INTERNATIONAL JOURNAL OF CONSERVATION SCIENCE
ISSN: 2067-533X Volume 13, Issue 3, July-September 2022: 991-1002 www.ijcs.ro

FEASIBILITY OF USING NON-INVASIVE SAMPLES FOR EARLY SEX DETERMINATION IN FALCON CHICKS BY POLYMERASE CHAIN REACTION

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Abstract

Captive breeding of falcons depends on accurate sex determination of adult birds and the owners often demand early sex determination of hatched chicks. The major objective of this study was to optimize PCR methods for the efficient sexing of falcon chicks using DNA sources other than blood and feathers. Eggshell and stool samples were collected from 24 hatching falcon chicks with furthur blood samples collected after the onset of sexual maturity. Experiment 1 was carried out to assess efficiencies of four various PCR protocols using whole blood from eight falcons. In Experiment 2, DNA contents from blood, stool, and eggshell membrane specimens of 24 falcons were assessed and subjected to modified PCR from Experiment 1. The optimized PCR protocol 2 (2550F/2718R primers) resulted in females' most distinctive electrophoresis patterns compared to males. The quantities of DNA extracted from stool samples were less than those extracted from blood and eggshell membrane in all species ($p < 0.05$); nevertheless, PCR of these samples resulted in accurate sex determination of the subjects, similar to that of blood. In conclusion, optimization of the previously described PCR methods facilitates accurate early sex determination of flcon chicks, eliminating the need for invasive sampling methods to provide DNA sources.

Keywords*: Chick sexing; Falcon; PCR; UAE*

Introduction

Falcons (Falconidae family, *Falco* species) are birds of prey with domestication potential. Breeding of these predators is popular in regions of the world such as the Arabian Peninsula, where they are trained to participate in racing and hunting sports. While sex determination generally serves as a vital tool for genetic, evolutionary, and ecological studies on avian [1], demands for sex determination in falcons are mainly linked to the fact that production of the offspring under captive conditions requires accurate sex determination for both natural mating and assisted reproduction programs. Moreover, early sex determination of falcon chicks offers practical advantages for designing rearing strategies that incorporate conserved genome resources into breeding schemes to decrease pressures on wild populations.

Avian physical sex determination is generally challenging due to inconspicuousness of the external genitalia, which can result in more than 50% misdiagnosis of sex in monomorphic species [2]. Several methods have been described to circumvent this problem, such as vent sexing, laparoscopic surgery, endoscopy, and measurement of sex steroids [3], but practical

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disadvantages linked to each of them limit their popularity within falconries. In addition to the invasive nature of these methods, they are not generally applicable in chicks and juveniles, and their accuracy varies with the operator's expertise. These disadvantages have created a trend for adopting more reliable noninvasive molecular approaches for sexing of the birds [4].

Molecular methods of bird sexing are mainly based on polymerase chain reaction (PCR) of paralogous copies of the highly conserved chromo helicase DNA binding protein gene (CHD1) located on Chromosomes W and Z (CHD1W and CHD1Z, respectively) using mismatch primers [5]. Amplification of these primers results in two amplicons in females and one amplicon in male birds. However, technical variations of the procedure and intrinsic variations in CHD genes in various birds urge further optimizations of this method when working with various species [6-9] and subspecies [10] to ensure maximum accuracy and repeatability of results. Numerous studies in avian genetics have used feathers or whole blood as the preferred sources of DNA [9, 11, 12]. While blood collection from adult falcons can partially be invasive, it is generally impractical in chicks, and plucking feathers from hatchlings is considered undesirable by the owners and, therefore, discouraged by the breeders. To the best of the authors' knowledge, no published studies are available on molecular sexing of falcons using DNA sources other than blood. Therefore, the major aims of the current study included: i) comparative optimization of PCR procedure for the sexing of falcon chicks using four various sets of primers to achieve accurate and repeatable results within common species of falcons, and ii) investigating the practicality of using eggshell membranes and stools as sources of DNA for PCR to eliminate the needs for invasive sample collection methods in chicks.

