Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 7912–7916, August 1994
Evolution

Phylogeny and evolution of the auks (subfamily Alcinae) based on mitochondrial DNA sequences

TRULS MOUM*†‡§, STEINAR JOHANSEN†, KJELL EINAR ERIKSTAD¶, AND JOHN F. PIATT‖

Departments of *Biochemistry and †Cell Biology, Institute of Medical Biology, University of Tromsø, 9037 Tromsø, Norway; ¶Norwegian Institute for Nature Research, Department of Tromsø, 9001 Tromsø, Norway; and ‖Alaskan Fish and Wildlife Research Center, Anchorage, AK 99503

Communicated by Charles G. Sibley, April 7, 1994

ABSTRACT **The genetic divergence and phylogeny of the auks was assessed by mitochondrial DNA sequence comparisons in a study using 19 of the 22 auk species and two outgroup representatives. We compared more than 500 nucleotides from each of two mitochondrial genes encoding 12S rRNA and the NADH dehydrogenase subunit 6. Divergence times were estimated from transversional substitutions. The dovekie (*Alle alle*) is related to the razorbill (*Alca torda*) and the murres (*Uria* spp). Furthermore, the Xantus's murrelet (*Synthliboramphus hypoleucus*) and the ancient (*Synthliboramphus antiquus*) and Japanese murrelets (*Synthliboramphus wumizusume*) are genetically distinct members of the same main lineage, whereas brachyramphine and synthliboramphine murrelets are not closely related. An early adaptive radiation of six main species groups of auks seems to trace back to Middle Miocene. Later speciation probably involved ecological differentiations and geographical isolations.**

The auks (subfamily Alcinae) are a distinct group of Northern Hemisphere seabirds that includes 22 extant and 1 recently extinct species [the nomenclature used throughout is that of Sibley and Ahlquist (1)]. The extant alcines are small- to medium-size sturdily built birds that pursue their prey by wing-propelled diving.

Earlier studies of the relationships of the Alcinae based on morphological characters, oology, and ecology have been summarized by Strauch (2). Still in dispute are the relationships among the murrelets (*Brachyramphus* spp. and *Synthliboramphus* spp.), the relationships of the guillemots (*Cepphus* spp.) and the dovekie (*Alle alle*) to other alcines, and the relationships among all of these groups. Strauch (2) used compatibility analysis to produce a phylogenetic representation based on morphology and natural history (Fig. 1*A*). His results indicated that the dovekie is related to the auks and that brachyramphine murrelets are not closely related to other murrelets. In another recent study, electrophoretic separation of enzymes was used to investigate the phylogenetic relationships among 12 alcine species from the Pacific (3). One finding of this study was a binary division of the Alcinae into a puffin/auklet clade and a clade of the remaining alcines (Fig. 1*B*).

Molecular sequence data offer new possibilities to resolve phylogenies and to improve quantitative estimates of genetic relationships. In the present study we used nucleotide sequence data from the mitochondrial gene-encoded 12S ribosomal RNA (12S) and NADH dehydrogenase subunit 6 (ND6). The 12S gene is known to evolve at a moderate rate. The ND6 protein-encoding gene is among the fastest evolving mitochondrial genes, and we have previously suggested that it might be useful for studies of closely related species (4). We have used two genes with different evolutionary rates in this study to allow the resolution of more (12S gene) or less (ND6 gene) distant relationships among the alcines.

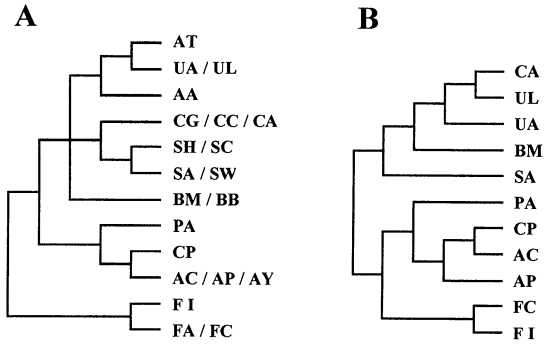

FIG. 1. Phylogenetic relations of the Alcinae hypothesized by two recent studies. Only genera covered by the present study are included. Species abbreviations are given in *Materials and Methods*. (*A*) Phylogeny derived from figure 18 in ref. 2, based on compatibility analysis of 33 characters from morphology and natural history. (*B*) Phylogenetic representation of 11 alcine species based on electrophoretic analysis of 24 protein loci (3).

