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Authors

Patton, JC
Avise, JC

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Evolutionary genetics of birds IV
Rates of protein divergence in waterfowl (Anatidae)

J. C. Patton & J. C. Avise
Department of Molecular and Population Genetics, University of Georgia, Athens, GA 30602, USA

Abstract

An electrophoretic comparison of proteins in 26 species of waterfowl (Anatidae), representing two major subfamilies and six subfamilial tribes, led to the following major conclusions: (1) the genetic data, analyzed phenetically and cladistically, generally support traditional concepts of evolutionary relationships, although some areas of disagreement are apparent; (2) species and genera within Anatidae exhibit smaller genetic distances at protein-coding loci than do most non-avian vertebrates of equivalent taxonomic rank; (3) the conservative pattern of protein differentiation in Anatidae parallels patterns previously reported in Passeriforme birds. If previous taxonomic assignments and ages of anatid fossils are reliable, it would appear that the conservative levels of protein divergence among living species may not be due to recent age of the family, but rather to a several-fold deceleration in rate of protein evolution relative to non-avian vertebrates.

Since it now appears quite possible that homologous proteins can evolve at different rates in different phylads, molecular-based conclusions about absolute divergence times for species with a poor fossil record should remain appropriately reserved. However, the recognition and study of the phenomenon of apparent heterogeneity in rates of protein divergence across phylads may eventually enhance our understanding of molecular and organismal evolution.

Introduction

Species and genera within several families of passeriform (perching) birds exhibit far smaller genetic distances at protein-coding loci than do most non-avian vertebrates of equivalent taxonomic rank (Avise *et al.*, 1980a, b, c; Barrowclough & Corbin, 1978; Corbin *et al.*, 1974; Martin & Selander, 1975; Smith & Zimmerman, 1976). In each of several passeriform families that have been extensively studied with multi-locus electrophoretic techniques, levels of genetic distance between congeneric species approximate those between conspecific populations of many fishes, mammals, and other non-avian vertebrates, and genetic distances between confamilial avian genera are typically lower than or equal to distances among very closely related species elsewhere (Avise & Aquadro, 1982). Comparable data for non-passerine birds are limited (Barrowclough *et al.*, 1981; Gutierrez *et al.*, 1983).

Several hypotheses (not mutually-exclusive) can be advanced to account for this conservative pattern of protein divergence in Aves: birds could be taxonomically 'oversplit' relative to other groups; hybridization and introgression between species could decelerate their differentiation; avian taxa could be younger than most other vertebrate taxa; avian proteins could be evolving more slowly, on the average, than those of other vertebrates. These hypotheses are difficult to test critically, in part because the fossil record for most passeriform groups is very poor. Avise *et al.* (1980c) tentatively conclude that protein evolution is decelerated in the wood warblers (Parulidae). This conclusion de-

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pends heavily upon the validity of estimated divergence times which were based primarily on zoogeographic considerations (Mengel, 1964).

In this report we describe and evaluate levels of protein divergence in a large assemblage of non-passeriform birds: the waterfowl (Anseriformes; Anatidae). We are primarily interested in two questions: (1) Is the conservative pattern of protein evolution in Passeriformes also exhibited by these non-Passeriforme birds?; (2) What are the rates of protein divergence in waterfowl relative to rates in other birds and in non-avian vertebrates? An ancillary goal is to refine estimates of the genetic and systematic relationships of a large number of species and genera of waterfowl. The basic rationale for choice of waterfowl, described in detail below, is that the family has been the subject of intense systematic study, and by 'bird-standards' is comparatively well-represented in the fossil record.

We have examined electrophoretic variation in proteins encoded by 17–19 loci in a total of 206 specimens of 26 species of ducks, geese, and swans. These samples represent several major lines of divergence in waterfowl, including two subfamilies and six distinct tribes.

The Anatidae

The well-delimited, presumably monophyletic family Anatidae contains approximately 150 living species of ducks, geese, and swans distributed worldwide. Sibley and Ahlquist (1972) thoroughly review the history of waterfowl classification. The family is generally divided into at least two major subfamilies, the Anserinae (tree ducks, geese, and swans) and Anatinae (most other ducks), and a variable number of subfamilial tribes. Important recent contributors to waterfowl taxonomy have been Delacour and Johnsgard, whose classifica-