Materials and Methods

Study design

The study included two sets of experiments. Experiment 1 was carried out to compare the efficiencies of four commonly used PCR protocols (P1–P4) for avian sex determination using whole blood samples of eight adult falcons with verified sex (four males and four females). Experiment 2 was carried out to develop the protocol optimized in Experiment 1 on using sources of DNA other than whole blood. Briefly, DNA samples from 24 subjects were extracted from the hatched chicks' eggshell membranes and stored at -80°C until use. Further blood and feces samples were collected from respective birds after verifying their sex during the following breeding season. All samples were extracted and used in a modified PCR protocol based on the results of *Experiment 1*.

Birds

A total number of 24 birds (12 males and 12 females) were used with 72 various specimens, including those of various falcon species and sexes, and collected biological specimens. Captive falcons included four major local species of Gyr (*F. rusticolus*), Peregrine (*F. peregrinus*), Saker (*F. cherrug*), and Gyr-Peregrine hybrid that were reared in Margham Breeding Center, Dubai, UAE, 2018–2020 (Figs. 1 and 2).

The falcon species were selected based on the bird's history and importance in the region [13]. These birds were bred and reared in the center under high standard conditions and were later subjected to morphometric evaluation by experts to sexual identify them based on sexual dimorphism.

Specimens

Briefly, EDTA blood specimens (maximum 200µL) were collected from cutaneous ulnar veins using 1.0mL disposable syringes and 27-gauge 1/2 disposable needles. Specimens were immediately transferred to the molecular laboratory in the cooling box for extraction of their DNA contents. Furthermore, stool specimens were collected from each falcon in 2.0mL microtubes and transferred to the lab. During the hatching season, the respective hatched chicks' inner shell membranes were carefully detached from the external shell using sterile forceps and

subjected to washing with the DPBS solution (Sigma Aldrich, USA) to eliminate possible cell contaminations and stored at -80°C until use.

Fig. 1. A mature white female Gyrfalcon

Fig. 2. A young white male Gyrfalcon

DNA extraction

In general, DNA of the whole blood and eggshell inner membrane specimens were extracted using DNeasy Blood and Tissue Kit (Qiagen, Germany) according to the manufacturer's instructions with minor modifications. In Step 1b, 25µL of the blood sample were transferred into a sterile 1.5mL microtube, and the volume was adjusted to 200µL by adding PBS. This was incubated at 58°C for 20min and then mixed with 150µL of elution buffer in Step 7. For the inner shell membrane, 50mg from each thawed specimen were cut into small pieces using sterile blades and mixed with a sufficient amount of sterile deionized water to make a final volume of 200µL before further processing. For the stool specimens, 200–300µL of each fresh sample were mixed with 100µL of sterile deionized water and homogenized before the extraction. Then, DNA of the mixture was extracted using QIAamp DNA Stool Mini Kit (Qiagen, Germany) according to the manufacturer's manual with minor modifications as follows: centrifugation time was extended to 6min in Step 6, incubation time increased to 15min in Step 11 and 100µL of the elution buffer was used instead of 200µL in Step 19. All DNA extracts were immediately stored at -80°C until use.

Polymerase chain reaction

In this study, four various protocols for DNA amplification were used to investigate the best protocol. Results were rechecked to ensure protocol accuracy and repeatability.