In most classifications of the past century, the auks, gulls, and waders have been placed in the order Charadriiformes. The classification of Sibley and Ahlquist (1) based on DNA·DNA hybridization retains the relationships among these groups in the infraorder Charadriides and presents evidence that auks and gulls are sister groups. A gull and a wader were consequently included as outgroup representatives in the present study.

MATERIALS AND METHODS

Tissue Samples and DNA Extractions. Tissue samples were collected from 21 species in the Atlantic and Pacific oceans. Nineteen alcine species were represented, including the common murre (*Uria aalge*, UA), the thick-billed murre (*Uria lomvia*, UL), the razorbill (*Alca torda*, AT), the dovekie (*Alle alle*, AA), the black guillemot (*Cepphus grylle*, CG), the pigeon guillemot (*Cepphus columba*, CC), the Japanese *murrelet* (*Synthliboramphus wumizusume*, SW), the ancient murrelet (*Synthliboramphus antiquus*, SA), the Xantus's murrelet (*Synthliboramphus hypoleucus*, SH), the Kittlitz's murrelet (*Brachyramphus brevirostris*, BB), the marbled murrelet (*Brachyramphus marmoratus*, BM), the Cassin's auklet (*Ptychoramphus aleuticus*, PA), the parakeet auklet (*Cyclorrhynchus psittacula*, CP), the least auklet

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: 12S, 12S ribosomal RNA; ND6, NADH dehydrogenase subunit 6; MP, maximum parsimony; ML, maximum likelihood; TV, transversion; MYA, million years ago. Twenty-three Two-letter species abbreviations are given in *Materials and Methods*.

‡Present address: Department of Medical Genetics, N-9038 University Hospital of Tromsø, Norway.
§To whom reprint requests should be sent at the present address.

**The sequences reported in this paper have been deposited in the GenBank data base (accession nos. X76345–X76362 and X76435).

(*Aethia pusilla*, AP), the whiskered auklet (*Aethia pygmaea*, AY), the crested auklet (*Aethia cristatella*, AC), the Atlantic puffin (*Fratercula arctica*, FA), the horned puffin (*Fratercula corniculata*, FC), and the tufted puffin (*Fratercula cirrhata*, FI). Among the commonly recognized genera only one (*Cerorhinca*; species *monocerata*) was missing. Two other species, the Craveri's murrelet (*Synthliboramphus craveri*, SC) and the spectacled guillemot (*Cepphus carbo*, CA) would have been needed to complete the subfamily. The two outgroup species included are the common gull (*Larus canus*, LC) and the purple sandpiper (*Calidris maritima*, CM). We isolated mtDNA-enriched fractions from muscle or heart tissues (5).

Amplification and Sequencing. PCR amplifications were carried out in 50-μl volumes of 10 mM Tris buffer (pH 8.3) containing 50 mM KCl, 2 mM $MgCl_2$, 1.5% glycerol, 0.2 mM of each dNTP, 0.5 μM of each primer, and 1.5 units of *Taq* polymerase (Perkin–Elmer/Cetus). Sequencing followed reported procedures (6, 7). The PCR primers for the 12S gene were H2306 (5′-GAGGGTATCTTTCAGGTGTA-3′) and L1267 (5′-AAAGCATGGCACTGAAGATG-3′), and the sequencing primers were L1547 (5′-AGGGTTGGTAAATCTTGTGCCAGC-3′) and L1755 (5′-TGGGATTAGATACCCCACTATGC-3′). L refers to light and H refers to heavy strands, and the numbers refer to the position of the 3′ nucleotide of the primer in the domestic fowl mtDNA sequence (8). A continuous stretch of ≈500 bp was sequenced from the 12S gene in all species except SH and SW. The complete nucleotide sequences of the ND6 gene were available for all species (4, 7).

Phylogenetic Analysis. Approximately 1020 nucleotides in each species were available for phylogenetic analysis. A phylogenetic tree was constructed by a composite alignment and distance matrix approach based on the neighbor-joining method [CLUSTAL by DNASTAR, (9, 10)]. Phylogeny reconstructions were also carried out by using the maximum-parsimony (MP) and maximum-likelihood (ML) methods of the PHYLIP package [DNAPARS; DNAML, version 3.51c, (11)]. In this case the nucleotide sequences were aligned by using the PILEUP option of the Genetics Computer Group (GCG) software package, version 7, and manual adjustment. MP trees were evaluated using the bootstrap procedure (12) based on 100 resamplings.

