

Part 12

Taxonomy, Phylogeny, Developments in Raptor DNA Studies and other Theoretical Aspects



Bat Hawk *Machaeramphus alcinus*

Conveners:

Michael Wink & Anthony van Zyl

Advances in DNA studies of diurnal and nocturnal raptors

Michael Wink

ABSTRACT

Sequence variations in the nuclear and mitochondrial genome of raptors offer a unique chance to elucidate their evolutionary past, phylogeography and population structure as DNA contains a blueprint of most evolutionary events. In this overview the ongoing efforts to study the evolution and phylogeny of diurnal and nocturnal birds of prey has been summarized. DNA sequencing of marker genes is undoubtedly the method of choice for this question. Considering the large number of species concerned, the present data base is still fragmentary and much more work (e.g., more taxa, additional genes) is needed to obtain a complete evolutionary scenario. Two other recent examples of DNA studies (e.g., molecular sexing and turnover assessment) represent new molecular tools to analyse the behaviour and ecology of raptors.

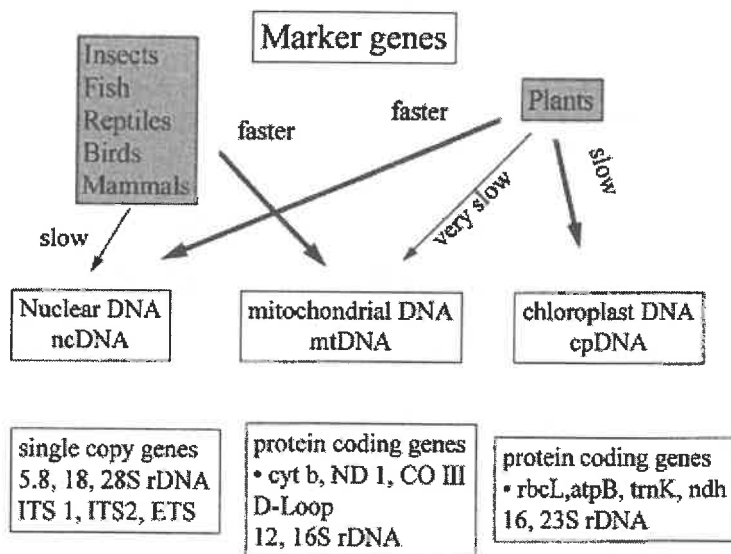
INTRODUCTION

Sequence variation in the nuclear and mitochondrial genome of animals (including birds) offers a unique chance to obtain a deeper knowledge of their evolutionary past, both in terms of space and time as DNA contains a blueprint of most evolutionary events. DNA studies have become a powerful tool in most areas of biology, in order to understand the phylogeny, systematics, and phylogeography of species, their ecology, population structure, breeding biology, paternity, and many other aspects (overviews in Avise 1994; Hoelzel 1992; Karp *et al.*, 1998; Hillis *et al.* 1996; Mindell, 1997; Nei 1996; Wink 1998).

The nuclear genome of vertebrates consists of approximately 3×10^9 nucleotides (in humans). Theoretically, this large size could be a base for over 3 million distinct genes. In reality however, the number of protein encoding genes ranges between 60000 and 100000 in humans and should be even lower in less developed animals. Thus, only 3% of the genome represent low or single copy genes (Fig. 1). The other 97% are rDNA repeats or non-coding introns, regulatory sequences, retroelements, transposons, degenerate copies and pseudogenes (remnants of past evolutionary events), tandemly repeated DNA sequences, such as telomeres, satellite DNA, minisatellites and microsatellites (Fig. 1). The mitochondrial DNA in animals and chloroplast DNA in plants have originated from bacterial endosymbionts which fused with early eukaryotic cells. Organelle DNA consists mainly of protein encoding and rRNA genes; introns and uncoding regions are rare (except for the D-loop in mitochondria) (Fig. 2).

cpDNA (Fig. 3). For a finer analysis of phylogeographic patterns, population structures, breeding systems and individual profiling in animal, more variable DNA sequences are needed. These can be found in the mitochondrial D-loop, in introns of protein encoding genes, and especially in mini- and microsatellites (Fig. 1). Whereas DNA sequencing is the major tool for phylogenetic studies, RFLP analysis, DNA-fingerprinting with single and multilocus probes, and microsatellite PCR are the methods of choice if a higher resolution is needed (overviews in Avise 1994; Hoelzel 1992; Karp *et al.*, 1998; Hillis *et al.* 1996; Wink & Wehrle, 1994; Wink 1998).

Figure 3 Schematic view of the use and evolutionary tempo of marker genes



This review summarizes several papers given at the 5th World Conference of birds of prey and owls with special focus on advances in molecular systematics and molecular ecology of raptors.

1. Molecular phylogeny and evolution of birds of prey and owls

Charles Darwin wrote to T.H. Huxley in 1857: "The time will come I believe, though I shall not live to see it, when we shall have fairly true genealogical trees of each kingdom of nature...". Sequencing of marker genes from mitochondrial and ncDNA has made fast progress in the last 10 years, after the development of polymerase chain reaction (PCR) which has revolutionized most areas of biology and medicine. Using sequences of marker genes, data on the molecular phylogeny and systematics of all groups of organisms can be generated and are published at a fast speed, so that Darwin's dream will probably become true within the next decades. DNA results will not replace studies in morphology, anatomy, behaviour or vocalizations, but will complement them. Sequence analysis of marker genes are helpful

- to elucidate the evolutionary past of most organisms
- to distinguish between convergent and phylogenetic traits
- to identify monophyletic groups which share common ancestry
- to understand speciation
- to elucidate phylogeographic patterns, migration and colonisation
- together with morphology, anatomy, behaviour, acoustics etc. to reconstruct the "true" phylogeny of a group of organisms
- to understand and maintain biodiversity

Of major importance is the selection of the appropriate marker gene for the relevant evolutionary time window. In order to study genetic relationships within families down to the species level, sequences of mitochondrial DNA (Quinn 1997; Moore & DeFilippis 1977) are very helpful, such as cytochrome b, 12S rRNA, ND2 and others (Fig. 2).