Protocol 1

In Protocol 1, simultaneous amplification of CHD-W (female-specific) and CHD-Z (male/female control) with similar amplicon sizes in males and females (345–390bp) was used on the whole blood specimens (Table 1). This protocol was originally developed by R. Griffiths [2] and used in the present study with modifications. The PCR amplification (Bio-Rad, USA) was carried out in a total volume of 20µL, including 1× reaction buffer (Invitrogen, USA), 2

mM of $MgCl_2$, 150µM of each dNTP, 0.1U/µL of Taq polymerase, and 150ng of each P2/P8 primer (Alpha DNA, Canada). Then, 250ng of the DNA sample was added to the reaction. The thermal cycling included initial denaturation of 94°C for 90s, followed by 35 cycles of 50°C for 40s, 72°C for 40s and 72°C for 5 min. The PCR products were separated using electrophoresis in 2.5% agarose gels in TBE buffer with Red Safe for 60 min at 100V and visualized under UV light.

Table 1. Specifications of the primers used in this study

Protocol	Primer	Sequence (5'-3')	Reference
1	P8	CTCCCAAGGATGAGRAAYTG	[2]
	P2	TCTGCATCGCTAAATCCTTT	[2]
2	2550F	GTTACTGATTCGTCTACGAGA	[2, 3]
	2718R	ATTGAAATGATCCAGTGCTTG	[2, 3]
3	NP	GAGAAACTGTGCAAAACAG	[1]
	P2	TCTGCATCGCTAAATCCTTT	[1]
4	P3	AGATATTCCGGATCTGATA	[5]
	P2	TCTGCATCGCTAAATCCTTT	[5]

Protocol 2

In this protocol, a pair of primers (2550F and 2718R) were used to amplify a conserved region between the two genes, resulting in a size difference of 150bp in amplification of CHD1W (450bp) and CHDIZ (600bp) genes (Table 1). Amplification (Bio-Rad, USA) was carried out in 20µL reactions, containing 1× reaction buffer (Applied Biosystems, USA), 2mM of $MgCl_2$, 200µM of each dNTP (Thermo Fisher Scientific, USA), 0.05U/µL of Taq polymerase (Thermo Fisher Scientific, USA) and 1.2µM of each 2550F and 2718R primers (Alpha DNA, Canada). Then, 200ng of the DNA template was added to the reaction. The thermal cycling protocol consisted of an initial denaturation step at 95°C for 4min, followed by 35 cycles of denaturation (95°C, 30s), annealing (52°C, 30s) and DNA extension (72°C, 45s) and a final extension step at 72°C for 5min. The PCR products were electrophoresed in 2% agarose gels in TBE buffer for 60min at 100V and visualized under UV light.

Protocol 3

Protocol 3 used NP and P2 primers for DNA amplification, first introduced by *H. Ito et al.* [1] and then used in the current study with changes (Table 1). Amplification was carried out in a total volume of 25µL. Reactions included 50mM of KCl, 10mM of Tris-HCl pH = 8.3, 1.5mM of $MgCl_2$, 0.2mM of each dNTP, 0.5µM of each primer (Alpha DNA, Canada) and 0.625U of DNA Taq polymerase (Invitrogen, USA). Moreover, 200 ng of the genomic DNA was used as a template. The thermal cycler (Bio-Rad, USA) program consisted of initial denaturation at 94°C for 90s, followed by 35 cycles of 72°C for 45s, 55°C for 45s and 94°C for 30s. The final extension was carried out at 48–50°C for 1.0min and 72°C for 5min. The PCR products were analyzed using electrophoresis in 3% agarose gels in 1× EtBr stained TBE buffer and UV light visualization. The expected PCR products included 350–390bp.

Protocol 4

In Protocol 4, simultaneous amplification of CHD-W (female-specific) and CHD-Z (male/female control) with similar amplicon sizes in males and females (110bp) was used on whole blood specimens (Table 1). This protocol was originally developed by *R. Griffiths* [5] and used in the present study with modifications. The PCR amplification (Bio-Rad, USA) was carried out in a total volume of 20 µl, including 1× reaction buffer (Invitrogen, USA), 2mM of $MgCl_2$, 100µM of each dNTP, 0.15U/µL of Taq polymerase, and 1µM of each P2/P3 primer (Alpha DNA, Canada). Then, 200ng of the DNA sample was added to the reaction. The thermal cycling included initial denaturation of 94°C for 90s, followed by 35 cycles of 56°C for 30s, 72°C for 30s and 94°C for 30s, followed by final extension of 72°C for 5min. The PCR products were separated using electrophoresis in 3% agarose gels in TBE buffer containing Red Safe for 70min at 100V and then visualized under UV light.