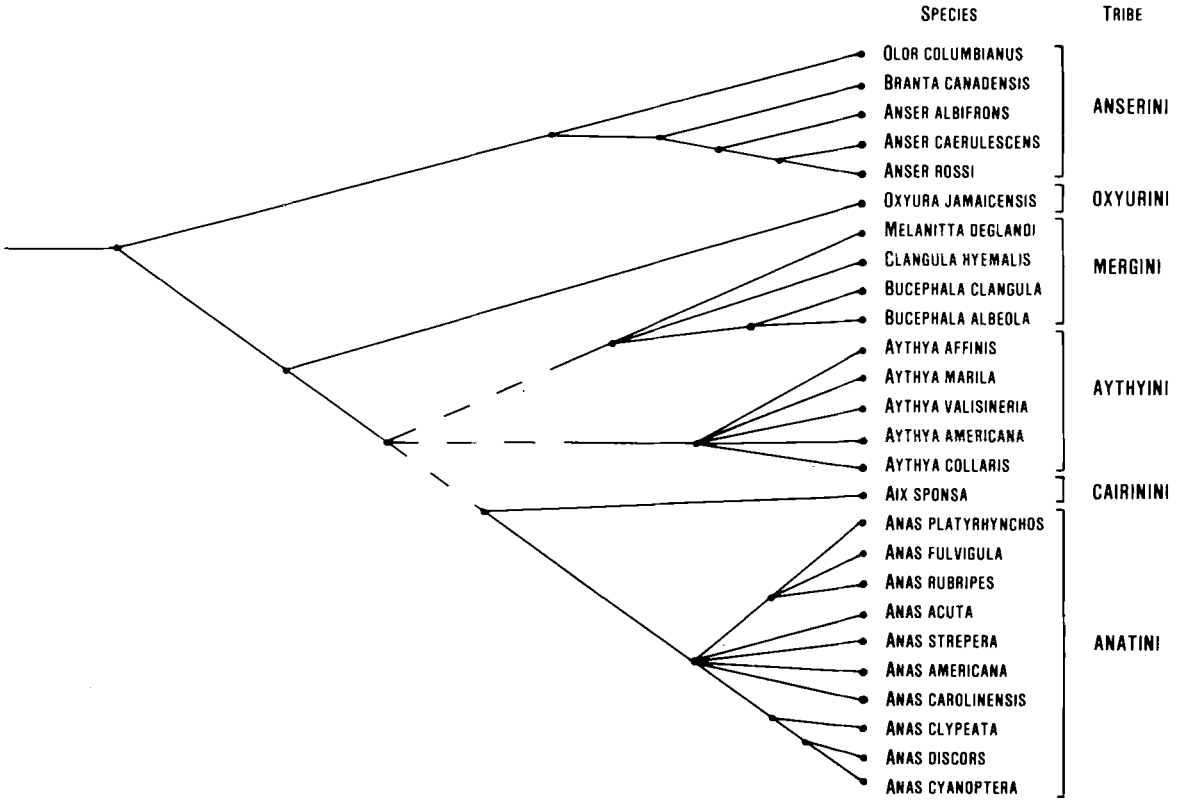

Fig. 1. 'Model' phylogeny of waterfowl based on behavioral and morphological considerations as discussed by Delacour and Mayr (1945) and Johnsgard (1968).

tions (reviewed in Delacour & Mayr, 1945; Delacour, 1954; Johnsgard, 1968; Bellrose, 1976) will form the basis for our present discussion.

For those 26 species examined by us, Delacour (1954) and Johnsgard (1968) are in fairly close agreement on taxonomic (and evolutionary) relationships. From their discussions and figures, we have distilled a summary phylogeny (Fig. 1) which constitutes a 'model' against which to evaluate the results of various methods of genetic data analysis. The higher classification underlying the model phylogeny (see also Morony *et al.*, 1975) is as follows:

Subfamily Anserinae
  Tribe Anserini - *Olor, Branta, Anser*
Subfamily Anatinae
  Tribe Anatini - *Anas*
  Tribe Aythyini - *Aythya*
  Tribe Cairinini - *Aix*
  Tribe Mergini - *Bucephala, Melanitta, Clangula*
  Tribe Oxyurini - *Oxyura*

Detailed relationships among species and genera, based on considerations of morphology and behavior, are discussed by Delacour and Mayr (1945).

A total of about 165 anseriform species are known from fossils, and the majority of these are no longer extant (Howard, 1964). An early but disputed (Olson & Feduccia, 1980) anseriform fossil (*Eonessa*) dates to the middle Eocene (Wetmore, 1938). By the Oligocene, representatives of both Anserinae and Anatinae (including the large extant genus *Anas*) were reportedly present. Additional living genera (including *Anser, Branta* and *Aythya*) date from the Miocene (Brodkorb, 1964; Howard, 1964; Romer, 1966). Thus several major evolutionary lines of waterfowl are probably *at least* as old as the mid-Tertiary (Olson & Feduccia, 1980). Several Pliocene remains appear indistinguishable from present-day species (Howard, 1964).

Using the fossil record as a rough time-scale, the basal split between Anserinae and Anatinae (Fig. 1) might be conservatively dated at about 25 million years ago, and many genera within the subfamilies

Table 1. Heterozygosity values determined by direct counts of proportions of individuals heterozygous per locus, averaged across the 17 – 19 assayed loci for twenty-six species of waterfowl.

Species	Common name	Sample size	$\bar{H}$
(1) *Olor columbianus*	Whistling Swan	2	0
(2) *Branta canadensis*	Canada Goose	8	0.050
(3) *Anser albifrons*	White-fronted Goose	11	0.014
(4) *Anser caerulenscens*	Snow and Blue Goose	14	0.025
(5) *Anser rossi*	Ross' Goose	4	0.038
(6) *Anas platyrhynchos*	Mallard	10	0.037
(7) *Anas fulvigula*	Mottled Duck	1	0
(8) *Anas rubripes*	Black Duck	4	0.028
(9) *Anas acuta*	Pintail	16	0.046
(10) *Anas strepera*	Gadwall	6	0.008
(11) *Anas discors*	Blue-winged Teal	1	0.056
(12) *Anas cyanoptera*	Cinnamon Teal	6	0.025
(13) *Anas carolinensis*[1]	Green-winged Teal	6	0.025
(14) *Anas americana*	Amerian Wigeon	16	0.016
(15) *Anas clypeata*	Northern Shoveler	17	0.028
(16) *Aix sponsa*	Wood Duck	7	0.045
(17) *Aythya americana*	Redhead	1	0.050
(18) *Aythya valisineria*	Canvasback	7	0.083
(19) *Aythya collaris*	Ring-necked Duck	3	0.018
(20) *Aythya marila*	Greater Scaup	15	0.028
(21) *Aythya affinis*	Lesser Scaup	23	0.048
(22) *Bucephala clangula*	Goldeneye	1	0.050
(23) *Bucephala albeola*	Bufflehead	5	0.032
(24) *Clangula hyemalis*	Oldsquaw	2	0.050
(25) *Melanitta deglandi*	Surf Scoter	7	0.015
(26) *Oxyura jamaicensis*	Ruddy Duck	14	0.030

[1] *crecca* in the most recent AOU checklist.

Table 2. Electromorphs (and their frequencies) observed for twenty-six species of Anatidae. Species are numbered as in Table 1. All samples appeared identically monomorphic at LDH-2, GOT-2, CK-3, IDH-2, PEPT-1, and IPO-1.

Protein	Species												
	O.c. (1)	*B.c.* (2)	*A.a.* (3)	*A.c.* (4)	*A.r.* (5)	*An.p.* (6)	*An.f.* (7)	*An.r.* (8)	*An.ac.* (9)	*An.st.* (10)	*An.d.* (11)	*An.cy.* (12)	*An.ca.* (13)
1) LDH-1	100	100	100	100	100	100	100	100	100	100	95	95	100
2) MDH-1	100	100 (0.95) 70 (0.05)	100	100	100	100	100	100	100	100	100	100	100
3) MDH-2	– 100	– 100	– 100	– 100	– 100	– 100	– 100	– 100	– 100	100 (0.03) – 100 (0.97)	– 100	– 100	– 100
4) IDH-1	125	120 (0.42) 90 (0.58)	120 (0.17) 90 (0.83)	120 (0.27) 90 (0.73)	120 (0.38) 90 (0.62)	200 (0.44) 100 (0.56)	100	200 (0.50) 100 (0.50)	200 (0.28) 160 (0.03) 100 (0.66) 50 (0.03)	200 (0.08) 100 (0.92)	200 (0.50) 100 (0.50)	200 (0.30) 100 (0.70)	200 (0.30) 100 (0.70)
5) 6PGD	200	200	200 (0.95) 100 (0.05)	200	200	100	100	100	130 (0.04) 110 (0.08) 100 (0.88)	100	100	110 (0.17) 100 (0.75) 10 (0.08)	100
6) αGPD	60	75	75	75 (0.96) 30 (0.04)	75	100	100	100	100	100 (0.84) 80 (0.08) 40 (0.08)	100	100	100
7) PGM	50	50	50	50	50	100	100	100	100 (0.97) 50 (0.03)	100	100	100	100
8) PGI	100	100	50	50	50	200 (0.20) 100 (0.80)	100	100	200 (0.03) 100 (0.97)	100	100	100	100
9) CK-2	-	100	140	140	140	100	100	100	100	100	100	100	100
10) GOT-1	80	130 (0.06) 100 (0.94)	100	100	100	100	100	100	100 (0.94) – 50 (0.06)	100	100	100	100
11) PEPT-2	-	100	100	100	100	100	100	100	100	100	100	100	100
12) HB	– 100	– 100	– 100	– 100	– 100	– 100	– 100	– 100	– 100	– 100	– 100	– 90	– 100
13) ALB	105	95 (0.06)	105	105	105	100	100	100	98	97	102	102	100