Nucleotide sequences of the mitochondrial cytochrome b gene have already been employed to study the systematics and evolution of diurnal raptors and owls (Avisé *et al.*, 1994; Griffiths 1997; Mindell 1997; from our laboratory: Seibold *et al.*, 1993, 1996; Heidrich & Wink, 1994, 1998; Heidrich *et al.*, 1995a,b, 1996; Helbig *et al.*, 1994; Wink 1995, 1998; Wink & Seibold, 1996; Wink *et al.*, 1996, 1998a,b; Wink & Heidrich, 1999; Seibold & Helbig, 1995, 1996).

In our studies, the mitochondrial cytochrome b gene was amplified and sequenced from approximately 35% of species and 50% of genera of diurnal raptors and 35% of species and 68% of genera of owls. Figures 4 and 5 provide a schematic overview on the various procedures between the starting point, a feather, tissue or blood sample and the final result as a phylogenetic tree. More details can be found in the following books and reviews: Avisé (1994); Hoelzel (1992); Karp *et al.*, (1998); Hillis *et al.* (1996); Wink & Wehrle, (1994); Sambrook *et al.* (1989), Swofford (1993), Kumar *et al.*, (1993) and Felsenstein (1993).

Figure 4. Schematic illustration of the steps from sample to DNA-sequence.

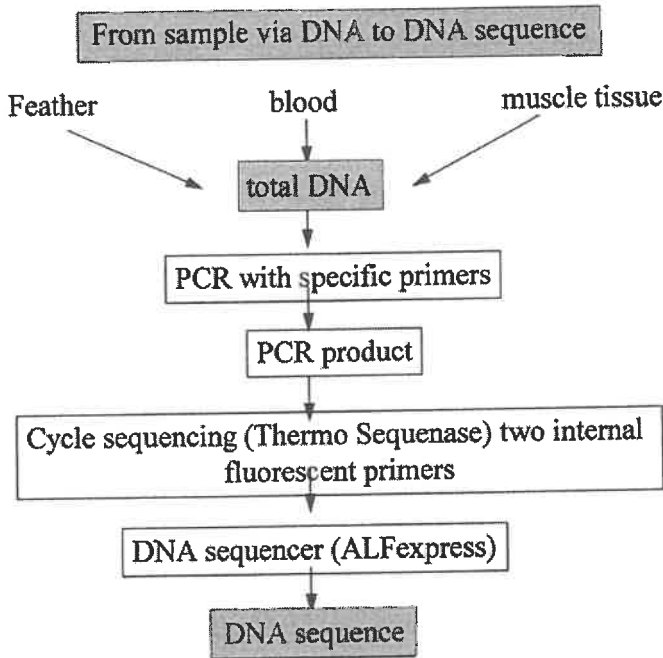
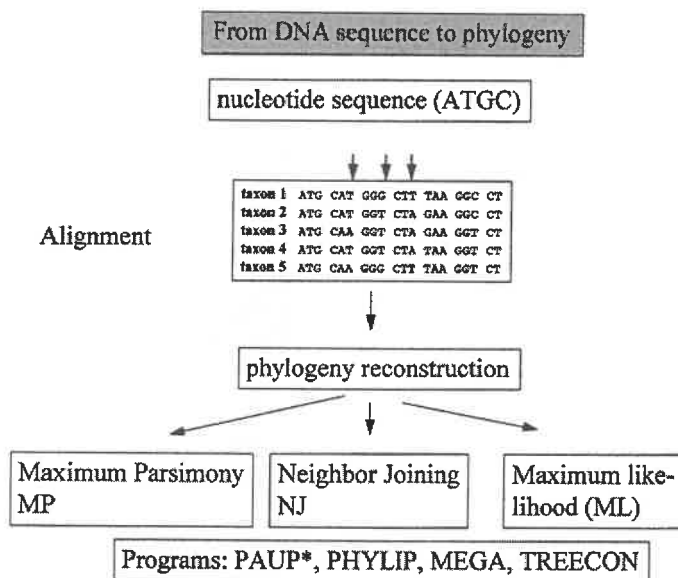


Fig. 6 represents a phylogenetic tree, reconstructed with Maximum Parsimony, for which we have analysed 1 to 3 taxa from each genus of diurnal and nocturnal raptors and members from a few other families. This tree should be regarded as a sort of "progress report" and not as a final result. A better resolution and higher reliability of the relationships between genera and families will be obtained when a complete data set is available, as long-branch attractions can be a major element of error (often found in incomplete data-sets) which could also distort some relationships between families in the tree shown in Fig. 6. Another reason, why we cannot resolve many of the deeper branches unambiguously is because of homoplasy due to multiple substitutions.