Highly variable nucleotide positions may add noise to phylogenetic inferences, in particular among ancient lineages (13). Therefore, various treatments were given that confined the analysis to more conservative changes. These included removal of the third codon positions of the ND6 gene using only transversions (TVs) in the third codon positions and allowing for TVs exclusively.

Approximately 22 nucleotide positions had to be removed from the analyses of the 12S gene to ascertain homology under strict rules (as determined by comparison of the avian sequences to those of several mammals). Hence, some of the runs were carried out on a curtailed data set of 1006 nucleotide positions. The ND6 and 12S data were also treated separately using the same methods as for the pooled data sets.

Molecular Clock Calculations. We provide tentative absolute datings for cladogenesis among alcines by calibrating mtDNA divergence against a molecular clock based on DNA·DNA hybridizations in birds. The genetic distance data based on DNA·DNA hybrid melting temperatures (Δ $T_{50}H$ values) have been worked out by Sibley and Ahlquist (1). We based the calibration on a Δ $T_{50}H$ 1.0 = 3.0 million years. The Δ $T_{50}H$ values used for the calibration were those of Galliformes versus Ciconiiformes: 28.0 [84 million years ago (MYA)], Scolopacidae versus Laridae: 15.6 (46.8 MYA), and Larinae versus Alcinae: 6.1 (18.3 MYA). Corrected divergence estimates of mtDNA based on TVs (14) were used for clock calculations. The domestic fowl (*Gallus gallus*; GG) mitochondrial sequence (8) was included in these calculations. The average divergence rate of mtDNA was estimated using regression analysis with the intercept fixed at origin.

RESULTS

Pairwise Comparisons. The alignment of 12S gene and ND6 gene sequences of 20 bird species totalled 1028 nucleotide positions. The number of shared nucleotides by any two species was typically 1021 (range, 1013–1022). Percent divergence using all substitutions (Table 1, upper diagonal) varied from 3.5 to 13.3 within the Alcinae. Divergence estimates based on TVs (Table 1, lower diagonal) ranged

Table 1. Pairwise divergence estimates in percentages of 20 avian species for 1028 nucleotide positions based on all substitutions (above diagonal) and on TVs alone (below diagonal)

	SA	FA	FC	FI	CG	CC	BB	BM	UA	UL	AT	AA	PA	AC	AY	AP	CP	LC	CM	GG
SA	—	11.9	11.4	11.6	12.3	11.9	12.9	12.2	11.5	12.1	11.5	11.9	11.0	12.2	11.7	13.3	12.3	12.8	15.0	17.8
FA	1.8	—	3.5	5.6	11.9	12.0	12.8	12.9	11.7	10.7	12.5	11.7	11.1	12.6	11.3	12.1	11.7	12.7	14.5	18.4
FC	1.7	0.1	—	5.6	10.9	11.0	12.0	11.8	11.3	10.5	11.4	10.8	9.8	11.3	10.6	10.6	10.5	11.3	13.6	18.0
FI	1.8	0.4	0.3	—	11.3	11.8	12.1	11.7	11.5	11.6	12.5	10.9	10.6	12.1	11.5	12.8	11.4	12.6	14.5	17.4
CG	2.0	1.8	1.7	1.8	—	5.7	11.4	10.3	10.8	11.3	10.5	11.1	10.7	12.3	10.5	12.6	11.2	12.9	15.1	17.8
CC	2.4	2.2	2.1	2.2	0.4	—	11.3	11.4	11.1	11.7	10.7	12.0	10.8	11.2	11.4	11.5	11.1	12.4	15.4	18.5
BB	2.4	2.4	2.3	2.4	2.0	2.4	—	7.8	11.3	11.6	11.3	11.7	11.0	11.7	10.9	12.3	11.3	12.6	15.1	18.3
BM	2.0	2.0	1.9	2.0	2.0	2.4	0.6	—	11.3	11.4	11.4	11.7	10.6	11.5	10.3	12.6	11.6	11.7	14.2	17.9
UA	1.7	1.5	1.4	1.5	1.7	2.0	2.3	1.9	—	5.9	8.8	10.2	10.8	11.2	11.4	13.1	11.8	13.3	16.2	18.5
UL	2.0	1.6	1.7	1.8	2.0	2.3	2.6	2.2	0.5	—	9.4	10.1	11.3	11.5	11.6	12.7	11.5	12.6	15.1	17.4
AT	1.8	1.4	1.3	1.4	1.6	2.0	2.2	1.8	0.9	1.2	—	8.2	11.0	11.8	10.2	12.5	10.3	12.4	15.2	18.9
AA	1.9	1.3	1.2	1.3	1.7	2.1	2.3	1.9	0.9	1.2	1.0	—	11.0	11.9	11.3	12.6	11.6	12.2	14.6	18.9
PA	2.0	1.4	1.3	1.4	1.6	2.0	2.0	1.8	1.7	2.0	1.8	1.7	—	7.3	7.0	7.7	7.0	11.7	15.3	17.8
AC	2.0	1.6	1.5	1.6	2.0	2.4	2.4	2.0	1.9	2.2	2.0	1.9	0.6	—	7.3	7.9	7.0	13.0	16.4	19.8
AY	2.5	1.9	1.8	1.9	2.3	2.7	2.5	2.1	2.2	2.3	2.3	2.2	0.7	0.7	—	7.6	6.1	11.1	15.6	18.5
AP	2.3	2.1	2.0	2.1	2.3	2.7	2.9	2.5	2.1	2.4	2.3	2.2	1.1	0.9	1.2	—	6.5	13.2	15.9	19.7
CP	2.3	1.7	1.6	1.7	2.1	2.5	2.5	2.1	2.0	2.3	1.9	2.0	0.5	0.5	0.6	1.0	—	11.9	15.0	18.1
LC	2.7	2.9	2.8	2.7	3.1	3.3	3.1	2.7	2.8	2.9	2.7	2.8	2.9	2.7	2.8	3.1	2.8	—	14.3	18.2
CM	4.6	4.8	4.9	4.8	4.8	5.3	5.5	5.0	5.0	5.1	4.8	4.9	5.0	5.0	5.4	5.4	4.9	5.4	—	18.0
GG	8.2	9.2	9.0	8.7	8.5	8.9	8.9	8.7	8.2	8.6	8.3	8.8	8.6	8.8	8.9	8.8	8.7	8.8	8.7	—