Table 2. Continued.

	Species													Buffer
	An.am. (14)	*An.cl.* (15)	*Ai.s.* (16)	*At.am.* (17)	*At.v.* (18)	*At.c.* (19)	*At.m.* (20)	*At.af.* (21)	*Bu.c.* (22)	*Bu.a.* (23)	*C.h.* (24)	*M.d.* (25)	*O.j.* (26)	
1)	100	95	100	100	180 (0.43) 100 (0.57)	100	100	100	100	100	100	100	100	a, b
2)	100	100 (0.95) 90 (0.03)	100	100	100	100	100	100	100	100	100	100	100	a
3)	– 100	– 100	– 100	– 100	– 100	– 100	– 100	– 100	– 100	– 100	– 100	– 100	– 100	a
4)	200 (0.12) 100 (0.88)	200 (0.44) 100 (0.56)	200 (0.63) 100 (0.24) 90 (0.13)	200	200 (0.64) 100 (0.36)	200 (0.17) 100 (0.83)	200 (0.77) 100 (0.23)	200 (0.39) 100 (0.61)	180 (0.50) 100 (0.50)	180 (0.50) 100 (0.50)	200 (0.25) 100 (0.75)	100	200 (0.11) 100 (0.89)	a
5)	100	100	100	120 (0.50) 100 (0.50)	100	100	100	100	100	120 (0.75) 100 (0.25)	100	100	100	a
6)	100 (0.96) 45 (0.04)	100	120 (0.07) 100 (0.93)	–	100	100	100	125 (0.02) 100 (0.85) 70 (0.13)	100	100 (0.70) 70 (0.30)	75	72	130 (0.96) 85 (0.04)	a, b
7)	100	100	50	50	50	50	50 (0.98) 10 (0.02)	50	50	50	50	50	140 (0.04) 120 (0.96)	a
8)	100	100	100	100	100	100	100	210 (0.02) 180 (0.02) 100 (0.96)	100	100	100	50	90 (0.05) 45 (0.95)	b
9)	100	100	100	100	100	100	100	100	100	100	100	100	200	a
10)	100	100	100	100	100	100	100	100	100	100	100	100	300 (0.93) 200 (0.07)	a
11)	100	100	100	100	100 (0.86) 95 (0.14)	100	100 (0.97) 95 (0.03)	100	100	100	100	100	100	b
12)	– 100	– 100	– 100	– 100	– 100	– 100	– 100	– 100	– 100	– 100	– 120	– 140	– 100	a
13)	93	101	104 (0.86) 101.5 (0.14)	103	103	103	103	103	103	103	110	106	107	b, c

[a] T.C. 6.7/6.3; [b] Poulik; [c] LiOH – Selander *et al.*, 1971.

are at least 15–20 million years old. As we will show, even with these conservative estimates of evolutionary age, protein differentiation in waterfowl appears to have proceeded at a very slow pace.

Materials and methods

Specimens were obtained through the cooperation of several state fish and game agencies and local chapters of Ducks Unlimited, and by personal collecting. Freshly-killed specimens, or those which had died on refuges from natural causes, were frozen and shipped to the laboratory for processing. Sample sizes of the species examined are listed in Table 1.

Extracts from heart, pectoral muscle, and liver were analyzed separately by horizontal starch-gel electrophoresis according to routine procedures described by Selander *et al.* (1971), Ayala *et al.* (1972), and Avise *et al.* (1980a). The proteins assayed in this study, listed by the standard abbreviations employed in the above references, are given in Table 2. All samples were compared on a side-by-side basis, and scoring was accomplished by designating mobilities relative to the common electromorph of the Mallard, which was arbitrarily labeled '100' (anodal migration) or '-100' (cathodal migration).

Mean genetic distances across loci were estimated by Nei's (1972) and Roger's (1972) formulas. Nei's $\bar{D}$ values were used to generate a phenogram by the Unweighted Pair-Group Method Analysis (UPGMA; Sneath & Sokal, 1973). Roger's $\bar{D}$ values were employed to develop a phylogenetic tree by the distance Wagner procedure (Farris, 1972). The raw data (presence or absence of electromorphs) were used to develop yet another tree by the qualitative method of Hennig (1966; see Patton & Avise, 1983). All heterozygosities (H's) were determined by direct counts of mean proportions of loci heterozygous per individual.

Results

Observed heterozygosities ranged from zero (*Olor columbianus* and *Anas fulvigula*) to 0.083 (*Aythya valisineria*). Because the two species exhibiting $H=0$ were represented by a total of only three specimens, our estimates do not imply that these species completely lack genetic variation. Across all species, mean heterozygosity equals 0.033, both unweighted and weighted by sample size. These heterozygosities are only slightly lower than mean values previously reported for birds and for other vertebrates (Powell, 1976).

Electromorphs and their frequencies are presented in Table 2. Since particular electromorphs can be thought of as qualitative characters, either present or absent in samples of a given taxon, they can be directly employed to generate an evolutionary tree according to Hennigian principles. A cladistic tree for the waterfowl is shown in Figure 2. This tree was developed by procedures detailed by Patton and Avise (1983), who also discuss strengths and weaknesses of this approach. Perhaps the most appealing aspect of this procedure is that individual character states (electromorphs in this case) are explicitly defined along all branches of the output tree. This in turn facilitates hypothesis testing because specific characters leading to points of ambiguity or discrepancy in the tree can be identified and potentially subjected to further study. Character states along branches of the cladistic tree in Figure 2 are presented in Table 3.