Figure 5: From DNA-sequence to phylogeny



A few phylogenetic relationships appear unequivocal already- at present. In most instances, the taxa which have been grouped in a common genus represent a monophyletic assemblage. In some instances we obtained good evidence for para- or polyphyletic groups, indicating that the systematics need a revision. Such examples are:

- *Aquila* and *Hieraeetus* form a paraphyletic clade; it would be plausible that *Hieraeetus* should be merged in *Aquila* (Wink and Sauer-Gürth, this volume).
- *Bubo*, *Ketupa*, *Nyctea*, and *Scotopelia* form a monophyletic assemblage; a common genus *Bubo* would be more appropriate for this complex (Wink & Heidrich, in press).
- Members of the genus *Otus* are polyphyletic and 3 clades can be distinguished. Here a split into the genera *Otus*, *Megascops* and *Ptilopsis* has been proposed (Wink & Heidrich, in press)

Members within defined terminal monophyletic groups should carry a common genus name. We have several instances, that monotypic genera have been created, although they derive from the same ancestor and form a closely related clade of birds. Examples are:

- Within Old world vultures, 3 lineages are evident, a) *Gypaetus*, *Neophron* and *Gypohierax*, b) *Gyps* and c) the *Aegyptius*-complex with *Aegyptius/Torgos/Trigonoceps*, and *Sarcogyps* as monotypic genera. Because vulture group c constitutes a monophyletic clade of species which share many morphological and behavioural characters, it would be plausible to place them in a single genus: The name *Aegyptius* has already been proposed for this assemblage (del Hoyo *et al.*, 1994; Mundy *et al.*, 1992).

The Black-shouldered kite, *Elanus caeruleus*, does not cluster within the Accipitridae, to which this taxon has been attributed (Fig. 6); *Elanus* falls between falcons and owls and seems to represent a unique group (family Elanidae?) of raptors. It would be interesting to find out whether *Elanoides*, *Gampsonyx*, and *Chelictinia* which share some characteristics with *Elanus* are true Accipitridae or more related to *Elanus*. The African Harrier-hawk *Polyboroides typus* shows many particular habits and morphological traits, which makes it difficult to classify this taxon correctly. *Polyboroides typus*, clusters at the base of the Accipitridae in the group of ancestral vultures of the *Gypaetus/Neophron/Gypohierax* group and honeybuzzards (Fig. 6).

do not share direct ancestry with Accipitridae and Pandionidae, indicating that the order Falconiformes or infraorder Falconides are apparently artificial units (Wink 1995; Wink *et al.*, 1998) which combine birds that share a common life style, especially behaviour and ecology.

For a more detailed discussion of phylogenetic relationships in diurnal and nocturnal raptors, see Wink & Ristow, Wink & Sauer-Gürth and Wink & Heidrich in this volume.

2. Molecular sexing of raptors

Falcons, hawks, and eagles show a high degree of sexual dimorphism, so that adult sexes are usually apparent. In vultures, however, a size or plumage dimorphism is not visible. But gender is important if ringing recoveries, recruitment rates or other ecological and behavioural data are to be analyzed. For reintroduction programs of Black vultures *Aegypius monachus*, Griffon vultures *Gyps fulvus* or Lappet-faced vultures *Torgos tracheliotus* knowledge of the sex of the birds is vital to create successful breeding pairs.

The gender of vultures can be determined macroscopically via endoscopy. But this procedure is stressful for the birds. Alternatively, sex chromosomes (female birds have ZW and males ZZ chromosomes) can be identified cytologically (Hatzofe & Getreide, 1990). If the size of the sex chromosomes differs, the DNA content of erythrocytes can be determined by flow cytometry (Nakamura *et al.* 1990).

A rapid and more flexible approach is molecular sexing (Ellegren and Sheldon 1997) by

- Hybridisation with oligonucleotide or W-specific DNA probes (Griffiths 1992, Griffiths & Holland 1990, Millar 1992, Millar *et al.* 1994, 1996, Rabenold *et al.* 1991)
- PCR-methods (Ellegren 1996, Ellegren & Sheldon 1997, Griffiths & Tiwari 1993, 1995; Griffiths *et al.*, 1992, 1996, Ogawa *et al.* 1997, Sabo *et al.* 1994). Whereas most methods are specific for a single species, the methods of Ellegren (1996), Griffiths *et al.*, (1996), and Ogawa *et al.* (1997) have a much wider application range.
- Instead of specific PCR primers RAPD have been applied that amplify part of the female W chromosome (Griffiths & Tiwari 1993).

Table 1. Molecular sexing of raptors by ISSR-PCR

<i>Species</i>	$(GACA)_4$	$(GACA)_4$ +RAPD	Kahn
Vultures			
<i>Gyps fulvus</i>	X	nd	X
<i>Aegypius monachus</i>	X	nd	X
<i>Torgos tracheliotus</i>	X	nd	X
<i>Neophron percnopterus</i>	no	no	X
Eagles			
<i>Aquila adalberti</i>	no	X	X
<i>Aquila nipalensis</i>	nd	nd	X
<i>Hieraaetus fasciatus</i>	X	nd	X
<i>Haliaeetus albicilla</i>	no	X	X
Falcons			
<i>Falco eleonorae</i>	no	no	X

X= method works successfully; no= method fails; n.d.= not determined; Kahn = Kahn *et al.*, (1998)

We have applied a new strategy using the single primer MW4 $(GACA)_4$ (Wink *et al.*, 1998). This method has been termed Inter Simple Sequence Repeat-PCR (ISSR-PCR). With $(GACA)_4$ several PCR products were obtained in DNA samples from *A. monachus*, *G. fulvus*, and *T. tracheliotus* of which one or more display a sex-specific pattern; i.e. they were only present in females but absent in males (Fig. 7,8). Specific PCR products of 450 or 550 nt length (marked with an arrow, Fig. 9), can be attributed to females only, but are absent in males. Since 65-75% of DNA in the W chromosome consists of repetitive sequences (Ellegren 1996, Ogawa *et al.* 1997, Rabenold *et al.* 1991), we assume

that the distinctive PCR products derive from DNA regions between microsatellite DNA. The advantage of this protocol is that PCR can be performed at higher, more stringent annealing temperatures as compared to RAPD primers of 10 nt length which reduces artifacts.

Figure 7. Schematic illustration of a PCR for molecular sexing with a single primer (ISSR-PCR).