Divergence estimates for all substitutions were calculated using the CLUSTAL method (9, 10). Divergences for TVs were calculated by using the equation $d = -1/2 \ln(1-2p)$, where p = TV/NS, and NS is the number of nucleotides shared by any two species (14). Species abbreviations are given in *Materials and Methods*.

from 0.1% to 2.9% within the Alcinae and overlapped slightly with those of alcines to the common gull (2.7%–3.3%).

Phylogeny. Phylogenetic analysis by CLUSTAL and by MP (consensus tree based on 100 bootstrap replications) yielded identical topologies when applied to the combined data of ND6 and 12S genes (Fig. 2). The ND6 data were analyzed separately to incorporate the SH and SW species for which 12S gene data were not available (Fig. 2 *Inset*). The overall phylogeny supports six major clades among alcines. These groups are represented by the synthliboramphine murrelets (SA, SW, and SH), the puffins (FA, FC, and FI), the auklets (PA, AY, AC, AP, and CP), the guillemots (CG and CC), the brachyramphine murrelets (BM and BB), and the auks (UA, UL, AT, and AA). The six groups were invariably sustained in the phylogenetic analyses. The robustness of these clades was also underlined by high bootstrap scores (95–100).

The branching pattern among the six major lineages could not be confidently resolved, as revealed by the low bootstrap scores (19–43) supporting these nodes. The ML method, while supporting the same major clades as MP and CLUSTAL, resulted in a different topology. The lengths of those branches defining the divergent topology were not significantly different from zero. A recurrent effect of the modifications that confined the analyses to conservative changes was a tendency for puffins to cluster with auklets (Fig. 3). The affinities of guillemots and both groups of murrelets were the least stable, and the affinity of each group tended to shift between the auks and each other.

The congeneric species BB and BM are clearly monophyletic, as are CC/CG and SA/SW. The latter cluster was supported by a bootstrap score of 98, and SH was the sister group of SA/SW (ND6 gene data only). A branching order was also clearly established among the puffins; FA and FC were the least divergent species pair among all alcines, and FI was the sister group of these.