DNA concentration

DNA concentrations of all specimens in Experiment 2 were assessed using Qubit 4 Fluorometer (Thermo Fisher Scientific, USA) and Invitrogen dsDNA BR Assay Kit (Invitrogen, USA). Briefly, 10µL of the extracted DNA were transferred into a sterile 0.5mL microtube and diluted with 190µL of the reagent. The microtube was transferred to the device chamber to quantify the DNA content of the sample.

Ethical approval

Full experimental procedures were carried out in compliance with guidelines from the Government of the United Arab Emirates Animal Care and Use.

Data analysis

Data of DNA concentration were examined for normality and subjected to logarithmic transformation before further processing. A generalized linear model was used to compare the effects of the bird species and specimens on DNA concentrations with sex included in the model as the random effect, and multiple comparisons were carried out using Bonferroni correction. Statistical analyses were carried out using SPSS Software v.20 (IBM Analytics, USA).

Results and discussion

Overall, genome extractions from various types of samples used in this study yielded enough DNA concentrations with good 260/280 and 260/230nm wavelength ratios. Results from *Experiment 1* demonstrated the superiority of Protocol 2 (2550F/2718R primers) for the sex differentiation of birds as compared to other three protocols, resulting in the most distinctive bands on electrophoresis gels (Fig. 3A-D). Female amplicons in the other three protocols (P2/P8, P2/NP, and P2/P) did not resolve well on gels due to the close band sizes and appeared as single stronger bands, creating difficulty in discerning differences between males and female s (Figs. 3A, C and D).

The same phenomenon was seen in single amplicons of the males due to the high purity and yield of the extracted DNA samples, which resulted in further confusion between the female fused bands and single male bands and possibly false reports. The use of 2550F/2718R primers for sexing of adult birds has previously been described in other Falconiformes such as white eagles, imperial eagles, and Egyptian vultures [9]. To the best of the authors' knowledge, this is the first report of optimizing this protocol for use in various falcons' species and comparing the results with other commonly used protocols of bird sexing under one experimental setup.

The necessity of comparative adjustments of PCR protocols for accurate sex determination of avian species lies in the fact that although the exonic regions of chromo helicase DNA binding protein genes are highly conserved within various bird species, including those in Falconiformes [1, 9-15], but intron polymorphism from length differences in noncoding regions limits PCR for sex determination in birds which necessitates further optimizations of the procedure when working on various species and subspecies [10,16, 17]. In falcons, *M.A. D'Aloia and C. Eastham* [13] used P2/P3 primers followed by site-specific restriction enzyme digestion for sexing; *Hae* III restriction enzyme successfully cut the CHD-Z PCR product (male/female control), and hence 110-bp CHD-Z amplicon was downgraded to two fragments. Nevertheless, the PCR product of P2/P3 did not provide discernible bands for males and females unless subjected to enzyme digestion (Fig. 3D). Therefore, the use of Protocol 2 (2550F and 2718R) seemed to provide a simple alternative solution for sexing of falcons, eliminating the need for further enzymatic digestion steps. In another report on Falconiformes, *H. Ito et al.*

[1] optimized P2/NP and P2/P8 for sexing of Peregrine falcon and reported that the former protocol resulted in two female amplicons with close band weights of nearly 392bp [1]. In the current study, PCR product of P2/NP primer appeared as a single band in both sexes of all four falcon species, including Peregrine (Fig. 3C), while 2550F and 2718R primers provided well-isolated bands, amplifying a conserved region between CHD1W (450bp) and CHDIZ (600bp) genes in all studied species (Fig. 3B). *H. Ito et al.* [1] further reported an allele-specific PCR, amplifying CHD1W by P2/P3 primer pair and simultaneously amplifying CHD1Z by P2/NP primer which resulted in two distinct amplicons in females of all species in Falconiformes. However, female samples' PCR products still appeared as two amplicons with close band sizes [1].