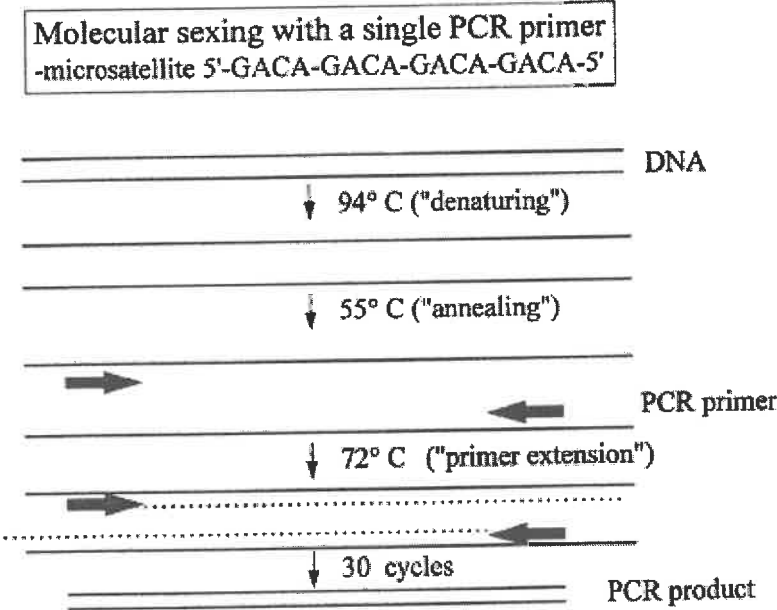
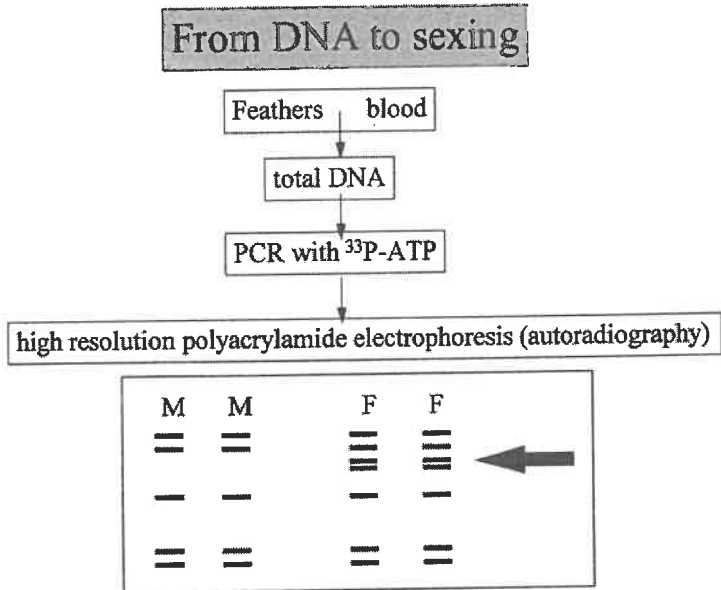


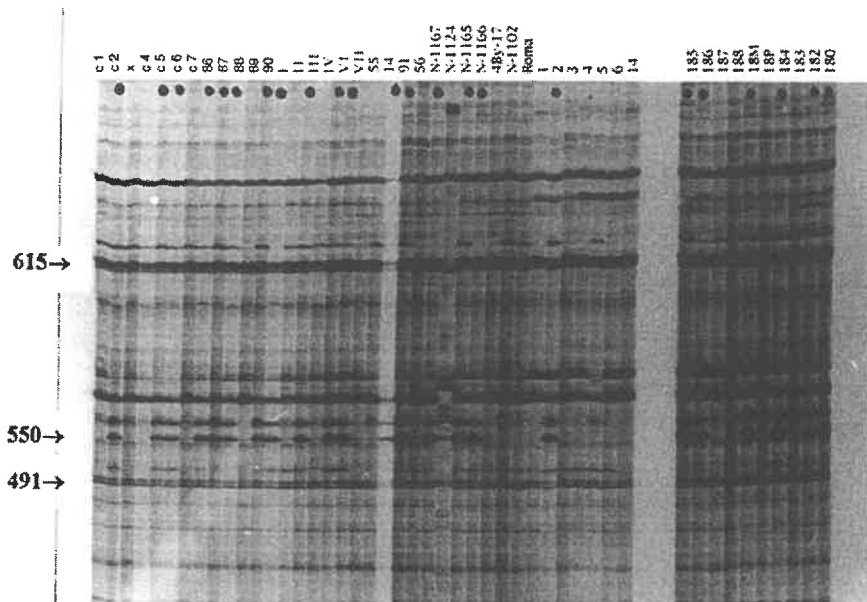
Figure 8: From DNA to molecular sexing



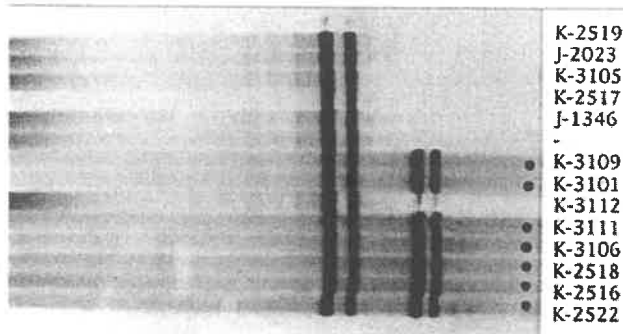
We have applied the (GACA)₄ PCR to a number of other species: It was successful with *Crex crex* (Wink, 1998), but failed with eagles (*Aquila adalberti*), sea eagles (*Haliaeetus albicilla*), terns, and bustards (*Chlamydotis undulata*). If the use of (GACA)₄ PCR fails then this primer can be used in combination with a RAPD primer according to the protocol outlined in (Matioli & de Brito 1995) (Table 1). Recently, Kahn *et al.* (1998) have published new PCR primers for molecular sex determination in birds. Preliminary analysis in our laboratory showed that these primers work in those species which could not be analyzed by our (GACA)₄-PCR (Table 1).

