

DNA-Fingerprinting of Eleonora's Falcon *Falco eleonora**

I. Swatschek, F. Feldmann, D. Ristow,
W. Scharlau, C. Wink & M. Wink

INTRODUCTION

Although many bird species seem to be monogamous according to observations and ringing recoveries, there is increasing evidence that extra-pair copulations (EPC) are not exceptional but occur at a certain rate in many populations. The study of pairing systems and the question of EPC became possible with the introduction of techniques which employ methods of molecular biology.

Especially the technique of DNA-fingerprinting is a powerful means to analyse close relationships within populations, e.g. pedigree analysis. Several protocols are available. They differ in the choice of a specific DNA-probe and/or the method of detecting hybridized DNA. Jeffreys *et al.* 1985; Ali *et al.* 1986; Nakamura *et al.* 1987; Georges *et al.* 1988; Zischler *et al.* 1989; Gyllenstein *et al.* 1989). In this study we used synthetic oligonucleotide probes [(GAA)₅, L18] and as a detection method, immunological procedures that avoid the use of radioactivity.

Eleonora's Falcon *Falco eleonora* breeds on isolated islands of the Mediterranean and winters in Madagascar. This species lives colonially and up to 400 breeding pairs can be found on rather small rocky islands. Eleonora's Falcon is territorial. Since space is limited on these islands, a breeding territory usually is small, with a radius of 10-50 m. As a consequence, breeding pairs nest in close proximity and usually have visual contact with each other. With respect to the known incidence of EPC in other bird species, our working hypothesis was that, although Eleonora's Falcon is a monogamous bird (Ristow *et al.* 1979) EPC should occur. To test this hypothesis we studied seventeen families of Eleonora's Falcon from an Aegean colony by DNA-fingerprinting.

MATERIAL AND METHODS

Collection of Blood

In 1991 we captured 78 adult female and 28 male falcons on an Aegean island (Ristow *et al.* 1991). Venous blood (c. 100 ml) was taken and stored in DNA-buffer (Arctander 1988). Blood was also collected from young falcons of which parent birds had been captured. Blood was stored at ambient temperature up to four weeks, then transferred to Heidelberg and stored at - 20°C.

Extraction of DNA

0.5 ml of the blood samples were mixed with 5 ml lysis-buffer (25 mM EDTA, 75 mM NaCl,

* Part 20 of a series on the biology of Eleonora's Falcon.

10 mM Tris, pH 7.5, 1% SDS) containing 1 mg proteinase K. After overnight incubation at 37°C, proteins were removed by precipitation with 2.5 ml of saturated NaCl solution. By adding 16 ml of 100% ethanol to the supernatant the DNA precipitated and was collected with a glass rod. The DNA was washed with 70% ethanol to remove salts, then airdried and dissolved in 1 ml H₂O.

Cleavage by Restriction Enzymes

30 µg DNA per sample were digested overnight with 25 units Hinf I (Pharmacia). Completeness of digestion was controlled by agarose gel-electrophoresis of 3 µg of the digested DNA.

Preparation of Oligonucleotide Probes

Oligonucleotide probe L18 was constructed synthetically according to the core sequence of a minisatellite for the Willow Warbler (*Phylloscopus trochilus*) (Gyllenstein *et al.* 1989), (GAA)₃, was purchased from Fresenius (Oberursel, FRG). The oligonucleotide probes were labelled with digoxigenin by using Dig-11-dUTP and terminal transferase. Internal standards and molecular weight markers were coupled with digoxigenin using a UV-sensitive digoxigenin derivate (Boehringer).

Electrophoresis

Genomic DNA which was treated with restriction enzyme was separated by agarose gel-electrophoresis: 0.7% agarose gels were run in 1xTAE-buffer at 4°C, 42V for 65 hours. The length of the gels was 30 cm.

Transfer of DNA from Agarosegels onto Nylon Membranes

When gel-electrophoresis was completed DNA was transferred from the gel to nylon membrane (Biodyne B) by capillary transfer as outlined by Maniatis *et al.* (1989). Nylon membranes were kept at 80°C for two hours to immobilize the DNA.

Hybridization and Detection

Detection employed immunological methods: DNA/DNA-hybrids were detected by an antibody which was raised against digoxigenin. This antibody was coupled to a phosphatase which in turn produced a coloured product at the sites of hybridization (Boehringer). After colour reactions were completed, nylon membranes were documented and processed by the Bioprofil system.

RESULTS AND DISCUSSION

We obtained blood samples of 106 breeding adult falcons. Some of them lost their clutch due to rat predation (Wink *et al.* 1985), so that no young became available. The chicks of other falcons were too young to obtain blood samples at the time we had to finish our field survey. So the final DNA-fingerprint analysis could only be performed on seventeen families of Eleonora's Falcon.