In developing the cladistic network, plesiomorphs (electromorphs ancestral to the assayed waterfowl) were disregarded in establishing genealogies within the tree. A total of 19 such plesiomorphs were identified by the criterion that they were shared by any members of the two basally related subfamilies, Anserinae and Anatinae. Electromorphs unique to a taxon or taxa within Anatinae, or within Anserinae, constitute derived character states (apomorphs), and form the basis for clade identification. A total of 63 apomorphs contributed to the structure of the tree.

The use of the qualitative phylogenetic approach proposed by Hennig (1966) is a subject of controversy for many reasons, including the possibility of producing alternative phylogenetic trees (see Sokal, 1975). The figured relationship of *Aythya–Bucephala* (Fig. 2) can serve as a case in point. Since no synapomorphic (shared-derived) character was found to define *Aythya*, several different branching sequences could be proposed to represent the relatedness of *Aythya* and *Bucephala*. Some of these representations would suggest a polyphyletic origin for *Aythya*, since minor synapomorphic alleles were shared by *A. americana–B.*

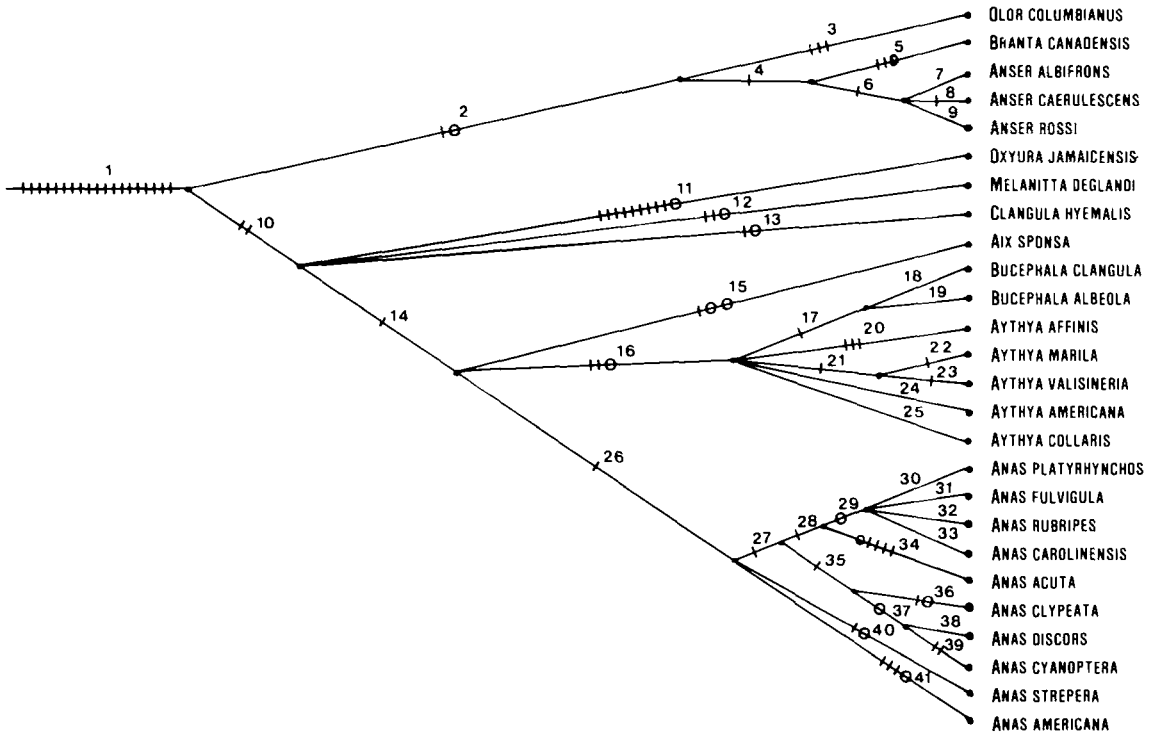

Fig. 2. Phylogenetic tree for assayed waterfowl generated from qualitative analysis of electromorphs using the method of Hennig (1966) and Patton and Avise (1983). Character states along branches of the tree are listed in Table 3. Lines crossing branches indicate derived electromorphs whose ancestral states can be hypothesized, and circles crossing branches denote electromorphs whose presumed ancestral states remain undetermined.

Albeola (Pgd^{120}), and by *A. affinis-B. albeola* (Gpd^{70}). In Fig. 2, we have opted to present a conservative interpretation which suggests that Pgd^{120} and Gpd^{70} were ancestral alleles independently retained in these species.

Two other 'trees', generated by Wagner analyses and UPGMA from genetic distance matrices, are presented in Figures 3 and 4 respectively. The results summarized in Figures 2–4 represent only a small sampling of numerous available methods of tree generation. Nonetheless, they do encompass a philosophically diverse array of approaches, ranging from cladistic to strictly phenetic, and utilizing both qualitative and quantitative data bases. There is no general concensus on the 'best' method of tree generation, nor even on criteria by which to recognize a 'best' tree. We prefer to employ conceptually diverse approaches, and to base our strongest conclusions on commonalities in results. The commonalities are further strengthened when they agree with 'model' phylogenies based on independent criteria. In the following sections, we discuss points of agreement and disagreement in the data analyses, and evaluate them against the 'model' phylogeny for waterfowl.

Commonalities

Anatinae and Anserinae

All methods of genetic data analysis recognize the basal split between the two subfamilies described by classical systematists (the exception is the UPGMA placement of *Oxyura*, discussed later). Anatinae and Anserinae cluster in UPGMA at a genetic distance of about 0.4, and they form major branches of the Wagner tree. Two synapomorphs ($Idh\text{-}1^{100}$ and $Idh\text{-}1^{200}$) define the Anatinae clade in the Hennigian analysis; Pgd^{200} and Alb^{105} were unique to the Anserinae and shared by all its members.

Table 3. Electromorphs defining branches of the phylogenetic tree in Figure 2. Line 1 lists characters presumed plesiomorphic (ancestral) to assayed waterfowl taxa, and successive lines list apomorphic (derived) characters. Electromorphs in brackets are apomorphs whose presumed ancestral states remain undetermined.

