

Sex Determination of Nestlings in Eleonora's Falcon *Falco eleonora*: plumage characteristics and molecular sexing¹

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ABSTRACT

Three basic colour morphs of Eleonora's Falcons ('ll', light; 'ID', dark heterozygous; 'DD', dark homozygous) can already be determined from nestling plumage. The colour pattern of the undertail-coverts is particularly suited to distinguish these morphs. Once the morph is known, sex can be determined from colour markings at the shaft near the tail end. With this procedure more than 85% of nestlings at >25 days of age were correctly sexed, when compared with results from molecular sexing using a PCR protocol. For 561 nests studied on an island near Crete between 1997-2001, the male-to-female ratio of fledglings was 544:490; the bias appears to be related to brood size: nests with two chicks have significantly more males, nests with three chicks are biased in favour of females.

INTRODUCTION

Eleonora's Falcon *Falco eleonora* is a medium sized falcon which shows only a slight sexual size dimorphism. Therefore the sex of nestlings cannot be determined as easily as in large falcons. However, a method for sexing fledglings based on reversed size dimorphism was described in Wink *et al.* (1982). The authors used weight of fledglings and width of mouth. As far as can be seen in the literature of the past two decades, this method is of limited use because handling of nestlings is usually done prior to fledging. As a consequence there is still a need for a convenient field method to sex nestlings of Eleonora's Falcon. It would be ideal for the field worker if sexing could be

¹ This contribution represents Part 27 of a series on Eleonora's Falcon.

² Ludger Witte tragically died during field work for this study in Sept. 2001

done by using feather patterns only. In addition, a PCR method has been developed that allows an unequivocal molecular sexing. As an application for the methods, the sex ratio in juveniles is reported in this communication in relation to brood size.

MATERIAL AND METHODS

Field studies

There are three basic colour morphs in Eleonora's Falcon. Approximately 70% are light coloured, 28% develop dark plumage when one year old, and 2% are already dark as fledglings. Morph is inherited in a Mendelian fashion, and 'dark' figures as the dominant trait (Wink *et al.* 1978). For simplicity, the abbreviations 'll' (light), 'lD' (dark heterozygous) and 'DD' (dark homozygous) are used to characterise these three morphs. It is likely that the genetics of morphs are more complicated than this simple model suggests since it neglects intermediate morphs (Wink *et al.* 1978, Ristow *et al.* 1998, Ristow *et al.* 2000).

A falcon colony of about 150 pairs on an island, less than 1 km in size, in the South Aegean Sea was visited by D. R. and L. W. in 1997-2001. Each year about 95% of the chicks were banded. If these were old enough, notes were taken on the undertail-coverts to determine morph, and about the markings near the tip of the rectrices to determine sexual differences. Blood samples of about 50 µl were taken to determine the sex in the laboratory later on.

DNA isolation and PCR-methods

Blood samples were preserved in an EDTA buffer (0.1 M Tris, pH 7.4, 10% EDTA, 1% NaF, 0.1% thymol) (Wink 1998) and stored at -20°C until processing. Total DNA was extracted from the blood samples by an overnight incubation at 37°C in lysis buffer (10 mM Tris [pH 7.5], 25 mM EDTA, 75 mM NaCl, 1% SDS) including 1 mg of Proteinase K (Merck, Darmstadt), followed by a standard phenol/chloroform protein extraction. DNA was precipitated from the supernatant with 0.8 volume of cold isopropanol, centrifuged, washed, dried and resuspended in TE buffer.

