Evolution of Mitochondrial Malate Dehydrogenase in Birds (II)*

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Abstract This is the second paper on our comparative study of electrophoretic mobility of mitochondrial malate dehydrogenase (M-MDH) in the pectoral muscle or red cells of 100 species of birds belonging to 34 families. Falconidae and Accipitridae showed the same mobility value of 140, while Cathartidae showed 100, which may suggest their terrestrial origin. In Alcidae two groups of the mobility value of 190 (*Uria*, *Cepphus*, *Brachyramphus* and *Synthliboramphus*) and 100 (*Ptychoramphus*, *Cyclorhynchus*, *Aethia* and puffins) were distinctively recognized.

In our previous paper (Kuroda *et al.* 1982), we suggested the possibility that the values of electrophoretic mobilities of mitochondrial malate dehydrogenase (M-MDH) reflected the higher categories of avian phylogeny. The present electrophoretic study of avian M-MDH involving 15 orders, 34 families, 84 genera, 100 species and 245 individuals confirmed and extended our earlier findings.

Materials and Methods

Animals. Birds were collected from December 1981 to July 1982 from the following localities.

Birds collected in North Pacific Ocean: *Fulmarus glacialis*, *Puffinus griseus*, *Puffinus tenuirostris*, *Uria lomvia*, *Synthliboramphus antiquus*, *Ptychoramphus aleuticus*, *Cyclorhynchus psittacula*, *Aethia cristatella*, *Fratercula corniculata* and *Lunda cirrhata* (H. Ogi). Birds collected in Hokkaido: *Oceanodroma leucorhoa*, *Cepphus carbo*, *Cerorhincha monocerata* (Akkeshi, July), *Brachyramphus marmoratus* and *Sterna hirundo* (Koshimizu, August) (R. Kakizawa); *Mergus serrator*, *Bonasa bonasia* (Koshimizu), *Uria aalge*, *Aethia pusilla* (Shari), *Histrionicus histrionicus* and *Clangula hyemalis* (Rausu) (M. Sekine & H. Nakagawa); *Porzana fusca* and *Pyrrhula pyrrhula* (Hakodate).

Birds from Hahajima, Ogasawara Islands: *Apalopteron familiare* (H. Morioka), from South Iwo Island: *Pterodroma hypoleuca* and *Bulweria bulwerii* (Y. Tsukamoto, South Iwo Island Expedition of Environment Agency, June, 1982), from Iriomote Island: *Amaurornis phoenicurus*, *Chalcophaps indica*, *Treron formosa*, *Motacilla cinerea*, *Pericrocotus divaricatus* and *Hypsipetes amaurotis* (O. Takahashi & M. Shoyama) and from East Ongul Island, Antarctica: *Pagodroma nivea* (Y. Naito).

Some other birds presented: *Anser fabalis* (Niigata Pref.), *Actitis hypoleucos* (Saitama Pref.), *Anas americana*, *Anas fulcata*, *Heteroscelus brevipes* and *Alauda arvensis* (Chiba Pref.), *Limosa limosa*, *Stercorarius parasiticus*, *Sterna albifrons* and *Bombycilla*

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japonica (Kanagawa Pref.). Blood samples were collected from birds kept in Zoos: *Vultur gryphus* and *Bubo bubo* (Nogeyama Zoo, Yokohama); *Cairina moschata* and *Aythya fuligula* (Tama Zoo, Tokyo); *Cygnus atratus*, *Cygnus melanocoryphus*, *Anser anser*, *Anser caerulescens*, *Branta leucopsis*, *Chlorophaga picta* and *Plectropterus gambiensis* (Inokashira Zoo, Tokyo). Other bird species given in Table 1 were obtained from dealers.

Electrophoresis. Samples of hemolysates or exudates from the frozen pectoral muscle were subjected to starch-gel electrophoresis at pH 7.0. The procedures used for electrophoresis and the detection of M-MDH mobilities were reported in the previous paper (Kuroda *et al.* 1982).

Results and Discussion

The relative values of M-MDH mobilities are shown in Table 1.