(1) *Mdh-1*^{100}, *Mdh-2* ^{100}, *Ldh-1*^{100}, *Ldh-2*^{100}, *Idh-1*^{90}, *Idh-2* ^{100}, *Pgd*^{100}, *Gpd*^{75}, *Pgi*^{100}, *Pgi*^{50}, *Pgm*^{50}, *Ck-2*^{100}, *Ck-3*^{100}, *Got-1*^{100}, *Got-2* ^{100}, *Ipo*^{100}, *Pept-1*^{100}, *Pept-2*^{100}, *Hb* ^{100}
(2) *Pgd*^{200} *[Alb*^{105}*]*
(3) *Idh-1*^{125}, *Gpd*^{60}, *Got-1*^{80}
(4) *Idh-1*^{120}
(5) *Mdh-1*^{70}, *Got-1*^{130}, *[Alb*^{95}*]*
(6) *Ck-2*^{140}
(7) (Speciation)
(8) *Gpd*^{30}
(9) (Speciation)
(10) Idh-1^{100}, Idh-1^{200}
(11) *Gpd*^{130}, *Gpd*^{85}, *Pgi*^{90}, *Pgi*^{45}, *Pgm*^{140}, *Pgm*^{120}, *Ck-2*^{200}, *Got-1*^{300}, *Got-1*^{200}, *[Alb*^{107}*]*
(12) *Gpd*^{72}, *Hb* ^{140} *[Alb*^{106}*]*
(13) *Hb* ^{120} *[Alb*^{110}*]*
(14) *Gpd*^{100}
(15) *Gpd*^{120} *[Alb*^{104}, *Alb*$^{101.5}$*]*
(16) *Pgd*^{120}, *Gpd*^{70} *[Alb*^{103}*]*
(17) *Idh-1*^{180}
(18) (Speciation)
(19) (Speciation)
(20) *Gpd*^{125}, *Pgi*^{210}, *Pgi*^{180}
(21) *Pept-2*^{95}
(22) *Pgm*^{10}
(23) *Ldh-1*^{180}
(24) (Speciation)
(25) (Speciation)
(26) *Pgm*^{100}
(27) *Pgd*^{110}
(28) *Pgi*^{200}
(29) *[Alb*^{100}*]*
(30) (Speciation)
(31) (Speciation)
(32) (Speciation)
(33) (Speciation)
(34) *Idh-1*^{160}, *Idh-1*^{50}, *Got-1* ^{50} *[Alb*^{98}*]*, *Pgd*^{130}
(35) *Ldh-1*^{95}
(36) *Mdh-1*^{90} *[Alb*^{101}*]*
(37) *[Alb*^{102}*]*
(38) (Speciation)
(39) *Pgd*^{10}, *Hb* ^{90}
(40) *Gpd*^{45} *[Alb*^{98}*]*
(41) *Mdh-2* ^{100}, *Gpd*^{80}, *Gpd*^{40} *[Alb*^{97}*]*

Olor

Except in the Wagner analysis, the Whistling Swan appears basally related to the geese which compose the remainder of Anserinae. Even in the Wagner tree, the earlier branching to *Branta* occurs only a small 2 distance units (Fig. 3) prior to the split of *Olor.* Three electromorphs (*Idh-1*^{125}, *Gpd*^{60}, and *Got-1*^{80}) were unique to *Olor*, and one synapomorph (*Idh-1*^{120}) defines the *Branta–Anser* clade.

Anser

All methods of analysis recognize the close genetic relationships among the three species of *Anser* reflected in the current classification. Baker and Hanson (1966) have previously reported only minor differences in hemoglobins and serum proteins among eight *Anser* (and three *Branta*) species. A synapomorph (*Ck-2*^{140}) defines the *Anser* clade.

Anas

This genus is also consistently delineated in all genetic summaries, as it is in morphological and behavioral characteristics. *Pgm*^{100} is synapomorphic for the *Anas* assayed, and the group forms a coherent assemblage in both the Wagner and UPGMA analyses. Genetic distances within the genus are very small (ranging from Nei's $\bar{D}=0.001$ between *Anas platyrhynchos* and *A. rubripes* to $\bar{D}=0.186$ between *A. strepera* and *A. cyanoptera*). Even so, some genetic groupings within the genus appear consistently and these are generally among species thought by systematists to be particularly closely related.

One such grouping involves *A. clypeata* (Northern Shoveler), *A. discors* (Blue-winged Teal) and *A. cyanoptera* (Cinnamon teal). Delacour and Mayr (1945) stress the 'extremely close relationship' among these species, evidenced by the almost identical and unique wing coloration, peculiar courtship methods, and shared feeding habits. Genetically, the three species share a unique allele (*Ldh-1*^{95}) which defines the clade, and they form distinct branches in the Wagner and UPGMA analyses. (Notwithstanding its common name, the Green-winged Teal is not particularly closely related to the 'blue-winged ducks', and is placed in a different group by Delacour and Mayr (1945).)

Another consistent genetic grouping involves *A. platyrhynchos* (Mallard), *A. fulvigula* (Mottled Duck), and *A. rubripes* (Black Duck). Some ornithologists consider this complex a 'superspecies', with the Black and Mottled Ducks representing sexually non-dimorphic forms of the Mallard; Graham (1979) suspects the forms may someday be tax-

