals by controlling basal metabolic rate. Thyroid have a biphasic influence on the metabolism of proteins and fats; at low physiological concentrations, they have anabolic effects, but at greater concentrations, they have catabolic effects. The thyroid promotes both growth and differentiation (or maturation) during development. These hormones may take permissive, indirect, or direct action. Thyroid appears to rely on binding to a nuclear thyroid hormone receptor (TR) for the majority of their effects (Decuypere et al. 2005). Koyama et al. (2019) further assert the crucial role of steroid hormones in sex differentiation in eutherian mammals and birds. Furthermore, hormones like triiodothyronine are known to influence beak angle disparity in the Eurasian Tree Sparrow (*Passer montanus* (Linnaeus, 1758)) (Nabi et al. 2020).

The variation in maxillary beak morphology still requires further understanding. Bird beaks exhibit a diverse array of shapes and curvatures, depending on the species. These variations are attributed to ecological adaptations in response to specific dietary requirements. The morphological diversity of bird beaks is underpinned by genetic and developmental influences (Bright et al. 2016). Various factors may influence curvature differences in bird beaks; for example, constant contact with hard substances during foraging in red junglefowl (*Gallus gallus* (Linnaeus, 1758)) leads to natural beak abrasion (Struthers et al. 2023). These findings suggest a complex interplay between genetics, hormones, and environmental factors in shaping beak morphology.

The upper beak is developed from the pair of maxilla and frontonasal primordia, while the lower beak is developed from the pair of mandibular primordia (Schneider and Helms 2003). Differences in beak shapes lead to variations in curvature angles. The beak angle can be further determined into two components, i.e., the beak axis angle and the beak tip angle. Both angle measures can be determined using the Pythagorean theorem (Baldwin et al. 1931). A prior study on the *Geospiza fortis* (Gould, 1837) bird demonstrated that bite force is substantially connected with morphological features like head and beak dimensions in addition to sex determination. Bite force and beak size

are connected because the beak transfers the forces from the jaw-closing muscles to the food. Therefore, the beak must be shaped to be able to endure the response pressures that the food item exerts (Herrel et al. 2005).

In general, the results of the morphometric study of the beak axis angle and the beak tip angle indicate that the size of the beak angle of the male birds is larger than that of the female birds. This aligns with external morphological observations that the male's beak is more pointed and slender than the female's beak, which is wider, so it looks flatter. A review of studies on biometric sexing in birds revealed sample sizes ranging from 42 to 449 birds and accuracy rates between 15% and 99.7% (with 60% reporting accuracy rates > 90%). Those studies cast doubt on the consistency of the results because all but six of the studies used sample sizes less than 200. However, not all of the smaller sample sizes were related to bigger or smaller predictive values (Hernández et al. 2011). According to Dechaume-Moncharmont et al. (2011), small sample sizes can lead to significant variability in classification estimates and, by chance alone, high or poor model accuracy. Given that the bottom bound of the bootstrapped 95% confidence interval stayed above a 70% classification rate, we are confident in the findings of our investigation. In our perspective, the use of geometric morphometrics, which analyses relationships between a whole collection of landmark points from our study and necessitates adjusting our developmental model to take centerline curvature into account, can overcome some of the limitations of these conventional measurements.

In conclusion, the application of beak curvature morphometrics and breeder predictions provides a reliable, convenient, non-invasive technique for determining the sex of juvenile Fischer lovebirds. These discoveries have a major impact on the avian breeding sector, as they offer an effective and precise way to determine sex early. Further investigations may be recommended by applying geometric morphometry methods complemented by Principal Component Analysis (PCA) to prove the authenticity of the beak morphometry and line analysis methods.

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REFERENCES

Akrom AM, Indarjulianto S, Yanuartono Y, Susmiati T, Nururrozi A, Raharjo S, Permana RGS, Govendan PN. 2020. Beak line and eye alignment as phenotypic sexing for domestic Canaries (*Serinus canaria*). Biogenesis Jurnal Ilmiah Biologi 8 (1): 89-93. DOI: 10.24252/bio.v8i1.11115.
Argarini AD, Nugrogo HA, Purwaningrum M, Haryanto A. 2020. Molecular bird sexing on fischeri Lovebird (*Agapornis fischeri*) by

using Polymerase Chain Reaction. Bio Web Conf 20: 04003. DOI: 10.1051/bioconf/20202004003.

Arnold AP, Itoh Y. 2011. Factors causing sex differences in birds. Avian Biol Res 4 (2): 10.3184/175815511X13070045977959. DOI: 10.3184/175815511X13070045977959.

Baehaqi I, Saraswati TR, Yuniwarti EYW. 2018. Sex determination in male and female *Melopsittacus undulates* using a Morphometric method. Biosaintifika J Biol Biol Educ 10 (3): 533-538. DOI: 10.15294/biosaintifika.v10i3.14067.

Baldwin SP, Oberholser HC, Worley LG. 1931. Measurements of Birds. Scientific Publications of the Cleveland Museum of Natural History, Cleveland, Ohio. DOI: 10.5962/bhl.title.60247.