Procellariiformes. Previously only two species of Procellariidae had been tested. Further 5 genera, 5 species (and 1 duplicate species*) were tested, with 1 species of Hydrobatidae. All these species showed the M-MDH mobility of 100.

Podicipedidae. Only one species had been tested previously, which showed the mobility of 100. This value for this family was confirmed by an additional species, *Podiceps cristatus*.

Ardeidae. Previously 10 species of 7 genera all showed the M-MDH mobility of

Table 1. Relative electrophoretic mobility of M-MDH in pectoral muscle (*) or red cells of various avian species.

	Relative mobility		Relative mobility
Procellariidae		<i>Uria aalge</i> (1)*	190
<i>Fulmarus glacialis</i> (3)*	100	<i>Cepphus carbo</i> (5)*	190
<i>Pagodroma nivea</i> (1)*	100	<i>Brachyramphus marmoratus</i> (5)*	190
<i>Pterodroma hypoleuca</i> (3)*	100	<i>Synthliboramphus antiquus</i> (5)*	190
<i>Bulweria bulwerii</i> (10)*	100	<i>Ptychoramphus aleuticus</i> (1)*	100
<i>Puffinus griseus</i> (6)*	100	<i>Cyclorhynchus psittacula</i> (2)*	100
<i>Puffinus tenuirostris</i> (4)*	100	<i>Aethia cristatella</i> (6)*	100
Hydrobatidae		<i>Aethia pusilla</i> (3)*	100
<i>Oceanodroma leucorhoa</i> (5)*	100	<i>Cerorhinca monocerata</i> (5)*	100
Podicipedidae		<i>Fratercula corniculata</i> (6)*	100
<i>Podiceps cristatus</i> (1)*	100	<i>Lunda cirrhata</i> (6)*	100
Ardeidae		Columbidae	
<i>Ixobrychus eurhythmus</i> (1)*	150	<i>Streptopelia risoria</i> (1)	140
Cathartidae		<i>Streptopelia tranquebarica</i> (1)	140
<i>Vultur gryphus</i> (2)	100	<i>Macropygia phasianella</i> (1)	140
Accipitridae		<i>Chalcophaps indica</i> (5)*	140
<i>Circus aeruginosus</i> (1)*	140	<i>Geopelia striata</i> (1)	190
<i>Accipiter nisus</i> (1)*	140	<i>Zenaidura macroura</i> (1)	140
<i>Buteo lineatus</i> (1)	140	<i>Columbina cruziana</i> (2)	140
<i>Spizaetus nipalensis</i> (1)*	140	<i>Gallinula chloropus</i> (1)*	220

Table 1. (continued)