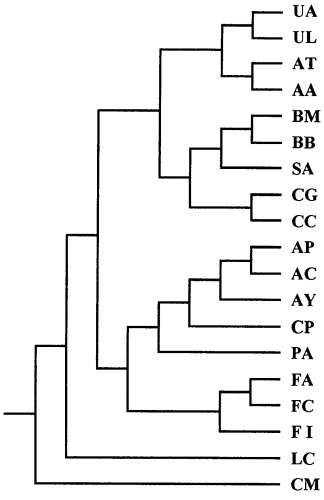

FIG. 3. ML tree for the Alcinae. A conservative approach was applied in which only transversional changes were counted in the third codon positions of the ND6 gene and 22 nucleotide positions of the 12S gene were omitted to ascertain that only homologous nucleotides were analyzed. Hence, 1006 nucleotide positions were analyzed in 17 alcine species and two outgroup representatives. The tree was arbitrarily rooted at CM. Species abbreviations are given in *Materials and Methods*.

In most phylogenetic reconstructions suggested by the molecular data, PA was the sister group of the remaining auklet species, although supported by a bootstrap score of

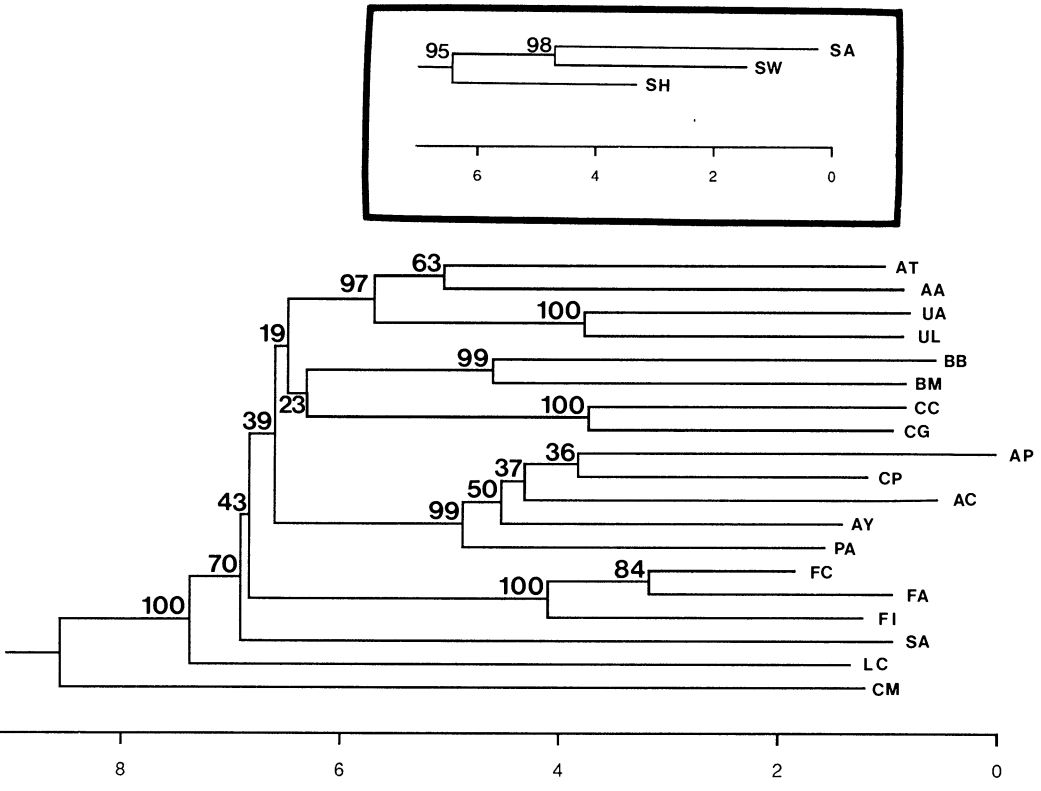

FIG. 2. Phylogenetic tree for the Alcinae based on sequence analysis using the CLUSTAL and MP methods. Seventeen alcine species and two outgroup representatives were included. All substitutions in 1028 nucleotide positions from the 12S and ND6 mitochondrial genes were analyzed. The scale indicates percent divergence and branch lengths were determined by using the CLUSTAL method. Values at the nodes represent bootstrap replication scores (based on 100 resamplings) obtained with the MP method. The phylogenetic tree was outgroup-rooted by the domestic fowl (*G. gallus*). (*Inset*) Relationships among synthliboramphine murrelets, based on ND6 gene data only (12S gene data were not available for SW and SH). Species abbreviations are given in *Materials and Methods*.

only 50 in the main analysis. In a separate MP analysis of the auklets using a puffin (FI) as the outgroup, this relation was supported by a bootstrap score of 83. This analysis suggested a second split-off of AC, then a third split between the AY and the AP/CP clade, but the bootstrap scores were still below 50.