Molecular sexing was modified (Becker & Wink, 2002) after the methods outlined in Kahn *et al.* (1998) which is based on the detection of the CHD gene on avian sex chromosomes. In most species, males produce one band and females two, presumably reflecting differing intron sizes of the W versus Z chromosomes (Kahn *et al.* 1998). PCR used were 1237L: GAG AAA CTG TGC AAA ACA G and 1272H: TCC AGA ATA TCT TCT GCT CC. PCR conditions: The PCR mix consisted of: 60 ng (2 µl) total DNA in 25 µl total volume, 0.12 µl 1272H Primer (97.45 pmol/µl), 0.103 µl 1237L Primer (83.1 pmol/µl), 1 µl nucleotide-mix (100 µM of GTP, CTP, TTP und ATP), 2.5 µl 10x buffer with 15 mM MgCl₂, 0.15 µl Taq-Polymerase (0.6 Units; Pharmacia Biotech; Freiburg), 0.1 µl ³³P α-dATP (1 µCi). PCR program: 2 min at 94°C; 31 cycles with 30 sec at 94°C, 1 min at 56°C, 2 min at 72°C and finally 10 min at 72°C. After 32 cycles the reaction temperature was maintained at 72 °C for 4 min and then lowered to 4 °C for further storage. PCR products were separated electrophoretically on a denaturing Sequagel matrix at 65 W for 1.5 h (length 40 cm). After drying, the gel was exposed to an X-ray film (Hyperfilm-MP,

Amersham) for 1-2 days, and developed (X-ray developer and fixer, Kodak). In females two PCR products were visible but only a single one in males.

RESULTS AND DISCUSSION

Feather patterns in Eleonora's Falcon depend on morph, of course. If feather patterns are to be used for sex identification, morph has to be determined in the first place. For the rare DD-nestlings, this is easily achieved by plumage and bill characteristics as described in Ristow *et al.* (2000). Morph of the common II and ID nestlings at >25 days of age can be unequivocally identified by the colour pattern of the undertail-coverts as described in Table 1. Additionally, there this pattern contains already some information on the gender of the birds, but especially in II individuals some uncertainty of sex determination remains. That is why we have additionally chosen another pattern as criterion.

Table.1. Colour pattern of undertail-coverts in Eleonora's Falcons in relation to age, plumage morph, and sex

Age	*Background	Marks	Description of marks, comments on sex
juv.II	Light ochre	Brown	Consisting of shaft-streaks, arrow-heads, or 3 mm broad bars, all end at least 5 mm from edge of web; marks can be arranged as if part of 10 mm spaced barring; marks may be almost absent. There is a trend amongst males that marks are smaller and less frequent than in females.
juv.ID	Ochre	Dark brown	Bars are vertical to the shaft and reach the edge of the webs. In males, their breadth is <4 mm, as compared to >6 mm of the ochre background between bars; in the ochre field, the shaft is partially ochre, too; the first subterminal mark is an arrow-head which does not reach the edge of the web. In females the bars are broader >5 mm as compared <5 mm of the ochre band; in the ochre field the shaft is brown; the subterminal mark is a triangle, which does not reach the edge of the web.
juv.DD	Dark ochre	Black-brown	Bars are vertical to the shaft and reach the edge of the webs. Their breadth in males is 5-6 mm as compared to 2-4 mm of the ochre bands in between. The breadth in females is 5-6 mm as compared to 3-5 mm of ochre in between. *In all three morphs, the ochre background in juvenile males has a pitch of orange which the respective females do not have.
immatures			Coloured and patterned like adults of the respective morph and sex, but with less prominent contrast.
ad.II	Cinnamon	Brown	Pattern variation same as in II juveniles. Again, there is a trend in males that marks are smaller and less frequent. The cinnamon in males is red-cinnamon, the subterminal marks are often blue-grey. Females have brown-cinnamon which turns ochre-yellow when bleached. Shafts may be lighter, of the same colour, or darker than webs. Few individuals are blank with no marks.
ad.ID	Brown	Blackbrown	Barring varies in contrast between female individuals. In males the background is also blackbrown, so no barring is discernible; an additional 1-2.5 cm grey tip creates a darker distal part of the web. Shaft in the distal half darker than webs.
ad.DD	Brown	Blackbrown	Same as in ID morph, but probably with a trend to be darker than the ID adults.

In Fig. 1-3 the tips of the rectrices for the six cohorts of juveniles are shown. The dark area along the shaft proximal from the subterminal band does not vary at random. The breadth of this dark line is smaller for males than for females, and this breadth increases from II ⇒ ID ⇒ DD morph juveniles. For convenience, we term this criterion the "stretch" rule. In some individuals a trend within the tail was observed that R2 and R5/R6 showed a broader dark

shaft line than R3 and R4. To be independent of this trend, the stretch rule is defined for R3 and R4:

- (i) in II males the shaft is coloured like the webs, or the dark colouration extends only on the shaft up to its proximal half within the ochre band
- (ii) in II females the shaft is dark within the whole ochre band
- (iii) in ID males the shaft is dark within the whole ochre band
- (iv) in ID females the dark colouration of the shaft spreads to the left and the right onto the web, resulting in a total breadth of 2 mm
- (v) in DD males the spread is 2-4 mm
- (vi) in DD females the central dark stripe spreads 5 mm or even more, so that the ochre band is almost obscured (Fig. 1 a-c).