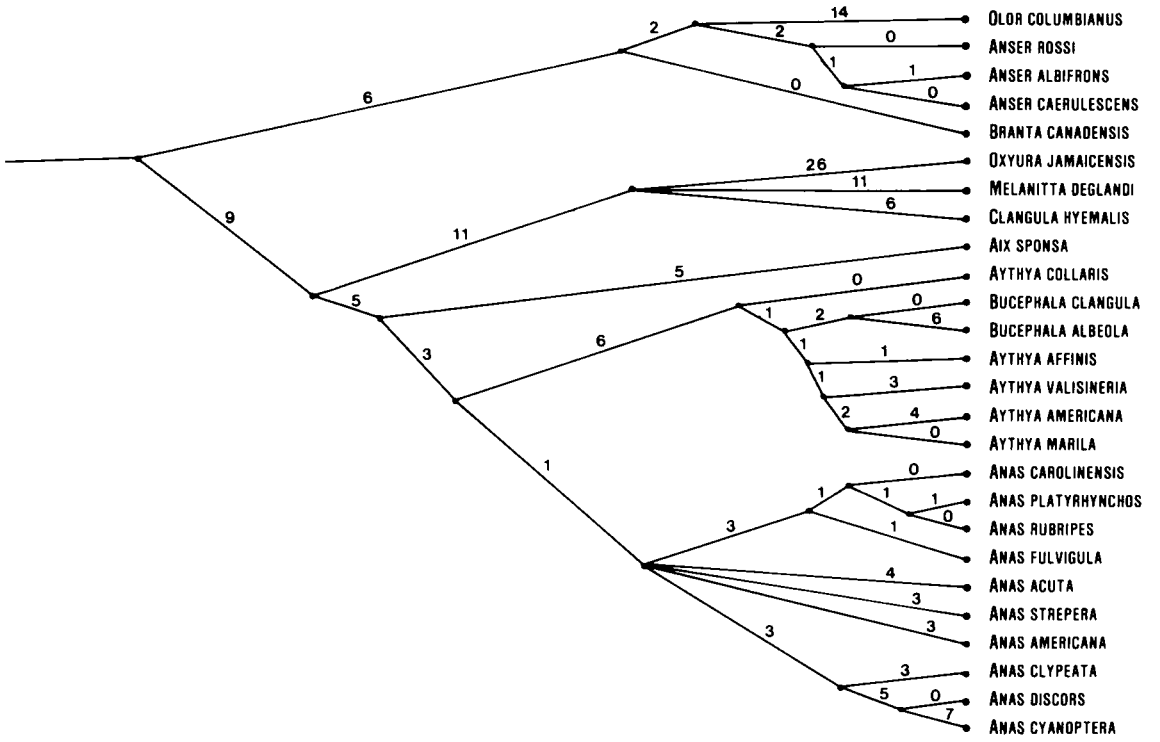

Fig. 3. Wagner tree for assayed waterfowl, generated from a matrix of genetic distances. Branch lengths are in Roger's $\bar{D}$ ($\times 100$).

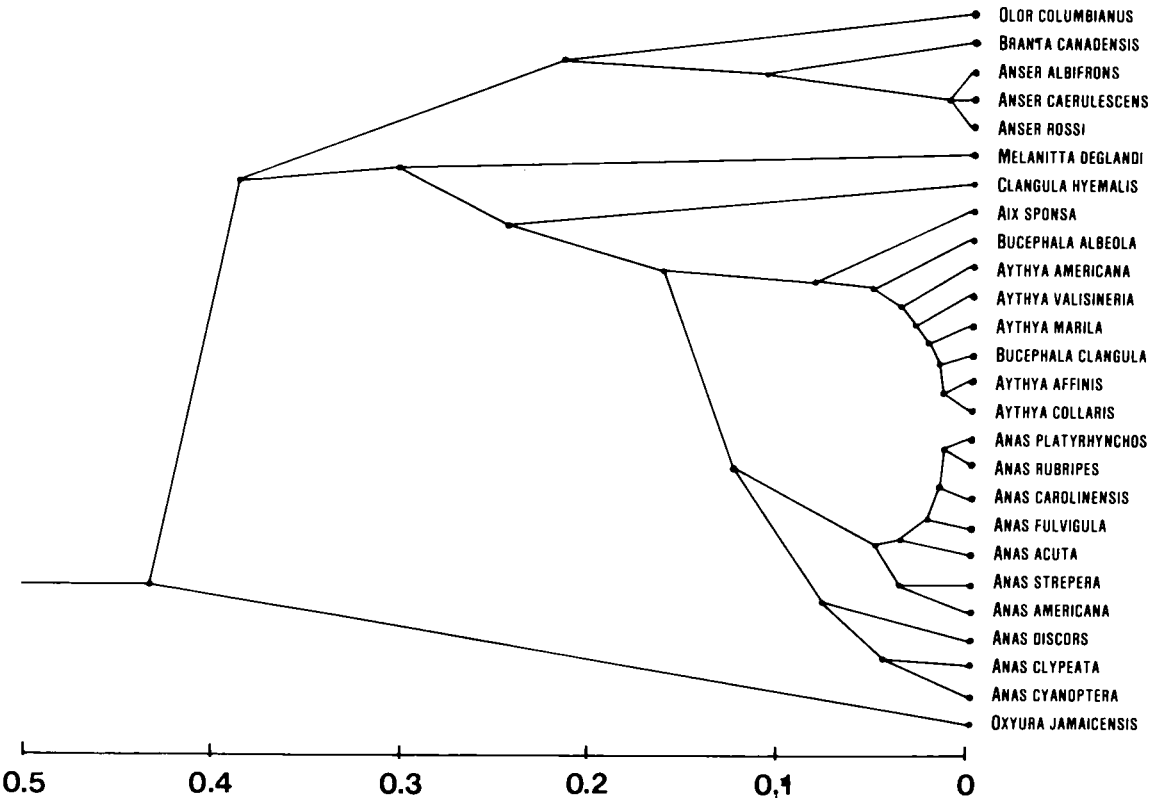

Fig. 4. UPGMA dendrogram for assayed waterfowl, generated from a matrix of genetic distances. The scale is in Nei's $\bar{D}$.

onomically merged. The three species, together with the Green-winged Teal, form a minor branch in each of the genetic trees, and they all share an electromorph (Alb^{100}) not observed in other waterfowl. The Green-winged Teal is not conventionally thought to be particularly close to the Mallard group, but based on our sample of 19 loci it could not be distinguished from the Mallard assemblage.

The other *Anas* species (*acuta*, *strepera*, *americana*) are placed in distinct subgeneric groups by Delacour and Mayr (1945), and apart from their obvious close genetic ties to other *Anas*, they show no consistent branching or clustering tendencies in Figures 2-4.

Aythya

Members of this compact genus of diving ducks or pochards are also very close in genetic composition. They congregate (with *Bucephala* - see beyond) in each method of genetic data summary. Within the genus, Nei's $\bar{D}$'s are very small and fall within a narrow range - 0.004 (*A. affinis* versus *A. collaris*) to 0.055 (*A. americana* versus *A. collaris*). No electromorph was found to define *Aythya*.

Bucephala

The two assayed species of this genus, *B. clangula* (Goldeneye) and *B. albeola* (Bufflehead), are very similar in genic composition: $\bar{D}=0.036$ (although in the UPGMA clustering they are intermingled with the *Aythya*). They do form a distinct minor branch in both the Wagner and Hennigian analyses, the latter determined by shared possession of a derived electromorph, $Idh\text{-}1^{180}$.

Discrepancies and ambiguities

Mergini

This tribe, which includes *Bucephala*, *Melanitta*, and *Clangula*, is thought by Delacour and Mayr (1945) to be well delineated, showing no close relationships to the pochards (*Aythyini*) or to other tribes. Therefore, it is surprising that *Bucephala* appears genetically close to *Aythya*, and furthermore that the three assayed Mergini genera exhibit no particular genetic ties to one another (Figs. 2-4). The Hennigian analysis suggests that Pgd^{120}, Gpd^{70}, and Alb^{103} are shared by *Bucephala* and *Aythya*, yet were not observed in other waterfowl. The mean genetic distance between *Bucephala*, *Melanitta*, and *Clangula* is rather high (by 'waterfowl and bird standards'): $\bar{D}=0.20$. Much of the genetic distinctness of *Melanitta* and *Clangula* from one another and from other Anatinae stems from their possession of several unique (autapomorphic) alleles (Fig. 2). The remainder of Anatinae (excluding *Oxyura*) are also united by possession of the synapomorph Gpd^{100} in high frequency in all species.