Ball GF, Wade J. 2013. The value of comparative approaches to our understanding of puberty as illustrated by investigations in birds and reptiles. Horm Behav 64 (2): 211-214. DOI: 10.1016/j.yhbeh.2013.05.002.

Bright JA, Marugán-Lobón J, Cobb SN, Rayfield EJ. 2016. The shapes of bird beaks are highly controlled by nondietary factors. Proc Natl Acad Sci USA 113 (19): 5352-5357. DOI: 10.1073/pnas.1602683113.

Carnes B, Ash A. 2023. Many Central American hummingbirds can be aged and sexed by molt patterns and beak corrugations. J Field Ornithol 94 (3): 11. DOI: 10.5751/jfo-00305-940311.

De Silva STD, Pagthinathan M, Bandara S, Pathirana IN. 2023. Sex identification methods of birds: A review. Asian J Med Biol Res 9 (4): 134-144. DOI: 10.3329/ajmbr.v9i4.69414.

Dechaume-Moncharmont F-X, Monceau K, Cezilly F. 2011. Sexing birds using discriminant function analysis: A critical appraisal. The Auk 128 (1): 78-86. DOI: 10.1525/auk.2011.10129.

Decuypere E, Van As P, Van der Geyten S, Darras VM. 2005. Thyroid hormone availability and activity in avian species: A review. Domest Anim Endocrinol 29 (1): 63-77. DOI: 10.1016/j.domaniend.2005.02.028.

Dewi CMS, Dhamayanti Y, Fikri F, Purnomo A, Khairani S, Chhetri S, Purnama MTE. 2024. An investigation of syrinx morphometry and sound frequency association in Lovebirds (*Agapornis fischeri*) chirps. F1000Res 11: 354. DOI: 10.12688/f1000research.108884.3.

Eck S, Fiebig J, Fiedler W, Heynes I, Nicolai B, Töpfer T, van den Elzen R, Winkler R, Woog F. 2011. Measuring Birds. Christ Media Natur, Hans-Josef Christ. Minden 83 (2): 117. DOI: 10.2989/00306525.2012.703802.

Grant BR, Grant PR. 1993. Evolution of Darwin's finches caused by a rare climatic event. Proc R Soc Lond B Biol Sci 251 (1331): 111-117. DOI: 10.1098/rspb.1993.0016.

Guzmaliza D, Puspita D. 2021. Penerapan metode Forward Chaining pada sistem pakar penyakit burung Lovebird. Jurnal Mahajana Informasi 6 (1): 31-40. DOI: 10.51544/jurnalmi.v6i1.1989. [Indonesian]

Habl C, Auersperg AMI. 2017. The keybox: Shape-frame fitting during tool use in Goffin's cockatoos (*Cacatua goffiniana*). PLoS One 12 (11): e0186859. DOI: 10.1371/journal.pone.0186859.

Hernández MÁ, Campos F, Martín R, Santamaría T. 2011. Usefulness of biometrics to analyse some ecological features of birds. In: Albert M (eds). Biometrics-Unique and Diverse Applications in Nature, Science, and Technology. IntechOpen, London. DOI: 10.5772/15031.

Herrel A, Podos J, Huber SK, Hendry AP. 2005. Bite performance and morphology in a population of Darwin's finches: Implications for the evolution of beak shape. Funct Ecol 19 (1): 43-48. DOI: 10.1111/j.0269-8463.2005.00923.x.

Jønsson KA, Fabre P-H, Fritz SA, Etienne RS, Ricklefs RE, Jørgensen TB, Fjeldså J, Rahbek C, Ericson PGP, Woog F, Pasquet E, Irestedt M. 2012. Ecological and evolutionary determinants for the adaptive radiation of the Madagascan vangas. Proc Natl Acad Sci USA 109 (17): 6620-6625. DOI: 10.1073/pnas.1115835109.

Klingenberg CP, Marugán-Lobón J. 2013. Evolutionary covariation in geometric morphometric data: Analyzing integration, modularity, and allometry in a phylogenetic context. Syst Biol 62 (4): 591-610. DOI: 10.1093/sysbio/syt025.

Klingenberg CP. 2013. Cranial integration and modularity: Insights into evolution and development from morphometric data. Hystrix 24 (1): 43. DOI: 10.4404/hystrix-24.1-6367.

Koyama T, Nakamoto M, Morishima K, Yamashita R, Yamashita T, Sasaki K, Kuruma Y, Mizuno N, Suzuki M, Okada Y, Ieda R, Uchino T, Tasumi S, Hosoya S, Uno S, Koyama J, Toyoda A, Kikuchi K,

Sakamoto T. 2019. A SNP in a Steroidogenic enzyme is associated with phenotypic sex in Seriola fishes. Curr Biol 29 (11): 1901-1909.e8. DOI: 10.1016/j.cub.2019.04.069.