	Relative mobility		Relative mobility
Falconidae		<i>Gallicolumba xanthonura</i> (1)	140
<i>Falco tinnunculus</i> (1)*	140	<i>Treron formosae</i> (2)*	140
<i>Falco subbuteo</i> (1)*	140	<i>Ducula aenea</i> (1)*	140
<i>Falco rusticolus</i> (1)*	140	<i>Ducula bicolor</i> (2)	140
Anatidae		Psittacidae	
<i>Cygnus atratus</i> (5)	100	<i>Neophema bourkii</i> (1)	360
<i>Cygnus melanocoryphus</i> (5)	100	<i>Psittacus erithacus</i> (2)	360
<i>Anser fabalis</i> (1)*	100	<i>Agapornis fischeri</i> (2)	320
<i>Anser anser</i> (2)	100	Cuculidae	
<i>Anser caerulescens</i> (3)	100	<i>Cuculus fugax</i> (1)*	200
<i>Branta leucopsis</i> (3)	100	Strigidae	
<i>Chloephaga picta</i> (3)	100	<i>Bubo bubo</i> (2)	200
<i>Plectropterus gambiensis</i> (2)	100	<i>Ketupa ketupu</i> (1)	
<i>Cairina moschata</i> (5)	100	<i>Strix aluco</i> (1)*	200
<i>Anas americana</i> (2)	100	Bucerotidae	
<i>Anas falcata</i> (2)	100	<i>Buceros bicornis</i> (1)*	200
<i>Aythya baeri</i> (1)*	100	Ramphastidae	
<i>Aythya fuligula</i> (14)	100	<i>Ramphastos toco</i> (1)*	250
<i>Histrionicus histrionicus</i> (1)*	100	Eurylaimidae	
<i>Clangula hyemalis</i> (1)*	100	<i>Calyptomena viridis</i> (1)	360
<i>Mergus serrator</i> (1)*	100	Alaudidae	
Phasianidae		<i>Alauda arvensis</i> (1)*	360
<i>Meleagris gallopavo</i> (3)	100	Motacillidae	
<i>Bonasa bonasia</i> (1)*	100	<i>Motacilla cinerea</i> (1)*	360
<i>Alectoris chukar</i> (4)	100	Campephagidae	
<i>Excalfactoria chinensis</i> (2)	100	<i>Pericrocotus divaricatus</i> (1)*	360
<i>Bambusicora thoracica</i> (1)	100	Pycnonotidae	
<i>Lophura nycthemera</i> (3)	100	<i>Hypsipetes amaurotis</i> (2) (2)*	340
<i>Chrysolophus pictus</i> (3)	100	Irenidae	
<i>Chrysolophus amherstiae</i> (4)	100	<i>Chloropsis cochinchinensis</i> (6)*	360
<i>Pavo cristatus</i> (4)	100	Bombycillidae	
<i>Pavo muticus</i> (1)	100	<i>Bombycilla japonica</i> (1)	360
Rallidae		Muscicapidae	
<i>Porzana fusca</i> (1)*	100	<i>Saxicola torquata</i> (1)*	360
<i>Amaurornis phoenicurus</i> (1)*	100	<i>Garrulax canorus</i> (2)	360
Charadriidae		Paridae	
<i>Vanellus indicus</i> (2)*	100	<i>Parus major</i> (1)*	360
Scolopacidae		Meliphagidae	
<i>Limosa limosa</i> (1)	200	<i>Apaloeteron familiare</i> (1)*	360
<i>Actitis hypoleucos</i> (1)*	250	Fringillidae	
<i>Heteroscelus brevipes</i> (12)	250	<i>Pyrrhula pyrrhula</i> (1)*	360
Stercorariidae		<i>Coccothraustes migratorius</i> (1)	360
<i>Stercorarius parasiticus</i> (1)*	100	Sturnidae	
Laridae		<i>Scissirostrum dubium</i> (1)	360
<i>Sterna hirundo</i> (2)*	100	Corvidae	
<i>Sterna albifrons</i> (1)	100	<i>Pyrrhocorax pyrrhocorax</i> (1)*	360
Alcidae			
<i>Uria lomvia</i> (4)*	190		

The numbers in parenthesis indicate the number of individuals examined.

150 and an additional species (duplicate genus*) tested gave the same value.

Falconiformes. So far 6 species of 6 genera of Accipitridae all had showed the mobility value of 140. This was confirmed by 4 additional species (1 new and 3 duplicate genera). Three species of *Falco* tested in the present study also had the mobility value of 140. This is of interest because it suggests that Falconidae and Accipitridae may be more closely related than are generally considered. The Cathartidae, on the other hand, may be a distinct and probably a primitive group, since two individuals of *Vultur gryphus* showed the M-MDH mobility of 100, which we think is the basic or primitive value, shown by terrestrial and aquatic bird groups. Therefore, Cathartidae is considered to be of terrestrial origin from which other Falconiform families may have evolved, and some fossil species, such as *Neocathartes*, could be said to suggest the remote connection with Ciconiiformes. The Accipitridae, at a high position in Fig. 4 of our previous paper, should be shifted to a lower position next to Ciconiiformes and arranged through Cathartidae (100) to Accipitridae (140) and Falconidae (140). Such relationships of Falconiformes with aquatic bird groups are implicit in the recent phylogenetic discussions on the biochemical (Sibley 1982) or ecop-hysiological (cf. Feduccia 1980) basis.

Anatidae. All the 16 additional species (5 new and 6 duplicate genera) showed the mobility value of 100, as in 29 species tested earlier.

Phasianidae. All the 10 additional species (7 new and 1 duplicate genera) showed the mobility value of 100, as in 17 species tested earlier.