In four families, the DNA of both male and female and their respective young was analysed (Fig. 1). Considering the bandsharing between females and their respective young it is obvious that the female was the real mother in all cases (see bands indicated by arrows in Fig. 1). The same conclusion can be obtained for the corresponding males, i.e. the male was the natural father in these families. In thirteen further families, DNA was available only from either the male or the female (Table 1). In all these cases the parentage of the parent whose DNA was available could be assessed unequivocally. Again, no evidence for EPC or extra-pair fertilization (EPF) could be detected.

More difficult was assessment of the parentage of the other parent bird, whose DNA was not available. However, we found in most cases that two to four bands which were not present in the profile of the known parent occurred concomitantly in those of the young. This is clear evidence that the unknown parent was identical for all the respective chicks. We cannot rule out, however, the unlikely case that a close relative of the unknown parent might have interfered. Therefore, we

Figure 1. Examples for DNA-fingerprinting of Eleonora’s falcons
a) DNA-profiles of 10 unrelated adult males
b) DNA-profile of a family with 2 young
c) DNA-profiles of a female with her 2 young
Arrows indicate significant bands

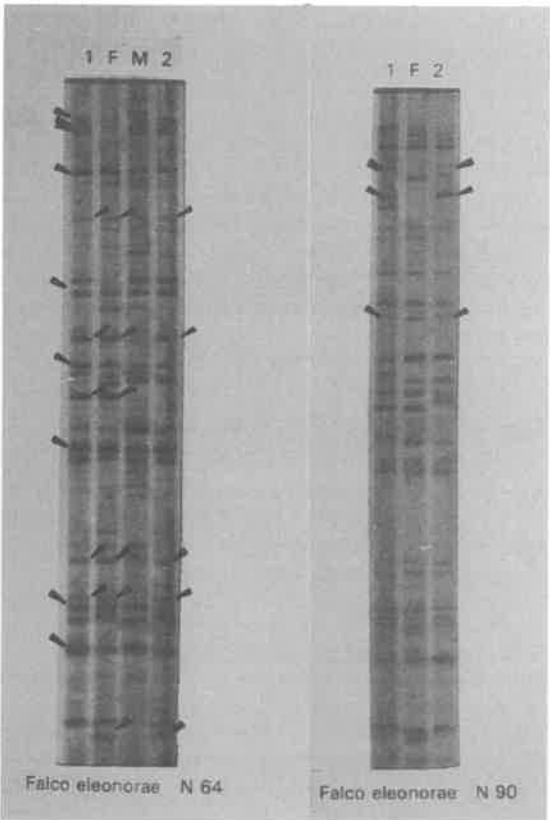
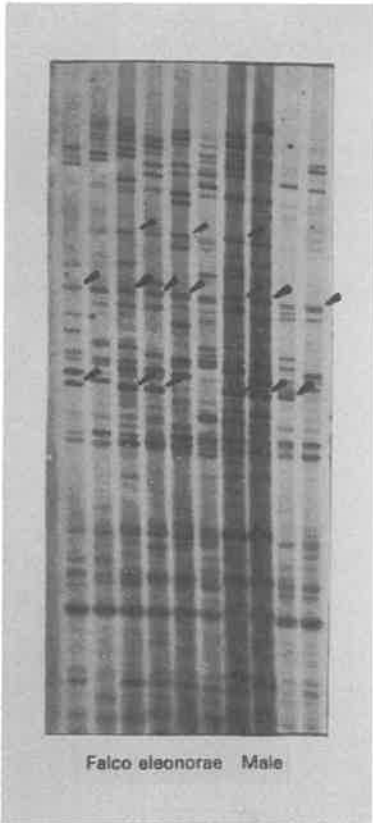


Table 1: Pedigree analysis in 17 families of *Falco eleonora*

Nest	Parentage Young					
	A		B		C	
	M	F	M	F	M	F
DNA of both parents available						
64	+	+	+	+		
83	+	+	+	+		
56	+	+	+	+		
11	n.d.		+	+	+	+
DNA of male available						
30	+	*	+	*		
85	+					
DNA of female available						
22	*	+	*	+		
26	*	+	*	+		
31	?	+	?	+		
45		+				
47		+				
90	*	+	*	+		
91	*	+	*	+		
101	*	+	*	+		
110	*	+	*	+	*	+
121		+				
125	*	+	*	+		

+ = parentage certain

* = parentage "very likely"

? = gels could not be read with certainty

Table 2. Summary of parentage assessments

	Number of young		
	certain	very likely	??
Female is the mother	27	3	1
Male is the father	11	15	5
Evidence for EPC	none	none	none

have classified these findings only as "very likely" (Tables 1 and 2). We could not make an unequivocal assessment because not all bands were clearly visible. However, these cases provide no evidence for EPC either.