Kurniawati DY, Puspitasari Y, Yudhana A, Saputro AL, Dhamayanti Y, Purnomo A, Bayram M, Purnama MTE. 2024. Sex determination in Japanese quails (*Coturnix japonica*) based on head morphometry variation among age. Biodiversitas 25 (8): 2740-2748. DOI: 10.13057/biodiv/d250847.

Le Gros A, Samadi S, Zuccon D, Cornette R, Braun MP, Senar JC, Clergeau P. 2016. Rapid morphological changes, admixture and invasive success in populations of Ring-necked parakeets (*Psittacula krameri*) established in Europe. Biol Invasion 18: 1581-1598. DOI: 10.1007/s10530-016-1103-8.

Lequitte-Charransol P, Le Saux E, Jiguet F. 2022. Sexing first-calendar-year Carrion Crows Corvus corone from biometrics reveals variation between years in post-fledging sex ratio. Ring Migr 36 (2): 82-90. DOI: 10.1080/03078698.2022.2098369.

Lerner HRL, Meyer M, James HF, Hofreiter M, Fleischer RC. 2011. Multilocus resolution of phylogeny and timescale in the extant adaptive radiation of Hawaiian honeycreepers. Curr Biol 21 (21): 1838-1844. DOI: 10.1016/j.cub.2011.09.039.

Mitchell MJ, Goswami A, Felice RN. 2021. Cranial integration in the ring-necked parakeet, *Psittacula krameri* (Psittaciformes: Psittaculidae). Biol J Linn Soc 133 (1): 47-56. DOI: 10.1093/biolinnean/blab032.

Nabi G, Hao Y, Liu X, Sun Y, Wang Y, Jiang C, Li J, Wu Y, Li D. 2020. Hypothalamic-Pituitary-Thyroid Axis crosstalk with the Hypothalamic-Pituitary-Gonadal Axis and metabolic regulation in the Eurasian tree sparrow during mating and non-mating periods. Front Endocrinol 11: 303. DOI: 10.3389/fendo.2020.00303.

Pecsics T, Laczi M, Nagy G, KoNdor T, Csörgő T. 2020. Skull morphometric characters in parrots (Psittaciformes). Ornis Hungarica 28 (1): 104-120. DOI: 10.2478/orhu-2020-0008.

Pohlen ZM, DeCicco LH, Buchanan JB, Tomkovich PS, Johnson JA. 2021. Sex determination of Red Knots *Calidris canutus roselaari* using morphometrics. Wader Study 128 (2): 183-188. DOI: 10.18194/ws.00241.

Pratama ASP, Ashori MA, Hadiwiyanto. 2021. Deteksi fertilitas telur burung Lovebird berbasis smartphone. Jurnal Jaringan Telekomunikasi 11 (2): 81-85. DOI: 10.33795/jartel.v11i2.65. [Indonesian]

Purwaningrum M, Nugroho HA, Asvan M, Karyanti K, Alviyanto B, Kusuma R, Haryanto A. 2019. Molecular techniques for sex identification of captive birds. Vet World 12 (9): 1506-1513. DOI: 10.14202/vetworld.2019.1506-1513.

Putra LAG, Yonathan CJ, Niedhatrata NI, Firdaus MHR, Yoewono JR. 2020. A review of the development of Polymerase Chain Reaction technique and its uses in scientific field. Stannum Jurnal Sains Terapan Kimia 2 (1): 17-30. DOI: 10.33019/jstk.v2i1.1619.

Schneider RA, Helms JA. 2003. The cellular and molecular origins of beak morphology. Science 299 (5606): 565-568. DOI: 10.1126/science.1077827.

Shao S, Quan Q, Cai T, Song G, Qu Y, Lei F. 2016. Evolution of body morphology and beak shape revealed by a morphometric analysis of 14 Paridae species. Front Zool 13: 30. DOI: 10.1186/s12983-016-0162-0.

Struthers S, Andersson B, Schmutz M, Matika O, McCormack HA, Wilson PW, Dunn IC, Sandilands V, Schoenebeck JJ. 2023. An analysis of the maxillary beak shape variation between 2 pure layer lines and its relationship to the underlying premaxillary bone, feather cover, and mortality. Poult Sci 102 (8): 102854. DOI: 10.1016/j.psj.2023.102854.

Szara T, Duro S, Gündemir O, Demircioğlu İ. 2022. Sex determination in Japanese quails (*Coturnix japonica*) using geometric morphometrics of the skull. Animals 12 (3): 302. DOI: 10.3390/ani12030302.

Turcu MC, Bel LV, Collarile T, Pusta DL. 2020. Comparative evaluation of two techniques of sex determination in Lovebirds (*Agapornis* spp.). Bull Univ Agric Sci Vet Cluj-Napoca Vet Med 77 (2): 106. DOI: 10.15835/buasvmcn-vm:2020.0030.

Wang S, Stiegler J, Wu P, Chuong C-M. 2020. Tooth vs. beak: The evolutionary developmental control of the avian feeding apparatus. Bull Am Mus Nat Hist 440: 205-228.