Other traditional classifications had considered the bay ducks (Aythyini of Johnsgard and Delacour) and sea ducks (Mergini) to be closely related and in a subfamily Aythyinae (i.e., Robbins *et al.*, 1966). Perhaps the close genetic ties of *Bucephala* and *Aythya* are compatible with this view. The genetic data also suggest that other sea duck genera, such as *Melanitta* and *Clangula* are only basally related to one another. A more comprehensive genetic investigation of the sea duck complex, including the Eiders (*Somateria*), Mergansers (*Mergus*) and Harlequin (*Histrionicus*), would now appear most desirable.

Aix

The Wood Duck (*Aix sponsa*) is the sole representative of the perching ducks, Cairinini, that we have examined. Both Johnsgard (1968) and Delacour and Mayr (1945) suggest close evolutionary ties between Cairinini and Anatini, based on coloration of the downy young and structure of the syrinx. Woolfenden (1961) urged that Cairinini be merged with Anatini. These relationships are not evident in the genetic data. In the UPGMA phenogram, *Aix* clusters slightly closer to *Aythya-Bucephala* than to *Anas*; in the Wagner tree, *Aix* falls just outside the Aythyini-Anatini complex; and in the Hennigian tree, *Aix* stems from a common branch point with Aythyini and Anatini.

Oxyura

The stiff-tailed ducks, Oxyurini, are thought to have branched very early from primitive Anatinae stock, and to be only distantly related to other tribes within that subfamily (Johnsgard, 1968; Delacour & Mayr, 1945). Our genetic data confirm that *Oxyura* is a highly divergent anatid, although different methods of genetic analysis do not entirely agree on its exact placement within Anatidae. Phenetically, *Oxyura* falls outside both the Anatinae and Anserinae clusters (Fig. 4), exhibiting a

mean genetic distance (Nei's $\bar{D}$) of about 0.43 to the other waterfowl species. In the Wagner and Hennigian analyses, *Oxyura* stems from a common basal branch point with *Melanitta-Clangula*, or with *Melanitta-Clangula*-Aythyini-Anatini, respectively. *Oxyura* does exhibit both *Idh-1*^{100} and *Idh-1*^{200}, electromorphs that were observed in most Anatinae species, but not in the Anserinae. These alleles account for its membership in the Anatinae clade in the Hennigian analysis. *Oxyura* also exhibits a large number of autapomorphic alleles (Table 3).

Overall, the agreement between the various genetic summaries of waterfowl relationships is quite good. The agreement between classical phylogenies and the genetic phylogenies of waterfowl is also very strong. The major area of potential disagreement concerns possible relationships of various sea ducks (Mergini) to one another and to the Aythyini. It must also be recognized, as discussed below, that *all* of the genetic distances in waterfowl are very small compared to genetic distances in most other families of non-avian vertebrates. The members of Anatidae are clearly a genetically close-knit assemblage.

Table 4. Summary of genetic distances (Nei's $\bar{D}$) in waterfowl, based on 17 – 19 loci.

Comparison	Number of species comparisons	$\bar{D}$	Range
Between species within genera			
Anser	3	0.002	(0.001 – 0.003)
Bucephala	1	0.036	
Aythya	10	0.023	(0.004 – 0.055)
Anas	45	0.092	(0.001 – 0.186)
Between genera within tribes			
Anserini	7	0.186	(0.116 – 0.262)
Mergini	5	0.238	(0.188 – 0.288)
Between tribes within subfamilies			
Anatinae	149	0.207	(0.010 – 0.565)
Between subfamilies			
Anserinae-Anatinae	105	0.386	(0.152 – 0.611)
Totals	*325*	*0.241*	*(0.001 – 0.611)*

Discussion

Conservative patterns of divergence

In discussions of comparative phylogeny, it is important to ask whether the taxonomies of the groups under consideration are equivalent. These questions can be best addressed when common scales for comparison are available. Such common scales for widely different phylads could be provided by times of divergence (where these are known or suspected) or by estimates of divergence in homologous molecules, for example. Concordance or non-concordance between results of comparisons utilizing different scales can provide insights into general patterns of evolutionary change.

Among the waterfowl, genetic distances between species at various levels of taxonomic recognition are summarized in Table 4. Since this is among the few large-scale estimates of such genetic distances in a group of non-Passeriforme birds, it is of interest to compare results with those previously published for several Passeriforme families. This is done in Table 5. It is apparent that the Anseriformes exhibit the same conservative pattern of

Table 5. Summary of genetic distances (Nei's $\bar{D}$) in Passeriformes and Anseriformes at comparable levels of the taxonomic hierarchy.

	Comparison[1]	Number of species comparisons	$\bar{D}$	Range
Anseriformes	Between species within genera	59	0.075	(0.001 – 0.186)
	Between genera within subfamilies	161	0.207	(0.010 – 0.565)
	Between subfamilies	105	0.386	(0.152 – 0.611)
Passeriformes[2]	Between species within genera	87	0.059	(0.000 – 0.279)
	Between genera within subfamilies	351	0.195	(0.011 – 0.636)
	Between subfamilies[3]	27	0.483	(0.350 – 0.693)

[1] According to the classification of Morony *et al.* (1975).

[2] Data from Avise *et al.*, 1980a, b, c; for additional data (which reflect the same trend), see Barrowclough and Corbin (1978) and Smith and Zimmerman (1976).

[3] Assumes *Icteria* is subfamilially distinct from other assayed Parulidae.

protein differentiation as do other birds. In many groups of Aves assayed to date, as a general rule mean genetic distances among congeners are typically about 0.10 or less; between subfamilial genera, $\bar{D}$s average about 0.20, and between subfamilies $\bar{D}$'s are commonly in the range of about 0.35–0.50. These statements should not be interpreted as hardened guidelines for future taxonomic assignments. They are simply empirical generalities from genetic data currently available.