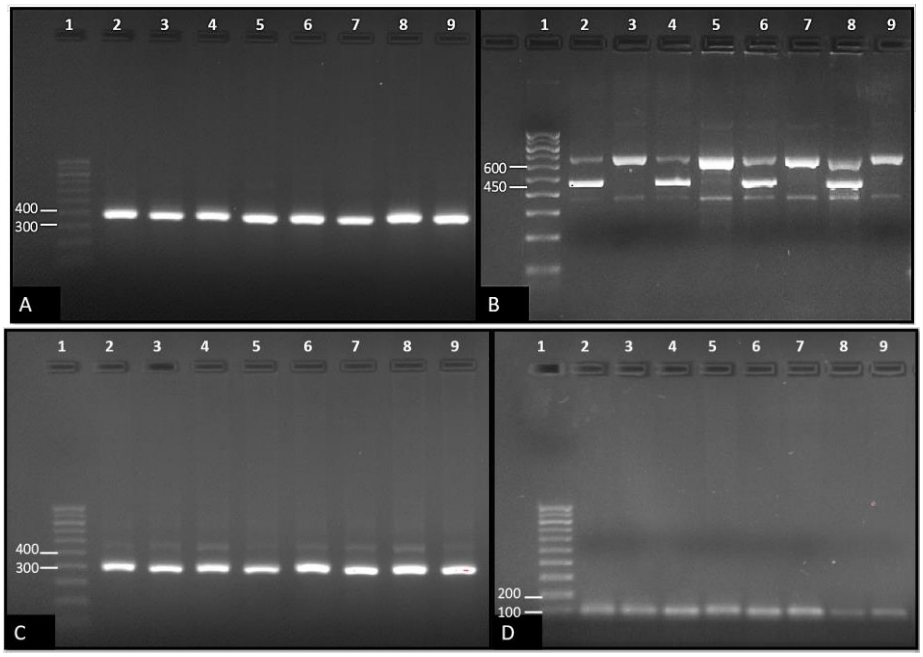

Fig. 3A-D. Gel electrophoresis of the PCR products on blood samples of various falcon species: A (P2/P8 B) 2550F/2718R; C. P2/NP; D. P2/P3 primers respectively and Lane 1, 100-bp ladder; Lane 2, female Gyr; Lane 3, male Gyr; Lane 4, female Saker; Lane 5, male Saker; Lane 6, female Gyr-Peregrine; Lane 7, male Gyr-Peregrine; Lane 8, female Peregrine; and Lane 9, male Peregrine

Concentrations of DNA extracted from whole blood samples did not show a significant different across the four species of falcons (Table 2). Nevertheless, stool samples yielded the lowest DNA concentration, compared to that of the embryonic membranes and whole blood across all four species ($p \leq 0.05$) (Table 2).

Table 2. Effects of falcon species and biological specimens on the extracted DNA concentrations (μg ml^{-1})

Species Specimen	Gyr	Gyr-Peregrine	Saker	Peregrine
Blood	52.95 $\pm$2.68^{Aa}	54.83 $\pm$1.85^{Aa}	54.60 $\pm$2.61^{Aa}	59.68 $\pm$1.87^{Aa}
EM	40.45 $\pm$3.08^{Ab}	50.25 $\pm$1.41^{Ba}	53.28 $\pm$1.53^{Ba}	57.23 $\pm$2.03^{Ba}
Stool	18.90 $\pm$0.91^{Ac}	22.10 $\pm$1.57^{ABb}	22.50 $\pm$1.37^{ABb}	26.13 $\pm$1.15^{Bb}

EM, embryonic membrane. Different superscript capital and small letters demonstrate

significant differences between and within the species, respectively

As shown in figure 4, the qualities of the extractions from whole blood, eggshell membranes, and stools were verified using automated assay and gel electrophoresis.