Figure 9. Examples of molecular sexing of raptors

A. Autoradiography of PCR products from (GACA)₄ PCR by high resolution gel electrophoresis. PCR products of *Gyps fulvus* with a size of 550 nucleotides are diagnostic for females (marked by arrow). Females of which the gender was established, are marked with a black dot.



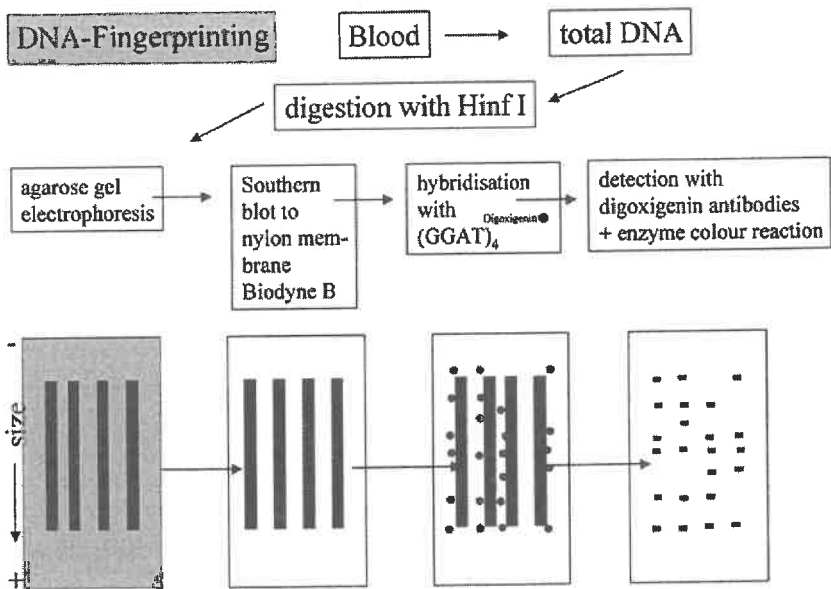
B. Autoradiography of PCR products from *Neophron percnopterus* using PCR primers for chromosome-specific intron size differences in the avian CHD gene (Kahn *et al.*, 1998). Females (marked with a dot) can be recognized by a second doublet of bands.



3. Determination of adult turnover and survival with DNA-Fingerprinting

Estimates for juvenile and adult survival are important population parameters in animals. Traditionally, survival data are obtained by marking juvenile and adult birds with tags, rings, or transmitters and to recover, trap, or monitor them during consecutive months and years. Reliable survival estimates are available for only a limited number of raptor species (Newton 1979) although knowledge of this parameter becomes increasingly important for conservation as many raptors are now rare or endangered. We have chosen multilocus DNA (Wetton *et al.*, 1987; Burke *et al.*, 1991, Hoelzel 1992) as a method for the determination of adult survival without the need to trap or mark the adult falcons (Wink *et al.*, 1999). As a first example, we have used blood samples from the Saker Falcon *Falco cherrug* from Kazakhstan. As there is a high demand for this falcon in falconry, the future of the species is uncertain, and survival estimates are of prime importance in assessing likely trends in its populations.

Figure 10. Schematic illustration of the steps involved in DNA fingerprinting using multilocus probes



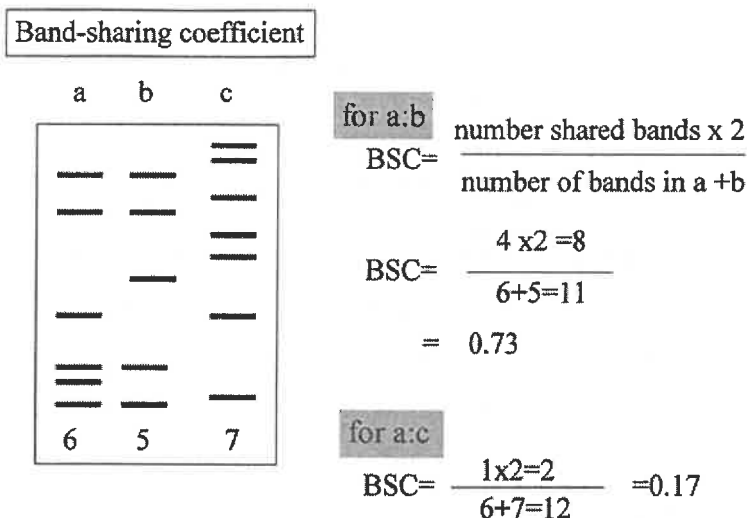
Our approach is based on the following assumptions: Many long-lived birds, especially raptors, show a high degree of site and mate tenacity and breed together in the same territory for consecutive years; this also applies to the Saker Falcon. Our strategy was to carry out multilocus fingerprinting (Fig. 10) of nestlings from the same territory in consecutive years as an indirect approach to estimate a minimal survival rate for adult Sakers.

- Nestlings of an individual brood are full sibs (band-sharing coefficients (BSC)= 0.5 to 0.8); accordingly, band-sharing coefficients (Fig. 11) between full sibs do not differ between years, but should differ significantly from those between unrelated birds (BSC=0.19)
- If the breeding adults are identical in a territory in consecutive years, the band-sharing coefficients of corresponding nestlings should not vary significantly between broods since all young are full sibs.
- If both partners have changed, then band-sharing coefficients between broods should be as low as between unrelated individuals (BSC=0.19) and differ significantly from the BSC value between broods (Fig. 11)

- In cases that one adult bird has been replaced, band-sharing coefficients between broods of the same territory should be significantly lower than those of full sibs but higher than those between unrelated birds.