A close relationship between the congeneric species UA and UL was maintained by the mtDNA data. Analysis of the 12S gene alone, the combined data, and conservative changes in general supported the dichotomy between UA/UL and AT/AA (Fig. 2), whereas the separate analysis of ND6 favored a first split-off of AA (bootstrap score 51).

Molecular Clock Calculations. The mtDNA divergence estimates used for the calibration (mean corrected values based on TVs) were 8.7% (GG versus ciconiiform species), 5.2% (CM versus larids), and 2.9% (LC versus alcines). Calibration against the DNA·DNA hybridizations indicated a mtDNA divergence rate based on TVs of 0.11% per million years. One instance of excessive rate heterogeneity was noted, that of AP (Table 1 and Fig. 2), and this species was consequently omitted from the clock calculations. The mean divergence estimates among the species groups (Table 2) suggest that an initial period of cladogenesis among the alcines lasted from about 20 to 14 MYA. Most subsequent speciations seem to have occurred less than about 5 MYA (Fig. 4).

DISCUSSION

Phylogeny. The mtDNA analysis clustered AA with AT, UA, and UL as a member of the auk lineage. Such a relation was hinted at by Storer (15) and was also supported by Strauch's (2) analysis. The latter study further suggested that AA is the sister group of the other auks. This possibility was supported by analysis of the ND6 gene alone, but taken together, the sequence data indicate a common ancestor of AA and AT.

The relationships among the five auklet species is less clear. The strongest support was for PA as the sister group of all other auklets, a relationship also indicated by other studies (refs. 2, 3, and 16; Fig. 1). AP clustered more readily with CP than with its congeners, AY and AC. The mtDNA analysis supports the conclusion of Strauch (2) that *Cyclorrhynchus* should be combined with *Aethia*, but additional analyses are needed to clarify the relationships among auklet species.

The phylogeny suggested by the mtDNA sequence data (Fig. 2) is mostly compatible with that of Strauch (Fig. 1*A*). The contention by Strauch and others that synthliboramphine and brachyramphine murrelets are not closely related was supported. Furthermore, the mtDNA analysis showed SA/SW and SH to be divergent lineages within the same clade (Fig. 2 *Inset*). The considerable divergence of SA/SW to SH seems to justify the retention of the genus *Endomychura* for SH, contrary to the suggestion of Strauch.

The unexpected finding of Watada *et al.* (3) that UL is genetically closer to *Cepphus* than to its congener UA was refuted by the present study. We found congeneric species (except *Aethia*) to cluster most closely. Such a result, in agreement with traditional classifications, lends some confidence to the mtDNA analysis.

Table 2. Mean corrected divergences in percent based on TVs among the six species groups recognized in this study

	Puffins	Auklets	Auks	Sy. murr.	Br. murr.
Auklets	1.7	—			
Auks	1.5	2.1	—		
Sy. murr.	1.8	2.2	1.9	—	
Br. murr.	2.2	2.3	2.2	2.2	—
Guill.	2.0	2.3	1.9	2.2	2.2

Sy. murr., synthliboramphine murrelets; Br. murr., brachyramphine murrelets; Guill., guillemots.

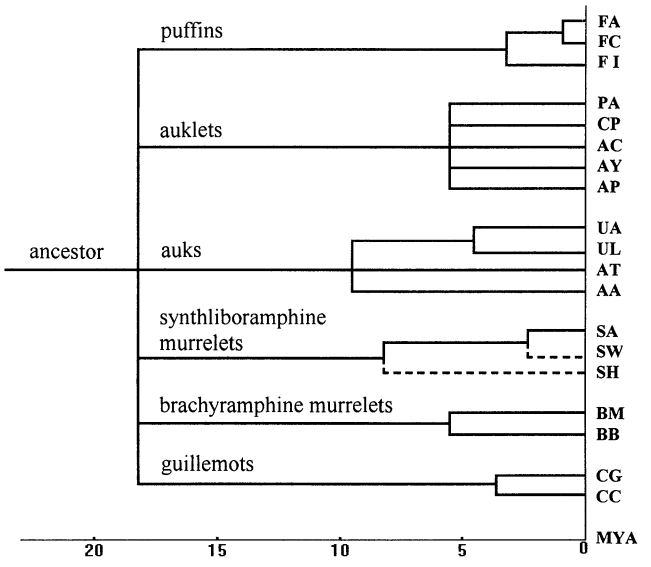

FIG. 4. Hypothetical reconstruction of alcine evolutionary history. The results of the phylogenetic analyses have been tentatively related to a time scale under the assumption of a divergence rate based on TVs of 0.11% per million years. In those cases for which the exact phylogeny could not be determined, the divergence estimates were averaged among the lineages involved. This pertained to the six species groups, to all auklets, and to the unresolved trichotomy among auks. The divergence times of SH and SW, for which 12S gene data were not available, have been indicated by dashed lines. These were estimated by assuming a ND6/12S gene ratio in TVs equal to the average among other alcines.