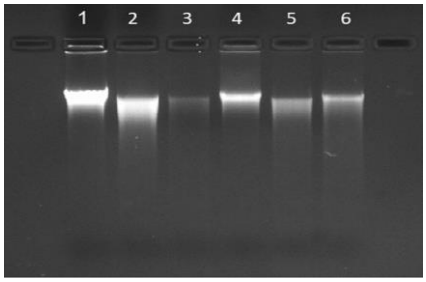

Fig. 4. Quality assessment of the extracted DNAs using agarose gel electrophoresis. Lane 1, male blood; Lane 2, male embryo membrane; Lane 3, male stool; Lane 4, female blood; Lane 5, female embryo membrane; and Lane 6, female stool

Results of *Experiment 2* showed that PCR of these specimens by the modified protocol accurately identified the sex of all subjects (100%) in the four studied species (Figs. 5 and 6; Table 3).

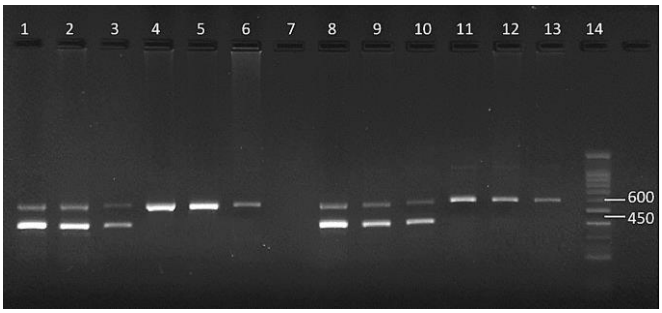

Fig. 5. Gel electrophoresis of the PCR products from CHD1W (450bp) and CHDIZ (600bp) genes of various falcon biological specimens in Saker and hybrid falcons. Lane 1, Saker female blood; Lane 2, Saker female embryo membrane; Lane 3, Saker female stool; Lane 4, Saker male blood; Lane 5, Saker male embryo membrane; Lane 6, Saker male stool; Lane 7, negative control; Lane 8, Gyr-Peregrine female blood; Lane 9, Gyr-Peregrine female embryo membrane; Lane 10, Gyr-Peregrine female stool; Lane 11, Gyr-Peregrine male blood; Lane 12, Gyr-Peregrine male embryo membrane; Lane 13, Gyr-Peregrine male stool; and Lane 14, 100-bp DNA ladder

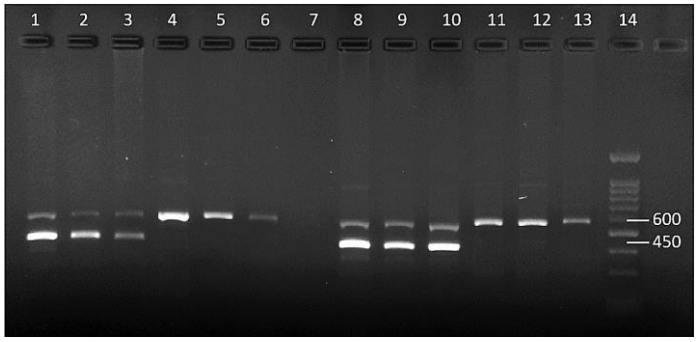

Fig. 6. Gel electrophoresis of the PCR products from CHD1W (450bp) and CHDIZ (600bp) genes of various falcon biological specimens in Gyr and Peregrine falcons. Lane 1, Gyr female blood; Lane 2, Gyr female embryo membrane; Lane 3, Gyr female stool; Lane 4, Gyr male blood; Lane 5, Gyr male embryo membrane; Lane 6, Gyr male stool; Lane 7, negative control; Lane 8, Peregrine female blood; Lane 9, Peregrine female embryo membrane; Lane 10, Peregrine female stool; Lane 11, Peregrine male blood; Lane 12, Peregrine male embryo membrane; Lane 13, Peregrine male stool; and Lane 14, 100bp DNA ladder