We have analyzed Saker broods of 16 territories between 1993 and 1997 and obtained a minimal estimate of 82% for adult survival. A value of 82% is in the range found in other falcons: For *F. eleonora* annual survival and turnover is 87% (Ristow *et al.*, 1991); for *F. columbarius* up to 70% (Oliphant *et al.*, 1993), for *F. peregrinus* 78-90% (Mearns & Newton, 1984; Court *et al.*, 1989), and for *F. mexicanus* 78-81% (Runde 1987).

Figure 11. Example of a DNA profiling of related (a,b) and unrelated birds (c) and the calculation of the corresponding band-sharing coefficients (BSC).



This new approach using DNA fingerprinting has a number of advantages and disadvantages:

Pros:

- no need to catch adult birds
- minimal survival rates can be obtained in a minimum of 2 years
- method probably works in other species with a high site tenacity and mate fidelity

Cons:

- data are reliable in case that no or a complete change took place
- exchange of one adult can lead to ambiguous results
- high "extra-pair paternity", high divorce, emigration, etc., increase the apparant mortality rate

However, the other methods used to determine turnover and survival, such as ringing, recapture and telemetry also provide only minimal estimates only and have their intrinsic shortcomings. We conclude that DNA fingerprinting provides a new and interesting alternative (Wink *et al.*, 1999; Kenward *et al.*, 1999).

CONCLUSIONS

In this overview I have shortly summarized our ongoing efforts to study the evolution and phylogeny of diurnal and nocturnal birds of prey. DNA sequencing of marker genes is undoubtedly

the method of choice for this question. Considering the large number of species concerned, our data base, although quite large at present, is still fragmentary and much more work (e.g., more taxa, additional genes) is needed to obtain a complete picture. The other two examples (e.g., molecular sexing and turnover assessment) are new molecular tools to study the behaviour and ecology of raptors, which will not necessarily replace the traditional methods but rather complement them.

ACKNOWLEDGEMENTS

I would like to thank my present and former coworkers H. Sauer-Gürth, H. Staudter, I. Seibold, P. Heidrich, and my collaborators R. Kenward, O. Hatzofe, G. Blanco, C. König for their help and support and many colleagues world-wide for supplying blood, tissue and feather samples of diurnal and nocturnal raptors. The Deutsche Forschungsgemeinschaft and The Land Baden-Württemberg supported parts of the study.

REFERENCES

- AVISE, J.C., W.S. NELSON & C.G. SIBLEY 1994. DNA sequences support for a close phylogenetic relationship between some storks and New World vultures. *Proc. Natl. Acad. Sci. USA*, 91, 5173-5177.
- AVISE, J.C. 1994. *Molecular markers, natural history and evolution*. Chapman and Hall, London.
- BURKE, T., O. HANOTTE, M.W. BRUFORD & E. CAIRNS 1991. Multilocus and single locus minisatellite analysis in population biological studies. In: Burke, T., Jeffreys, A.J., & Wolff, R. (eds): *DNA fingerprinting approaches and applications*: 154-168. Basel.
- COURT, G.S., D.M. BRADLEY, C.C. GALES & D.A. BOAG 1989. Turnover and recruitment in a tundra population of Peregrine falcon (*Falco peregrinus*). *Ibis* 131: 487-496.
- DEL HOYO, J., A. ELLIOTT & J. SARGATAL 1994. *Handbook of the birds of the world*. Vol. 2, Lynx edition, Barcelona.
- ELLEGREN, H. 1996. First gene on the avian W chromosome (CHD) provides a tag for universal sexing of non-ratite birds. *Proceedings of the Royal Society of London Series B Biological Sciences* 263, 1635-1641.
- ELLEGREN H. & B.C. SHELDON 1997. New tools for sex identification and the study of sex allocation in birds. *Trends Ecology & Evolution* 12, 255-259.
- FELSENSTEIN, J. 1993. PHYLIP, version 3.5c, Department of Genetics, Univ. Washington, Seattle.
- GRIFFITHS, R., S. DAAN & C. DIJKSTRA 1996. Sex identification in birds using two CHD genes. *Proceedings of the Royal Society London B* 263, 1251-1256.
- GRIFFITHS, R. & P.W.H. HOLLAND 1990. A novel avian W chromosome DNA repeat sequence in the lesser black-backed gull (*Larus fuscus*). *Chromosoma* 99, 243-250.
- GRIFFITHS, R., B. TIWARI & S.A. BECHER 1992. The identification of sex in the starling *Sturnus vulgaris* using a molecular DNA technique. *Molecular Ecology* 1, 191-194.
- GRIFFITHS, R. & B. TIWARI 1995. Sex of the last wild Spix's macaw. *Nature* 375, 454.
- GRIFFITHS, R. 1992. Sex -biased mortality in the Lesser Black-Backed Gull. *Ibis* 134, 237-244.
- GRIFFITHS, T. & B. TIWARI 1993. The isolation of molecular genetic markers for the identification of sex. *Proceedings National Academy of Sciences, USA* 90, 8324-8326.
- GRIFFITHS, C.S. 1997. Correlation of functional domains and rates of nucleotide substitution in cytochrome b. *Mol. Phylog. Evol.* 7, 352-365.
- HATZOFE, O. & S. GETREIDE 1990. Sex determination in vultures and other mono-morphic raptors. *Torgos* 8: 27-29.
- HEIDRICH, P. 1998. Untersuchungen zur molekularen Phylogenie ausgewählter Vogelgruppen anhand von DNA-Sequenzen des mitochondrialen Cytochrom b-Gens. PhD thesis, Universität Heidelberg.
- HEIDRICH, P. & M. WINK 1994. Tawny owl (*Strix aluco*) and Hume's tawny owl (*Strix butleri*) are distinct species, Evidence from nucleotide sequences of the cytochrome b gene. *Z. Naturforsch.*, 49c, 230-234.
- HEIDRICH, P. & M. WINK 1998. Phylogenetic relationships in holarctic owls (Order Strigiformes): Evidence from nucleotide sequences of the mitochondrial cytochrome b gene. In *Holarctic Birds of Prey*. (R.D. Chancellor, B.-U. Meyburg, J.J. Ferrero, eds), ADENEX & WWGBP; pp. 73-87.
- HEIDRICH, P., C. KÖNIG & M. WINK 1995a. Molecular phylogeny of the South American Screech Owls of the *Otus atricapillus* complex (Aves, Strigidae) inferred from nucleotide sequences of the mitochondrial cytochrome b gene. *Z. Naturforsch.* 50c: 294-302.
- HEIDRICH, P., C. KÖNIG & M. WINK 1995b. Bioakustik, Taxonomie und molekulare Systematik amerikanischer Sperlingskäuze (Strigidae: Glaucidium spp.). *Stuttgarter Beiträge zur Naturkunde A*, 534, 1-47.