Divergence estimates based on TVs (Table 2) revealed that puffins and auks are the least divergent species groups. There is no other evidence suggesting a close relationship between puffins and auks. A probable explanation is that puffin and auk mtDNAs evolve at a lower-than-average rate among alcines, possibly caused by the elevated generation times in these species groups (17). If this is taken into account, a relationship between puffins and auklets seems likely. This is also supported by the phylogenetic analyses of conservative nucleotide sites (Fig. 3). The binary division of the Alcinae into a puffin/auklet and an auk/murrelet/guillemot clade is supported by the study of Watada *et al.* (ref. 3; Fig. 1*B*) and to some extent by the present one (Fig. 3). This dichotomy apparently represents an early split of the alcines into a lineage characterized by adaptations for plankton-feeding (highly specialized auklets and intermediate puffins) and a lineage consisting mainly of fish-feeders (see ref. 18).

Evolutionary History. The comparisons based on TVs among all species groups are remarkably similar (Table 2). A likely reason why the sequence data were unable to resolve the branching order among the alcine species groups is that a period of rapid cladogenesis occurred early in alcine evolution. This is a situation similar to that observed in pecoran ruminants (19) and bovids (20). The same consideration could apply to the assembly of auklet species.

Transversional substitutions in mitochondrial genes have been shown to accumulate approximately linearly with time and have been used in clock calculations involving vertebrate species (19, 20). A poor fossil record makes clock calibrations in birds inherently difficult. A calibration based on DNA·DNA hybridizations was suggested by Sibley and Ahlquist (1). The original notion of a simple relationship between $\Delta T_{50}H$ values and time has been modified by the authors themselves. It is now thought that the average rate of molecular evolution may be correlated to generation times and possibly to other demographic factors (1, 21). Therefore, the present calibrations must be considered provisional. For

bird species with an age of more than 2 years at first breeding, a calibration based on a $\Delta T_{50}H$ 1.0 = 3.0 million years is tentatively suggested by C. G. Sibley (personal communication). We calibrated mtDNA divergence against the molecular clock based on DNA·DNA hybrid melting temperatures. This approach is admittedly flawed by possible errors associated with calibration of two molecular clocks instead of one but has the advantage of being derived from the same group of organisms (22).

A mean divergence of all intergroup comparisons of 2.0% indicates that an initial period of alcine cladogenesis occurred about 18 MYA in Early or Middle Miocene. This is later than the period that Bédard (23) suggested as the most probable for alcine radiation (Middle and Late Oligocene). According to Bédard, ecological opportunities in Late Oligocene were presumably conducive to adaptive radiation of efficient diving animals that could exploit the increase in oceanic productivity caused by the cooling of ocean surface temperatures. Bédard claims that this was less likely in the warmer Miocene. However, a comprehensive summary by Warheit (24) suggests that improved ecological conditions also occurred in Middle Miocene. A permanent ice cap in East Antarctica formed between 15 and 13 MYA, which steepened the latitudinal thermal gradient. Tectonic events restricted equatorial flow in both the eastern and western Pacific. These events caused upwelling of nutrient-rich water in California and an increase in planctonic productivity at about 13–11 MYA. During the same time period (13–8 MYA), alcine fossil diversity in the Pacific increased dramatically (24). Therefore, convincing evidence is in favor of an adaptive radiation of alcines somewhat later than suggested by the molecular datings. Refinement of the molecular datings or other evidence is required before we can make any conclusions on this matter.

Subsequent alcine evolution has probably involved geographical isolations and ecological specializations. The existence of alcine exchanges through the Bering Strait probably had a major effect (23). The Bering Strait opened for a brief period toward the end of Miocene and later closed and opened several times in concordance with the Quaternary glaciations, thereby allowing faunal exchanges. On the basis of the divergence times alone (Fig. 4), about 10 speciation events dating to Pliocene and Pleistocene may be related to interoceanic exchanges. Also, much of the late differentiation probably occurred in Pacific refugia created by changing sea levels, varying ice cover, and associated landmass barriers to dispersal (15, 25). The most notable refugia were the Sea of Okhotsk, the Japan Sea, and the Bering Sea.