Table 3. Results of falcon sexing using various bird specimen and species

Species	Falcon ID	SQ			Sex	Phenotyping	Sexual behavior	PCR	%
		EM	B	S					
Gyr	G1, G2, G3	3	3	3	Male	Male	Male	Male	100
	G4, G5, G6	3	3	3	Female	Female	Female	Female	100
Peregrine	P1, P2, P3	3	3	3	Male	Male	Male	Male	100
	P4, P5, P6	3	3	3	Female	Female	Female	Female	100
Saker	S1, S2, S3	3	3	3	Male	Male	Male	Male	100
	S4, S5, S6	3	3	3	Female	Female	Female	Female	100
Gyr-Peregrine	GP1, GP2, GP3	3	3	3	Male	Male	Male	Male	100
	GP4, GP5, GP6	3	3	3	Female	Female	Female	Female	100

SQ, sample quantity; EM, eggshell membrane; B, blood; S, stool; PCR, polymerase chain reaction using 2550F/2718R primers; %, result identity proportion

In general, the most desirable time of sexing for the highlighted species is immediately after hatching or during the early days of birdlife. A major disadvantage of using commonly used blood and feather samples to provide DNA from chicks is linked to the invasive nature of these sampling methods, which is critically discouraged by the bird owners. Experiment 2 coroborated the feasibility of using readiliy available samples such as egg-shell membrane and stool as DNA sources and hence eliminating the need for aggressive handling of the chicks.

The use of noninvasive samples for PCR creates several challenges the success in facing of which depends mainly on retrieving sufficient target DNA from those samples. In 2008, *F. Hogan et al.* [15]. optimized protocols for the use of shed feathers in genetic analyses and showed that successful amplification of multilocus genotypes depended on the starting materials with good DNA samples and specimens with low DNA qualities were associated with a higher risk of amplification failure, as previously suggested by *G. Segelbacher* [18]. In the current study, various results reported for DNA concentrations highlight the uasefulness of these DNA sources for genetic studies in birds and for early sex determination of various falcon species in particular. An interesting observation in this study was that the lowest concentration of extracted DNA from both embryonic membrane and stool samples was observed in Gyrfalcons, indicating possible species' effects on the extraction quality ($p \leq 0.05$) (Table 2). For the concentrations of DNAs from male and female birds, the mean concentrations ($ng \cdot mL^{-1}$) respectively included 55.60±6.15 and 55.45±5.48 in blood, 51.36±7.47 and 49.24±8.65 in embryonic membranes and 23.83±3.81 and 20.75±3.54 in stools of the males and females, regardless of the bird species or collected biological specimens. Concentrations of DNAs were usually higher in males than those in females, with no significant differences ($p > 0.05$) (Table 4).

Table 4. Mean DNA concentrations ($\mu g \cdot mL^{-1}$) of various falcon specimens

Sex	Blood	EM	Stool
Female	55.45 ±5.48	49.24 ±8.65	20.75 ±3.54
Male	55.6 ±6.15	51.36 ±7.47	23.83 ±3.81