- HELBIG, A.J., I. SEIBOLD, W. BEDNAREK, H. BRÜNING, P. GAUCHER, D. RISTOW, W. SCHARLAU, D. SCHMIDL & M. WINK 1994. Phylogenetic relationships among falcon species (genus *Falco*) according to DNA sequence variation of the cytochrome b gene. in *Raptor conservation today* (B.-U. Meyburg & R.D. Chancellor eds.), 593-599.
- HILLIS, D.M., C. MORITZ & B.K. MABLE 1996. *Molecular systematics*. 2nd ed., Sinauer Assoc. Sunderland.
- HOELZEL, A.R. 1992. *Molecular genetic analysis of populations*. IRL-Press, Oxford.
- KAHN, N.W., ST. JOHN & T.W. QUINN 1998. Chromosome-specific intron size differences in the avian CHD gene provide a simple and efficient method for sex determination in birds. *Auk* 115, 1074-1078.
- KARP, A., P.G. ISAAC & D.S. INGRAM 1998. *Molecular tools for screening biodiversity*. Chapman & Hall, London.
- KENWARD, R., M. WINK, V. MARCSTROM, S.S. WALLS, M. KARLBOM, R.H. PFEFFER, E.A. BRAGIN, K.H. HODDER & A. LEVIN. Rapid modelling of sustainable use for raptors with radio-tags and DNA fingerprints. (Ecology Letters submitted)
- KUMAR, S., K. TAMURA & M. NEI. *MEGA-Molecular Evolutionary Genetics Analysis*. Version 1.0. Pennsylvania State University.
- MATIOLI, S.R. & R.A. DE BRITO 1995. Obtaining genetic markers by using double-stringency PCR with microsatellites and arbitrary markers. *BioTechniques* 19, 752-755.
- MEARNS, D. & I. NEWTON 1994. Turnover and dispersal in a Peregrine falcon (*Falco peregrinus*) population. *Ibis* 126: 347-355.
- MILLAR, C.D., I. ANTHONY, D.L. LAMBERT, P.M. STAPLETON, C.C. BERGMANN, R.A. BELLAMY & E.C. YOUNG 1994. Patterns of reproductive success determined by DNA fingerprinting in a communally breeding oceanic bird. *Biological Journal of the Linnean Society* 52, 31-48.
- MILLAR, C.D., D.M. LAMBERT, S. ANDERSON & J.L. HALVERSON 1996. Molecular sexing of the communally breeding pukeko: an important ecological tool. *Molecular Ecology* 5, 289-293.
- MILLAR, C.D. 1992. Sex-specific restriction fragments and sex ratios revealed by DNA fingerprinting in the Brown Skua. *Journal of Heredity* 83, 350-355.
- MINDELL, D.P. (ed.) 1997. *Avian molecular evolution and systematics*. Academic Press San Diego.
- MOORE, W.S. & V.R. DEFILIPPIS 1997. The window of taxonomic resolution for phylogenies based on mitochondrial cytochrome b. In: Mindell, D.P. (ed.) *Avian molecular evolution and systematics*. Academic Press San Diego, pp. 83-119.
- MUNDY, P.J., D. BUTCHARD, J. LEDGER & S. PIPER 1992. *The vultures of Africa*. Academic Press, London.
- NAKAMURA, D., T.R. TIERSCH, M. DOUGLASS & R.W. CHANDLER 1990. Rapid identification of sex in birds by flow cytometry. *Cytogenetics Cell Genetics* 53, 201-205.
- NEI, M. 1996. Phylogenetic analysis in molecular evolutionary genetics. *Annu. Rev. Genet.* 30, 371-403.
- NEWTON, I. 1979. *Population ecology of raptors*. Berkhamstead.
- OGAWA, A., I. SOLOVEI, N. HUTCHISON, Y. SAITOH, J.E. IKEDA, H. MACGREGOR & S. MIZUNO 1997. Molecular characterization and cytological mapping of a non-repetitive DNA sequence region from the w chromosome of chicken and its use as a universal probe for sexing Carinatae birds. *Chromosome Research* 5, 93-101.
- OLIPHANT, L.W., I.G. WARKENTIN, N.S. SODHI & P.C. JAMES 1993. Ecology of urban Merlins in Saskatoon. In: *Biology and conservation of small falcons* (M.K. Nicholls & R. Clarke, eds), pp 42-44, Hawk & Owl Trust, London.
- QUINN, T.W. 1997. Molecular evolution of the mitochondrial genome. In: Mindell, D.P. (ed.) *Avian molecular evolution and systematics*. Academic Press San Diego, pp. 3-28.
- RABENOLD, P.P., W.H. PIPER, M.D. DECKER & D.J. MINCHELLA 1993. Polymorphic minisatellite amplified on avian W chromosome. *Genome* 34, 489-493.
- 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*: 199-212. Basel.
- RUNDE, D.E. 1987. Population dynamics, habitat use and movement patterns of the Prairie falcon (*Falco mexicanus*). PhD thesis, University of Wyoming.
- SABO, T.J., R. KESSELI, J.L. HALVERSON, I.C.T. NISBET & J.J. HATCH 1987. PCR-based method for sexing roseate terns (*Sterna dougallii*). *Auk* 111, 1023-1027.
- SAMBROOK, J., E.F. FRITSCH & T. MANIATIS 1989. *Molecular Cloning. A Laboratory Manual*. 2nd ed. CSHL, Cold Spring Harbour, New York.
- SEIBOLD, I. 1994. Untersuchungen zur molekularen Phylogenie der Greifvögel anhand von DNA-Sequenzen des mitochondrialen Cytochrom b-Gens. Dissertation University Heidelberg, Hartung-Gorre Verlag, Konstanz.