The auk lineage represents a special case in which speciation events seem to have occurred in the intervening period between the two main periods of speciation depicted in Fig. 4. The presence of almost all living and fossil specimens of *Alca* and *Alle* in the North Atlantic indicates that the auk lineage (except *Uria*) evolved in the Atlantic Ocean. The fossil allied to *Alca* (*Miocepphus mclungi*) from Mid-Miocene deposits of Maryland supports this interpretation (26). According to the divergence times suggested by the molecular data, *Miocepphus* was probably close to the common ancestor of this lineage. If Bédard (23) is correct that *Uria* evolved in the Pacific, it is probable that its ancestor found a route from the Atlantic to the Pacific Ocean in Late Miocene that predates the opening of the Bering connection.

We thank C. G. Sibley for comments and criticism and R. Barrett for improving the English. This study was supported by the Norwegian Research Council.

1. Sibley, C. G. & Ahlquist, J. E. (1990) *Phylogeny and Classification of Birds* (Yale Univ. Press, New Haven, CT).
2. Strauch, J. G., Jr. (1985) *Auk* **102,** 520–539.
3. Watada, M., Kakizawa, R., Kuroda, N. & Utida, S. (1987) *J. Yamashina Inst. Ornithol.* **19,** 79–88.
4. Moum, T. & Johansen, S. (1992) *Genome* **35,** 903–906.
5. Jones, C. S., Tegelström, H., Latchman, D. S. & Berry, R. J. (1988) *Biochem. Genet.* **26,** 83–88.
6. Hultman, T., Ståhl, S., Hornes, E. & Uhlén, M. (1989) *Nucleic Acids Res.* **17,** 4937–4946.
7. Moum, T., Willassen, N. P. & Johansen, S. (1994) *Curr. Genet.* **25,** 554–557.
8. Desjardins, P. & Morais, R. (1990) *J. Mol. Biol.* **212,** 599–634.
9. Higgins, D. G. & Sharp, P. M. (1989) *Comput. Appl. Biosci. Commun.* **5,** 151–153.
10. Saitou, N. & Nei, M. (1987) *Mol. Biol. Evol.* **4,** 406–425.
11. Felsenstein, J. (1993) *PHYLIP: Phylogeny Inference Package*, Version 3.5c (Dept. of Genet., Univ. of Washington, Seattle).
12. Felsenstein, J. (1985) *Evolution* **39,** 783–791.
13. Edwards, S. V., Arctander, P. & Wilson, A. C. (1991) *Proc. R. Soc. London B* **243,** 99–107.
14. Tajima, F. & Nei, M. (1984) *Mol. Biol. Evol.* **1,** 269–285.
15. Storer, R. W. (1952) *Univ. Calif. Publ. Zool.* **52,** 121–222.
16. Storer, R. W. (1945) *Ibis* **87,** 433–456.
17. Hudson, P. J. (1985) in *The Atlantic Alcidae*, eds. Nettleship, D. N. & Birkhead, T. R. (Academic, London), pp. 233–261.
18. Bédard, J. (1969) *Ibis* **111,** 189–198.
19. Kraus, F. & Miyamoto, M. M. (1991) *Syst. Zool.* **40,** 117–130.
20. Allard, M. W., Miyamoto, M. M., Jarecki, L., Kraus, F. & Tennant, M. R. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 3972–3976.
21. Martin, A. P. & Palumbi, S. R. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 4087–4091.
22. Hillis, D. M. & Moritz, C. (1990) in *Molecular Systematics*, eds. Hillis, D. M. & Moritz, C. (Sinauer, Sunderland, MA), pp. 502–515.
23. Bédard, J. (1985) in *The Atlantic Alcidae*, eds. Nettleship, D. N. & Birkhead, T. R. (Academic, London), pp. 1–51.
24. Warheit, K. I. (1992) *Paleobiology* **18,** 401–424.
25. Udvardy, M. D. F. (1963) in *Pacific Basin Biogeography: A Symposium*, ed. Gressit, J. L. (Bishop Museum Press, Honolulu), pp. 85–111.
26. Olson, S. L. (1985) in *Avian Biology*, eds. Farner, D. S., King, J. R. & Parkes, K. C. (Academic, Orlando, FL), Vol. 8, pp. 79–238.