Rallidae. Two additional genera and species showed the mobility value of 100 as in one species tested earlier.

Charadriidae. One species of *Charadrius* earlier showed the value of 100 and this was confirmed by one species of *Vanellus*.

Scolopacidae. In the previous data, *Tringa*, *Xenus*, *Arenaria*, *Phalaropus* and *Calidris* showed the M-MDH mobility of 250, with the exception of 100 in *Numenius phaeopus*. This time, we added *Actitis hypoleucos* and *Heteroscelus brevipes* (12 birds) to the mobility value of 250, while *Limosa limosa* showed the value of 200. *Numenius* and *Limosa* are large-bodied genera with distinct foraging habits and adaptive bill shape. It is of interest to conjecture that their low M-MDH mobility values might reflect some ecophysiological correlations or older (primitive) phylogenetical origin.

Stercorariidae. This is a newly tested family, represented by *Stercorarius parasiticus*, which showed the mobility value of 100.

Laridae. So far, 2 genera 6 species had showed the mobility of 100. One new genus and 2 species *Sterna albifrons* and *S. hirundo* were added to this list.

Alcidae. So far, one species *Synthliboramphus antiquus* had been tested, showing the mobility value of 190. This value was confirmed with 5 individuals of the same species, as well as with large bodied *Uria lomvia* and *U. aalge*, middle-sized *Cephus carbo* and small-sized *Brachyramphus marmoratus*. The other small to middle-sized species, *Ptychoramphus*, *Cyclorhynchus*, *Aethia* (*crisatella* and *pusilla*), *Cerorhinca*, *Fratercula* (*corniculata*) and *Lunda*, all showed the mobility of 100. This clear grouping

* Duplicate genus or species means the genus or species used both in previous and present studies.

well fit the Ogilvie-Grant's (1898) basic classification: Alcinae—Feathering on the lores extending at least to the posterior border of the nasal opening which is exposed or overhung by feathers, and Fraterculinae—Feathering on the lores not extending to the posterior border of the nasal opening, which is overhung by a horny scale. This grouping is also supported osteologically; Alcinae having a long sternum, with short clavicles, and Fraterculinae a shorter sternum, with developed clavicles and with posterior plate (Kuroda 1954). The only exception is *Cepphus* which was confirmed to be Alcinae species by M-MDH. Osteologically *Cepphus* resembles to Fraterculinae type (Kuroda 1955). This may be attributed to its adaptational deviation from Alcinae group in its food habits and breeding activity.

Columbidae. So far, 4 genera and species had showed the mobility value of 140, with the exception of 190 in *Geopelia cuneata*. In the new tests, *Geopelia striata* showed the mobility of 190 while *Gallicolumba luzonica* had 220 and 10 other species (6 new and 2 duplicate genera), including *Gallicolumba xanthonura*, had 140.

Psittacidae. The very high M-MDH mobility of 300–360 in parrots have been discussed in the previous paper. Three species (*Psittacus* and duplicate genera *Neophema* and *Agapornis*) were added in the present study.

Cuculidae and *Strigidae*. In Cuculidae, *Cuculus fugax* was added to two other congeneric species so far tested, all showing the mobility of 200. In Strigidae, 3 species *Ketupa ketupu*, *Bubo bubo* and *Strix aluco* (duplicate genus) was added, showing the value of 200 as in the other species already examined (*Otus* showing only 130).

Bucerotidae. This is a new family to be tested. *Buceros bicornis* showed the mobility value of 200. However, related Alcedinidae marked 220 and 250, and Coraciidae 220.

Ramphastidae. Previously *Ramphastos sulfuratus* showed the mobility value of 230, while the newly tested congeneric species *R. toco* marked 250.

Passeriformes. All the Passeres species previously tested had showed the value of 360 which was confirmed by 13 families (4 duplicate families), but only in Pycnonotidae (*Hypsipetes amaurotis*) the value of 340 was obtained.

In conclusion, some of the results confirmed our previous view with similar data of additional species, but in some groups, such as Falconiformes, Scolopacidae, Alcidae, Bucerotidae and Ramphastidae, new data proved to be useful for deducing phylogenetic history or to elucidate adaptive radiation among taxa.

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