Summarizing this analysis, we thus found no evidence for EPC. Also the incidence of egg-dumping by an unrelated female must be a rare event, which nevertheless occurs. In 1979 we found a clutch of four eggs and their measurements indicated that this clutch was produced by two females contributing two eggs each (Wink & Ristow, unpublished).

Our DNA-fingerprint analysis clearly indicates, that parent birds seen at a nest of Eleonora's Falcon are usually the natural fathers and mothers of the respective young. This result shows that a general statement on the abundance of EPF in birds of prey cannot be given. Even in likely candidates for EPF, such as the colonially breeding Eleonora's Falcon, strictly monogamous behaviour was determined. The analysis by DNA-fingerprinting thus confirms our previous results obtained by observation and ringing recoveries.

Figure 1 reveals another phenomenon. A number of bands are shared by both male, female and the young of one nest. In a population in which all adults are genetically unrelated, the number of these bands shared should be rather low whereas it is higher when animals are related. For example, in colonies of the eusocial naked Mole-rat (*Heterocephalus glaber*), DNA-fingerprints show an extremely high level of bandsharing indicating the close relationship of the animals (Reeve *et al.* 1990). Following a similar argument, we preliminarily assume, that the adult Eleonora's Falcons of the breeding colony studied are genetically related because of the relatively high degree of bandsharing (see markings in Fig. 1).

ACKNOWLEDGEMENTS

Our research was supported by grants of the Deutsche Forschungsgemeinschaft (We 719/7-1). We thank Ch. Langlotz for technical assistance, and the Greek government for permission to do research on Eleonora's Falcon.

SUMMARY

Seventeen families of Eleonora's Falcon were analysed by DNA-fingerprinting using oligonucleotide probes. Although the birds breed colonially and in close proximity, no evidence was obtained for EPF, indicating that male and female seen at a falcon's nest are usually the natural parents of the respective young.

REFERENCES

- ALI, S. D., R. MÜLLER, & J. T. EPPLER 1986. DNA fingerprinting by oligonucleotide probes specific for simple repeats. *Hum. Genet.* 74: 239-243.
- ARCTANDER, P. 1988. Comparative studies of avian DNA by restriction fragment length polymorphism analysis: Convenient procedures based on blood samples from live birds. *J. Orn.* 129: 205-216.
- GEORGES, M., A.-S. LEQUARRE, M. CASTELLI, R. HANSET & G. VASSART 1988. DNA fingerprinting in domestic animals using four different minisatellite probes. *Cytogenet. Cell. Genet.* 47: 127-132.
- GYLLENSTEN, U. B., S. JAKOBSSON, H. TEMRIN & A. C. WILSON 1989. Nucleotide sequence and genomic organization of bird minisatellites. *Nucleic Acids Research* 17: 2203-2214.
- JEFFREYS, A. J., V. WILSON & S. L. THEIN 1985. Hypervariable minisatellite regions in human DNA. *Nature* 314: 67-73.
- MANIATIS, T., J. SAMBROOK & E. F. FRITSCH 1989. *Molecular cloning: A laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor (1989).
- NAKAMURA Y., M. LEPPERT, P. O'CONNELL, R. WOLFF, T. HOLM, M. CULVER, C. MARTIN, E. FUJIMOTO, M. HOPF, E. KUMLIN & R. WHITE. 1987. Variable number of tandem repeat (VNTR) markers for human gene mapping. *Science* 235: 1616-1622.

- REEVE, H. K., D. F. WESTNEAT, W. A. NOON, P. W. SHERMAN, C. & F. AQUADRO 1990.** DNA "fingerprinting" reveals high levels of inbreeding in colonies of the eusocial naked mole-rat. *Proc. Natl. Acad. Sci. USA*. 87: 2496-2500.
- RISTOW, D., C. WINK & M. WINK 1979.** Site tenacity and pair bond of the Eleonora's Falcon. *Il-Merill* 20, 16-18.
- RISTOW, D., F. FELDMANN, W. SCHARLAU, C. WINK & M. WINK 1991.** Population dynamics of Cory's Shearwater (*Calonectris diomedea*) and Eleonora's Falcon (*Falco eleonora*) in the Eastern Mediterranean. In A. Seitz & V. Loeschke (eds). *Species conservation: A population-biological approach*, p. 199-212, Birkhäuser Verlag, Basel.
- WINK, M., D. RISTOW & C. WINK 1985.** Biology of Eleonora's Falcon: 7. Variability of clutch size, egg dimensions and egg coloring. *Raptor Research* 19: 8-14.
- ZISCHLER, H., I. NANDA, R. SCHÄFER, M. SCHMID & J. T. EPPLER 1989.** Digoxigenated oligonucleotide probes specific for simple repeats in DNA fingerprinting and hybridization in situ. *Hum. Genet.* 82: 227-233.

Prof. Dr. M. Wink
 Institut für Pharmazeutische Biologie
 Universität Heidelberg
 Im Neuenheimer Feld 364
 D-6900 Heidelberg