EM, embryonic membrane

The usefulness of eggshell membranes as sources of DNA for avian population genetics was reported in a study by *K.B. Trimbos et al.* [19], by which they demonstrated DNA amplification success rates of 99.1% for eggshell membranes and 97.7% for blood specimens. They further demonstrated that DNA from eggshell membranes was not affected by degeneration or possible cross-contamination, as shown for the blood specimens. In the current

study, DNA yield from egg-shell membrane was similar to blood and consistently higher than stool across all studied species (Table 3). Lower concentrations of the extracted DNA in stool samples can generally be attributed to the presence of high concentrations of uric acid, fats, and proteins in stools, as well as other biochemicals such as oligosaccharide contents and phenolic compounds (Table 3) [20] which make DNA extraction from stool specimens a real challenge, sometimes necessitating the use of further sensitive techniques [21]. In this study, despite lower concentrations of DNA from stool samples (Table 3), automated assessment of the extracted DNAs using agarose gel electrophoresis verified the quality of extraction from stool, compared to other samples (Fig. 4). It must be noted that DNA from stool of herbivore birds can can lead to trouble due to the presence of numerous PCR inhibitors with plant origin [22], it can be hypothesized that extracted DNAs from stool of carnivore birds may include the genome of the pray as well and consequently lead to false results. However, the PCR results from stool samples of all 24 birds in this study were in complete agreement with those of other samples, and the accuracy of these findings was further verified by examining the respective birds after the onset of maturity. However, studies with larger sample sizes are necessary to further substantiate the usefulness of stool samples for large-scale application in genetic studies on falcons.

Protocol 2 of the current study used the primer pair of 2550F as forward and 2718R as reverse primers. *Fridolfsson and Ellegren* [23] originally synthesized this primer pair in 1999. Very recently, *A. Mataragka* [8] comparatively assessed the performance of PCR assays commonly used for sexing of avian species and reported it as a reliable method for most cases of bird sexing using various specimen resources. They recommended preliminary studies when a bird species was assessed for the first time, as carried out in the current study. Previously, *L. Begovic et al*. [7] used CHD-based 2550 and 2718 primers to optimize molecular methods in sex-typing of heron species with various DNA sources of feathers, eggshells, and eggshells swabs. They demonstrated successful amplification of the CHD gene in heron species. Similarly, they suggested the necessity of optimizing laboratory procedures, especially when various noninvasive tissue specimens were available, as suggested by the present study. However, parameters other than good optimizations of the laboratory procedures still interrupt bird sexing, threatening the results' reliability. Examples of these parameters include individual variations, contaminations, and sampling, and/or laboratory errors. The error rate may surprisingly be as high as 100% [24]. Therefore, careful reviews and setups of the optimization protocols must be carried out to avoid uncertainties that increase the molecular methods' accuracy and feasibility.

Conclusions

In summary, results from various experiments in this study have shown that optimizing the current PCR protocols can lead to further reliable and simpler identification of the target genes with fewer false results in popular falcon species in the Arabian Peninsula. The use of P2 (2550F/2718R) primers simplifies PCR procedure by eliminating the need for further processing of PCR products and eases the result interpretation. Captive breeding of falcon’s benefits from early sex determination of chicks and hatchlings in this species; hence, access to good-quality DNA material through non-invasive sampling methods is of significant practical value. Results of this study have indicated for the first time that good-quality DNA samples can be obtained from eggshell membranes of hatching chicks, and when necessary, stool samples

can alternatively be used for the accurate sex determination of falcons. Further studies with larger sample sizes are necessary to better optimize the current molecular methods of falcon sexing.

Acknowledgments

The authors especially thank Dr. Mirjam Ricarda Hampel, who kindly provided falcon blood and stool specimens, and Prof. Arash Ghalyanchi for his valuable advice and providing the primer sets. Authors deeply appreciate the support from the Zaabeel Office for H. H. Sheikh Mohammed Bin Rashid Al Maktoum, Vice-President and Prime Minister of the UAE and Ruler of Dubai, during the course of this study.

Funding Body

This study was generously sponsored by the Zaabeel Office for H. H. Sheikh Mohammed Bin Rashid Al Maktoum, Vice-President and Prime Minister of the UAE and Ruler of Dubai.

Abbreviations

PCR: polymerase chain reaction
CHD: chromo helicase DNA binding protein

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Received: April 22, 2021
Accepted: August 20, 2022