

Phylogenetic Differentiation of the Osprey *Pandion haliaetus* inferred from nucleotide sequences of the mitochondrial cytochrome b gene

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ABSTRACT

The mitochondrial cytochrome b gene of three of the four subspecies of the Osprey (*P. h. haliaetus*, *P. h. carolinensis*, and *P. h. cristatus*) was amplified by PCR and sequenced. Ospreys cluster as a monophyletic group within the Falconiformes and show a clear and unequivocal phylogeographic pattern according to the recognised subspecies. Genetic distances between the subspecies are between 1.9 and 3.8%, i.e. in the range of "good" species. Together with morphological characters and distribution pattern the genetic data indicate that the three osprey subspecies appear to represent distinct species.

INTRODUCTION

The Osprey *Pandion haliaetus* is a unique raptor that feeds entirely on fish and has a world-wide distribution. It has been placed in a monotypic family, the Pandionidae, within the Falconiformes (Sibley & Monroe 1990) and has been subdivided at present into four geographically defined subspecies:

- nominate *P. h. haliaetus* (Palearctic, incl. Cape Verde Islands; migratory),
- *P. h. carolinensis* (North America; migratory),
- *P. h. ridgwayi* (Caribbean subregion; non-migratory),
- *P. h. cristatus* (Australia, from Sulawesi and Java to New Caledonia; non-migratory)

When Ospreys were first described between 1822 (Vieillot 1822) and 1837 (Gould 1837), three species were recognised that were placed in the genus *Pandion*. Lumping started in 1874 (Sharpe 1874) and ended with Knowlton & Ridgway (1909) (summary Preyost 1983). In the following decades disputes mainly concerned the recognition of additional subspecies.

Ospreys vary in size, both geographically and by sex. Usually females are larger than males, about 20% larger in body mass, and 5-10% larger in wing length. Some overlap is possible, but this is limited; in North America, for example, nearly all males weigh less than 1600 grams, while nearly all females weigh more. Yet at a distance such differences are obscured and it is often difficult to distinguish between the sexes in the field, even though females tend to have darker breast plumage.

The overall habits and behaviour of the four subspecies are very similar, although a few characters have been described that differ (del Hoyo *et al.* 1994). The subspecies show only slight differences, mostly in plumage and size, with *haliaetus* and *carolinensis* the largest and darkest, and *ridgwayi* essentially a pale form of *carolinensis*. The Australian *cristatus* is a small osprey with dark breast band and a relatively pale crown (del Hoyo *et al.* 1994).

Table 1 summarises a few plumage characters and Table 2 differences in wing length as an indicator of size (Weick 1980; Prevost 1983; del Hoyo *et al.* 1994). The ospreys of the northern hemisphere appear to share a number of characters and differ from the Australasian subspecies *P. h. cristatus*, which is the smallest of the four subspecies.

Table 1. Morphological characters of the *Pandion* taxa

Sample sizes are in brackets (after Prevost 1983).

	Male		Female	
	Breast ¹ Average	Crown ² average	Breast average	Crown average
Palearctic	2.7 (72)	2.5 (32)	3.4 (65)	2.7 (41)
Cape Verde	2.2 (6)	2.5 (6)	2.3 (4)	2.5 (4)
Red Sea	1.6 (5)	2.0 (5)	1.7 (3)	1.7 (3)
North America	1.4 (49)	2.6 (49)	2.4 (47)	2.8 (46)
Bahaman	1.0 (4)	1.0 (3)	1.0 (3)	1.0 (3)
Australasian	2.9 (42)	1.5 (42)	3.7 (50)	1.3 (50)

¹ The breast markings were scored from 1 to 4: 1=the breast almost white; 2=a band of partially brown feathers < 3 cm wide; 3=a band of partially brown feathers > 3 cm wide; 4=a wide band of entirely brown feathers

² Crown markings were scored from 1 to 3: 1=less than 10% black feathers; 2= from 10 to 50% black feathers; 3=over 50% black feathers

Another character is larger underwing coverts. All Palearctic adult ospreys, and only these, have the large underwing coverts (one half rufous-brown, one half white). Although North American ospreys have underwings similar to those of Palearctic ospreys, their larger underwing coverts are barred dark brown. In Bahamian ospreys these coverts are nearly white, whereas in Australasian birds they are nearly all ashy brown (Prevost 1983).

In this communication we report on a molecular phylogeny and phylogeography of ospreys based on nucleotide sequences of the mitochondrial *b* gene covering three of the subspecies. Experimental evidence is presented that the three taxa represent genetic lineages isolated for probably more than 1 million years.