In some DD fledglings tail barring exists only on the inner webs, so that the stretch criterion cannot be applied. But these cases present no problem because sex is easily determined by general plumage and bill colour. - The tail end becomes visible for applying the stretch criterion when the chicks are 25 days old. With this tool, other minute sexual plumage differences may be noted in juveniles. For example, the light fringes of body feathers are broader in males than in females. So, when a male and a female of the same morph are sitting next to each other, the lighter breast and crown of the male is discernible.

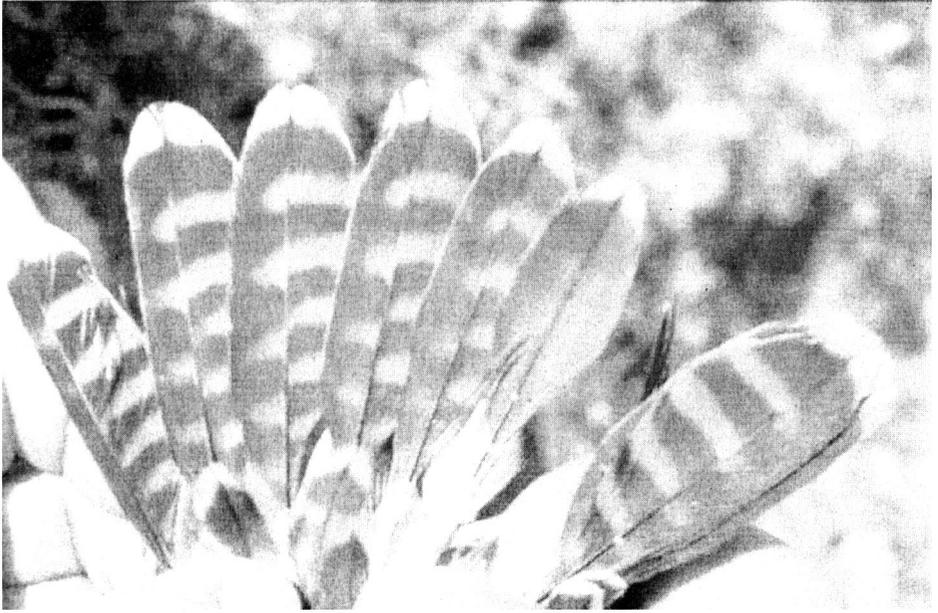
A few juveniles show a tail pattern of intermediate structure within the above rule. Very few show a displaced grid pattern of the dark bars on the outer web relative to those on the inner web so that the rule cannot be applied at all.

Table 2. Distribution of male and female fledglings of Eleonora's Falcon in broods with 1, 2 or 3 young per nest.

The observed totals were 544 males and 490 females in 561 nests (Crete; 1997-2001). Expected nest numbers for the different male/female cohorts were calculated with the theoretical probability of 0.5 for male and female birth, respectively.

brood size	number of nests		sub number of nests	expected nest number	chi square test
1	160	with 1 m	79	$1/2 \times 160 = 80$	n. s.
		" 1 f	81	$1/2 \times 160 = 80$	
2	328	with 2 m	102	$1/4 \times 328 = 82$	p<0.001
		" 1 m + 1 f	163	$1/2 \times 328 = 164$	
		" 2 f	63	$1/4 \times 328 = 82$	
3	73	with 3 m	7	$1/8 \times 73 = 9.1$	n.s.
		" 2 m + 1 f	23	$3/8 \times 73 = 27.4$	
		" 1 m + 2 f	32	$3/8 \times 73 = 27.4$	
		" 3 f	11	$1/8 \times 73 = 9.1$	
[561		with 544 m juv.		$1/2 \times 1034 = 517$ m	p<0.1
	and 490 f juv.		$1/2 \times 1034 = 517$ f		

Figure 1. Tail end of light morph (II) juvenile Eleonora's Falcon. The ochre bands may reach the edge of the outer web. The area at the shaft in the ochre band proximal to the subterminal band helps to sex juveniles, as in these nest siblings. (a) Male, with simple ochre band. (b) Female, a dark stretch at the shaft interrupts the ochre band.