- SEIBOLD, I., A. HELBIG, B.-U. MEYBURG, J. NEGRO & M. WINK 1996.** Genetic differentiation and molecular phylogeny of European *Aquila* eagles according to cytochrome b nucleotide sequences. pp. 1-15, *In: Eagle studies*. B.-U. Meyburg & R. Chancellor (eds). WWGBP, Berlin, London & Paris.
- SEIBOLD, I., A. HELBIG, & M. WINK 1993.** Molecular systematics of falcons (family Falconidae). *Naturwissenschaften* 80, 87-90.
- SEIBOLD, I., A. HELBIG, B.-U. MEYBURG, J.J. NEGRO & M. WINK 1995.** Genetic differentiation and molecular phylogeny of European *Aquila* eagles (Aves: Falconiformes) according to cytochrome b nucleotide sequences. *In: Eagle Studies* (B. Meyburg & R. Chancellor, eds.). WWGBP, Berlin, London & Paris.
- SEIBOLD, I. & A.J. HELBIG 1995.** Evolutionary history of New and Old World vultures inferred from nucleotide sequences of the mitochondrial cytochrome b gene. *Phil. Transact. Roy. Soc. London Series B*, 350, 163-178.
- SEIBOLD, I. & A.J. HELBIG 1996.** Phylogenetic relationships of the sea eagles (genus *Haliaeetus*): Reconstructions based on morphology, allozymes and mitochondrial DNA sequences. *J. Zool. Syst. Evol. Res.* 34, 103-112.
- SIBLEY, C.G. & B.L. MONROE 1990.** *Distribution and taxonomy of birds of the world*. Yale University Press, New Haven.
- SWOFFORD, D.L. 1993.** PAUP-Phylogenetic analysis using parsimony. Version PAUP.
- WETTON, J.H., R.E. CARTER, D.T. PARKIN & D. WALTERS 1987.** Demographic study of a wild house sparrow population by DNA fingerprinting. *Nature* 327: 147-149.
- WINK, M. 1995.** Phylogeny of Old and New World vultures (Aves: Accipitridae and Cathartidae) inferred from nucleotide sequences of the mitochondrial cytochrome b gene. *Z. Naturforsch.* 50c, 868-882.
- WINK, M. 1998.** Application of DNA-markers to study the ecology and evolution of raptors. *In: Holarctic birds of prey*. (R.D. Chancellor, B.-U. Meyburg & J.J. Ferrero, eds), pp. 49-72, ADENEX-WWGBP.
- WINK, M., P. HEIDRICH & C. FENTZLOFF 1996.** A mtDNA phylogeny of sea eagles (genus *Haliaeetus*) based on nucleotide sequences of the cytochrome b gene. *Biochemical Systematics and Ecology* 24, 783-791.
- WINK, M., & P. HEIDRICH 2000.** Molecular evolution and systematics of owls (Strigiformes). *In: C. König, F. Weick, & J.H. Becking (eds) Owls of the world*. Pica Press.
- WINK, M., H. SAUER-GÜRTH, F. MARTINEZ, G. DOVAL, G. BLANCO & O. HATZOFE 1998.** Use of GACA-PCR for molecular sexing of Old World vultures (Aves: Accipitridae). *Molecular Ecology* 7, 779-782.
- WINK, M. & H. SAUER-GÜRTH (this volume).** Advances in the molecular systematics of African raptors.
- WINK, M. & I. SEIBOLD 1994.** Molecular phylogeny of Mediterranean raptors (Families Accipitridae and Falconidae). *In: Biology and Conservation of Mediterranean raptors*, 1994. (Muntaner, J. & J. Mayol, eds), SEO/BirdLife, Madrid 1996, Monografia 4: 335-344.
- WINK, M., I. SEIBOLD, F. LOTFIKHAH & W. BEDNAREK 1998.** Molecular systematics of holarctic raptors (Order Falconiformes). *In Holarctic Birds of Prey*. (R.D. Chancellor, B.-U. Meyburg, J.J. Ferrero, eds), ADENEX & WWGBP; pp. 29-48.
- WINK, M., H. STAUDTER, Y. BRAGIN, R. PFEFFER & R. KENWARD (in press).** The use of DNA fingerprinting to determine annual survival rates in Saker falcons (*Falco cherrug*). *Journal Ornithologie* (1999 in press).
- WINK, M. & H. WEHRLE (eds.) 1994.** *PCR im medizinischen und biologischen Labor*. GIT-Verlag, Darmstadt.

Prof. Dr. Michael Wink
 Universität Heidelberg
 Institut für Pharmazeutische Biologie
 INF 364, D-69120 Heidelberg
 Germany