Table 2. Wing lengths (mm) of the *Pandion* taxa (after Weick 1980)

	<i>females</i>	<i>males</i>
<i>P. h. haliaetus</i>	470-510	450-510
<i>P. h. carolinensis</i>	488-518	462-506
<i>P. h. ridgwayi</i>	455-495	433-483
<i>P. h. cristatus</i>	425-490	426-431

MATERIAL AND METHODS

We have isolated total DNA from feather, blood or tissue samples (see Wink 2000) which had been kindly supplied by several colleagues (B. Bed'Hom, J. Haapala, D. Ristow, J. Penhallurick, J.-C. Thibault, W. E. Boles, R. Schodde, O. Hadzofe). The cytochrome b gene was amplified by PCR (primer sequences in Wink & Sauer-Gürth 2000) and sequenced by using AlfExpress (Amersham Pharmacia Biotech) or ABI 3100 (Applied Biosystems) instruments. Sequences were aligned manually and analysed with the software packages PAUP* (Swofford, 2002) and MEGA2 (Kumar *et al.* 2001) (see Wink 2000; Wink & Sauer-Gürth 2000; Wink *et al.* 2002; Broders *et al.* 2003 for further details).

RESULTS AND DISCUSSION

Ospreys form a well-supported monophyletic clade (Fig. 1) (Wink & Sauer-Gürth 2000) and cluster within the basal groups of the Accipitridae; in other reconstructions *Pandion* takes a basal place outside the Accipitridae (which would support its status as a monotypic family). Several taxa of kites that had not been included into previous phylogenetic studies (Wink 2000; Wink & Sauer-Gürth 2000) take an even more basal position (Fig. 1) than hitherto seen for *Pandion*. However, longer mitochondrial sequences and nuclear sequences are needed to identify the position of *Pandion* unequivocally.

Table 3. Informative characters in the *Pandion* data set

Taxon/Node	11111111
	1122223344445555666667778888999990000000
	992424499292468239128995694699144890012457
	369756917799759274845049223448829306810831
<i>P.h. haliaetus</i> FL	GGGAATCCCGCGTCGATCGTGTGCACCACTTCGCTTAGTACC
<i>P.h. haliaetus</i> IsA...A.....
<i>P.h. haliaetus</i> Co
<i>P.h. haliaetus</i> PoA.....
<i>P.h. haliaetus</i> F20425
<i>P.h. haliaetus</i> F20429
<i>P.h. haliaetus</i> F20430
<i>P.h. haliaetus</i> F9263
<i>P.h. carolinensis</i> USA 4252	A...GCATTAAACGAG.TA..C.T...GT....CC.A.GTA
<i>P.h. carolinensis</i> USA 4253	AA..GCATTAAACGAG.TA.ACAT....T...A.CC.A.CTA
<i>P.h. carolinensis</i> USA 4255	AA..GCATTAAACGAG.TA..C.T...GT...AACC.A.GTA
<i>P.h. carolinensis</i> USA 4254	A...GCATTAAACGAG.TA.ACAT....T...AACC.A.GTA
<i>P.h. cristatus</i> AUS 7011	.AAG....TA..C.AGCTAC.C..G.T...CCT..CC.....
<i>P.h. cristatus</i> AUS 21009	.AAG....TA..C.AGCTAC.C..GTT..CCT..CCG.....
<i>P.h. cristatus</i> AUS 21008	.AAG....TA..C.AGCTAC.C..G.T...CCT..CCG.....

Within the ospreys a clearcut phylogeographic pattern was found, irrespective of the methods or outgroups used for phylogeny reconstructions (MP, ML, NJ) (Fig. 2). The Australasian *P. h. cristatus* usually takes a basal position, whereas *P. h. carolinensis* and *P. h. haliaetus* cluster as sister taxa in MP reconstructions (this would agree with morphological characters). No phylogeographic pattern was found between different European populations of the osprey. Table 3 illustrates the comparably large number of phylogenetically informative characters in the *Pandion* data set.

Figure 1. A molecular phylogeny of ospreys based on almost complete nucleotide sequences of the cytochrome b gene