A much larger literature exists on genetic distances among non-avian vertebrates. Some phylads do exhibit a conservative level of genetic differentiation comparable to that summarized for Aves above, but these are in the minority. Far more typically, even closely related congeners exhibit $\bar{D}$'s as large or larger than those characteristic of avian genera or subfamilies. The protein electrophoretic literature is summarized by Avise and Aquadro (1982).

In referring to comparisons of heterozygosity levels in different organisms, Selander (1976) argues that 'the major determinant of the span of variation in estimates of polymorphism is the laboratory in which the survey was conducted!' This argument cannot account for the emerging avian/non-avian difference in mean level of genetic differentiation. First, the conservative Aves pattern has been reported independently by several labs (Barrowclough & Corbin, 1978; Smith & Zimmerman, 1976). Second, in our own laboratory we have assayed other vertebrates concurrently with members of Aves, employing similar buffers and electrophoretic conditions to analyze comparable sets of proteins. Results for 16 species and 9 genera within the single rodent subfamily Cricetinae (Patton *et al.*, 1981) are contrasted with the avian results in Table 6. Both at the species and generic levels, mean genetic distances among rodents are 6–7 times as large as mean genetic distances among birds.

Rate of protein evolution

One hypothesis to account for these trends is that species of Anatidae are evolutionarily younger than most other vertebrates. This seems unlikely given the fossil evidence presented earlier. An alternative hypothesis is that protein evolution is decelerated in birds relative to non-avian vertebrates. This possibility has proved difficult to evaluate in the Passeriformes because of the poor fossil record, but with caution we can address the issue in the Anatidae.

Nei (1971, 1975) has shown that his measure of genetic distance can be related to time of divergence of species by the formula $\bar{D}=t\,r$, where r is a variable determined by several considerations about protein structure and variation: c, the proportion of amino acids which are electrophoretically detectable; n, the average number of amino acids per protein; and λ, the rate of amino acid substitution per polypeptide per site per year ($r=2cn\lambda$). Suppose we wish to compare two phylads, A and B, which exhibit genetic distances D_A and D_B. Then

$$\frac{\bar{D}_A}{\bar{D}_B}=\frac{t_A c_A n_A \lambda_A}{t_B c_B n_B \lambda_B} \qquad (1)$$

There is no evidence to suggest that c's or n's are consistently different in birds than in other vertebrates (it is perhaps conceivable that for some reason the electrophoretic buffers yield lower c's in birds, and this possibility may be worth experimental test). Thus formula (1) simplifies to

Table 6. Summary of genetic distances (Nei's $\bar{D}$) in birds versus rodents at comparable levels of the taxonomic hierarchy.

	Comparison	Number of species comparisons	$\bar{D}$	Range
Birds[1]	Between species within genera	146	0.065	(0.000 – 0.274)
	Between genera within subfamilies	512	0.199	(0.010 – 0.636)
Rodents[2]	Between species within genera	120	0.393	(0.002 – 1.002)
	Between genera within subfamilies	83	1.256	(0.398 – 2.238)

[1] Data from Avise *et al.*, 1980a, b, c, and this paper.

[2] Data from Avise *et al.*, 1974 (and reference therein), and Patton *et al.*, 1981.

$$\frac{\bar{D}_A}{\bar{D}_B} = \frac{t_A \lambda_A}{t_B \lambda_B} \quad (2)$$

As mentioned in the introduction, the fossil record suggests that many genera of waterfowl are about 20 million years old. Hibbard (1968) suggests that the earliest divergence of the genera of the cricetine rodents examined by Patton *et al.* (1981 – Table 6) also occurred about 20 million years ago. If we accept these estimates, that anseriform and rodent genera are of roughly equivalent age, the ratio λ_A/λ_B equals about 6; rodents are diverging in protein composition at rates several times faster than waterfowl. If the rodent genera are in fact younger (or the waterfowl older) the ratio of rates of genetic divergence in the two groups would be even greater than this.

The problem can be looked at in another way. Mean genetic distance between the waterfowl subfamilies Anserinae and Anatinae is 0.386 (Table 5). In a large survey of protein comparisons in other organisms, Nei (1975) estimates r to be roughly 2×10^{-7} per year for electrophoretically detectable protein substitutions. Substituting these values in the formula $t=\bar{D}/r$, and assuming that waterfowl proteins evolve at the standard rate, the subfamilies should be about 2 million years old. Even if we plug these values into the slowest calibrated 'protein-clock' of which we are aware, that proposed in 1977 by Sarich (and which assumes *all* assayed proteins belong to a 'slowly-evolving' class), estimated divergence time would be about 12 million years. These estimates still contrast with the literal fossil interpretation which places the Anserinae-Anatinae divergence in waterfowl at perhaps 25 million years. Again, at face value, it appears that protein divergence is proceeding much more slowly than in most other organisms. Gutierrez *et al.* (1983) report very similar conclusions for another group of non-Passeriforme birds. Protein clocks previously suggested for some non-avian vertebrates proved to be far to rapid (by a factor of five) to yield divergence times compatible with observed genetic distances and with the fossil record for the Galliformes.

In view of the inevitable uncertainties about true divergence times in waterfowl, and in most other organisms, we are hesitant to draw firm conclusions about the exact magnitude of any possible slowdown in protein evolution in Aves. Further uncertainties involve the reliability of estimates of $\bar{D}$, and the representativeness of protein data to divergence in the total avian genome. From immunological comparisons of albumins and transferrins, Prager and Wilson (1975) conclude that these proteins 'have evolved about three times as slowly in birds as in other vertebrates'. Wilson *et al.* (1977) later suggested that this conclusion may have been premature. Nonetheless, the evidence for an avian protein deceleration remains sufficiently compelling to warrant further study. In the future it will be especially valuable to assay other portions of the avian genome as well as to develop and test hypotheses about the causal process responsible for any possible deceleration of protein divergence in birds.

One of the important observations of research in molecular evolution is that the pace of evolution among non-homologous proteins can vary tremendously (Sarich, 1977). Rates of amino acid substitution per polypeptide site differ by more than 100-fold from rapidly evolving immunoglobulins and fibrinopeptides to slowly evolving histones (Dayhoff, 1972; Wilson *et al.*, 1977). Such observations have stimulated a great deal of research leading to an increased recognition of the significance of functional constraints (or lack thereof) on protein divergence (Wilson *et al.*, 1977). If indeed it eventually proves true that homologous or analogous proteins also evolve at distinct rates in different phylads, our ability to use molecular information to reach definitive conclusions about absolute divergence times for species with a poor fossil record will be comprised. In that case, an exploration of possible reasons for heterogeneous rates across phylads could ultimately lead to a fuller understanding of the factors governing protein differentiation.

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