H000636 male L
080 22.9.99

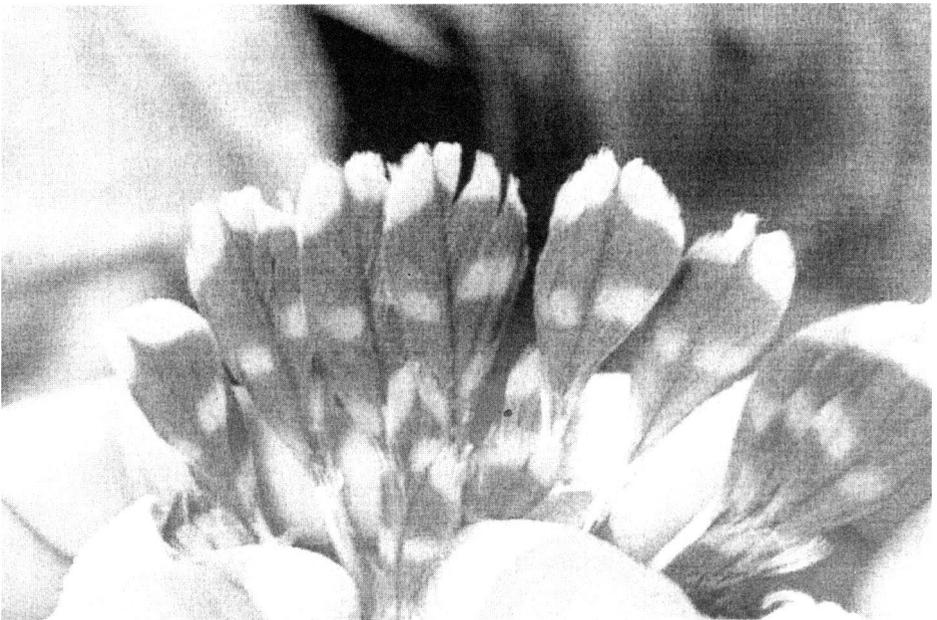


H000637 female L
080 22.9.80

Figure 2. Tail end of heterozygous dark morph (ID) juvenile Eleonora's Falcon. The stretch is broader than in the light morph, in (a) males thinner than in (b) females.



5323764D male
046 21.9.99 OSD



5323765DI female
046 21.9.99 OSD

Figure 3. Tail end of homozygous dark morph (DD) juvenile Eleonora's Falcon. Nest siblings, male on the left and female on the right. Typically, the stretch is even broader than in ID juveniles. - There is a higher frequency of barring indication on the central pair R1 in this rare morph as compared to the other two morphs.



M470 female
20.9.98

M469 male
20.9.98

In order to estimate the robustness and error rate of the morphological sexing, the gender was determined from blood samples by PCR analysis. An overall accuracy of >85% for the shaft criterion was found. This value will be improved in the future, if necessary by a combination with other criteria such as head shape, mouth width or weight of nestlings (Wink *et al.* 1982)

The application of sexing nestlings offers some research opportunities. For example, in the investigated colony during 1997-2001, the observed distribution of male and female young in nests of various brood sizes deviates from the expected sex ratio of 1:1. Overall, more males fledged than females. But there is no common trend between cohorts (Table 2): nests with 2 chicks have significantly more males, nests with 3 chicks are biased in favour of females.

CONCLUSIONS

In an Eleonora's Falcon colony on a small island the investigated population is well defined, well sized, fairly well accessible, and the very constant Mediterranean climate allows only for small scale variations of breeding performance between years. Such a plot is ideal for sex ratio studies. However, the situation as shown in Table 2 appears to be complex. The sex ratio apparently depends on several unknown factors which are not separated when investigating brood size alone.

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