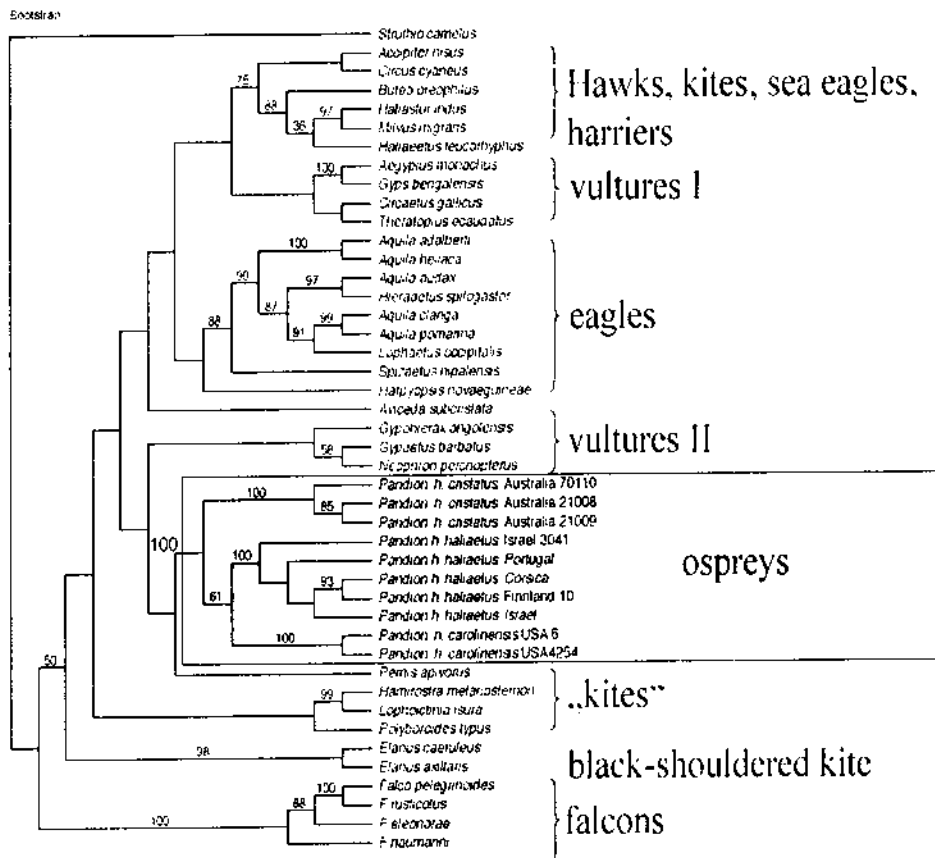


Table 4 summarises the genetic distances between the three osprey taxa. P-distances are between 2 and 4%; assuming a molecular clock of 2% = 1 million years of divergence (Tarr *et al.*, 1993; Wilson *et al.* 1987), the taxa have diverged 1 to 2 million years ago.

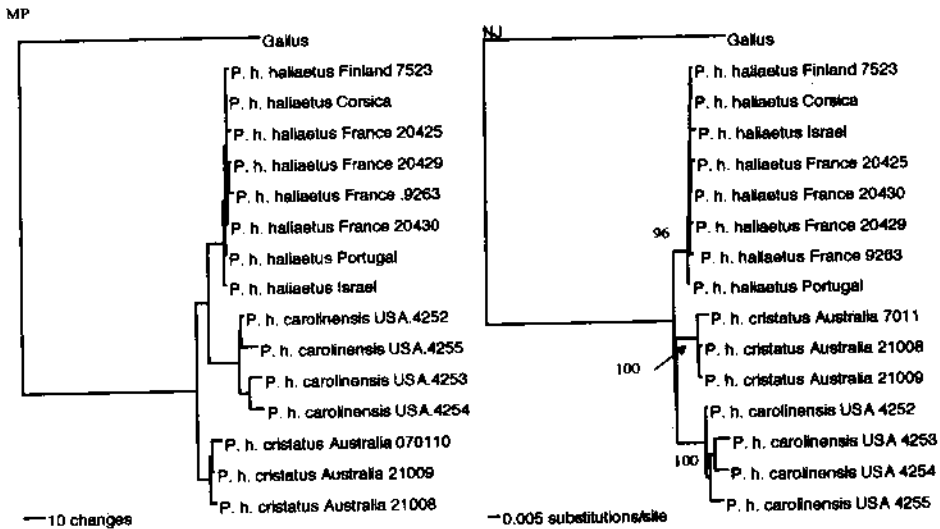
Table 4. Genetic distances between *Pandion* taxa

	1*	2	3**
1. <i>P. h. haliaetus</i>	-	2.1-3.5	1.9-2.6
2. <i>P. h. carolinensis</i>	-	2.9-3.8	
3. <i>P. h. cristatus</i>	-		

*distances within European ospreys: 0.1-0.5%; **within Australian birds: 0.3-0.8%

Figure 2. Phylogenetic differentiation within *Pandion haliaetus*

MP= Maximum parsimony; NJ= Neighbour Joining (Kimura2-distance algorithm)
Numbers at branches are bootstrap values in % (from 1000 replications)



Genetic distances between closely related sister species of some eagles (*A. clanga*/*A. pomarina*; *A. heliaca*/*A. adalberti*; *H. fasciatus*/*H. spilogaster*) are in the range of 1.7 to 2.1% (Table 5). Since the distances within ospreys clearly exceed this threshold, we suggest that the *Pandion* taxa represent "good" species although the morphological differences are small. They are also very small in case of the sister species mentioned in Table 5. Thus the old concept of the early 19th century (Vieillot 1822; Gould 1837), of defining at least three distinct osprey species, might not have been so wrong at all.

Table 5. Distance between closely related sister pairs of eagles (in % sequence divergence)

	1	2	3	4	5	6
1 <i>Aquila clanga</i>	-	1.7	7.1	7.5	7.2	6.7
2 <i>Aquila pomarina</i>		-	7.3	7.7	7.1	6.6
3 <i>Aquila heliaca</i>			-	1.8	7.4	6.6
4 <i>Aquila adalberti</i>				-	7.8	7.2
5 <i>Hieraaetus fasciatus</i>					-	2.1
6 <i>Hieraaetus spilogaster</i>						-

CONCLUSIONS

Genetic distances between osprey taxa are in a range that is typical for distinct raptor species. Morphological and genetic differences exist and support the suggestion, that the geographically defined subspecies may be recognised as distinct species:

- *P. haliaetus*
- *P. carolinensis*
- *P. ridgwayi*
- *P. cristatus*

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