A PARTIAL CLASSIFICATION OF WATERFOWL (ANATIDAE) BASED ON SINGLE-COPY DNA

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ABSTRACT.—Single-copy DNA-DNA hybridization was used to establish phylogenetic relationships among 13 species of waterfowl (Anatidae) chosen from 10 tribes. On the basis of UPGMA clustering of ΔT_m distances, we suggest that the tribes Anatini, Aythyini, Tadornini, Mergini, and Cairinini diverged more recently than the Anserini, Stictonettini, Oxyurini, Dendrocygnini, and Anseranatini. The Freckled Duck (*Stictonetta naevosa*, tribe Stictonettini) is only distantly related to the other Anatidae. Presumably the lineage diverged very early. The sheldgeese (tribe Tadornini) and the true geese (Anserini) are only remotely related. The Oxyurini, considered to be in the subfamily Anatinae, is remotely related to the other Anatidae. The Dendrocygnini form an isolated tribe with no close relationship to the swans and geese (subfamily Anserinae). We found that the screamers (Anhimidae) are distantly related to the Anatidae.

A method to estimate missing cells in a data matrix of pairwise distances is presented.
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RECENTLY, nucleic acid sequence analysis has been used to estimate relationships of many organisms. The analysis can include direct sequencing of individual cloned genes or gene products, restriction enzyme analysis of homologous DNA fragments, and general analysis of unique DNA by single-copy DNA-DNA hybridization. In higher eukaryotes, perhaps only 5–10% of the DNA (including gene-coding regions and a small fraction of the single-copy DNA) is under selection (Britten 1986). The major proportion of DNA, unique as well as repetitive, consists of nucleotide sequences that are never expressed as proteins, have no known function, and are therefore presumably free to evolve (Britten 1986).

Although both single-copy and repeated DNA comparisons have been used in studies of phylogenetic relationships in birds (Shields and Strauss 1975; Eden et al. 1978; Sibley and Ahlquist 1982, 1983), the total DNA of higher eukaryotes is composed of many subsets of DNA that show varying degrees of reiteration (Britten and Kohne 1968). These include the extremes of specific sequences, present as only one copy per genome through sequences repeated millions of times. The repeated families show sequence as well as copy-number evolution. Sequences that occur only a few times in one organism may occur many times even in a closely related species (Zwiebel et al. 1982). Depending on reassociation conditions, the repetitive DNA fraction in bird genomes makes up about 10–40% of the total DNA (Eden et al. 1978) but contains only a small percentage of the total sequence diversity. The remaining 60–90% are unique sequences and represent about 98% of the total sequence complexity (Sibley and Ahlquist 1983).

The most convenient procedure for sequence comparison of total DNA of different species involves measurement of the thermal stability of reannealed heteroduplex single-stranded DNA. Because repetitive DNA sequences reanneal first, such sequences might contribute in a disproportionate way to the thermal stability of resulting duplexes (Sibley and Ahlquist 1983). For this reason the repetitive DNA fraction is usually removed. Nevertheless, repetitive sequences, because of rapid evolution, can also provide valuable data on the relationship of recently evolved species (Gillespie 1977, Arnason and Widegren 1986).

Although the fossil record of birds is incomplete, that of waterfowl appears better than in many other families (Olson and Feduccia 1980). Paleontological investigations show that the major waterfowl radiation probably took place within the last 60 million years (Delacour and Mayr 1945, Olson and Feduccia 1980). These events fall within the sensitivity range of DNA-DNA hybridization proposed by Sibley and Ahlquist (1983).

We propose a phylogeny of the Anatidae based on single-copy DNA relationships among samples from 13 of the 148 species of waterfowl

belonging to 10 traditionally defined tribes. Single-copy DNA sequence relationships among these waterfowl and three other avian species (Northern Screamer, *Chauna chavaria*; Spur-winged Plover, *Vanellus spinosus*; and domestic chicken, *Gallus gallus*) were investigated.

Materials and Methods

Material came from Mergus Waterfowl Farms (Tallahassee, Florida). Species were the White-faced Whistling-Duck (WW) (*Dendrocygna viduata*), Plumed Whistling-Duck (*D. eytoni*), Bar-headed Goose (BG) (*Anser indicus*), Red-breasted Goose (*Branta ruficollis*), Ruddy Duck (RD) (*Oxyura jamaicensis*), Orinoco Goose (OG) (*Neochen jubata*), Hooded Merganser (HM) (*Lophodytes cucullatus*), Wood Duck (WD) (*Aix sponsa*), White-eyed Pochard (WP) (*Aythya nyroca*), Mallard (ML) (*Anas platyrhynchos*), White-cheeked Pintail (WC) (*Anas bahamensis*), and the nonanatid Spur-winged Plover and domestic chicken. Northern Screamer (SC) material came from Mary Dam (Haines City, Florida), the Magpie Goose (MG) (*Anseranas semipalmata*) and Trumpeter Swan (TS) (*Cygnus buccinator*) from Michael Lubbock (Sylva, North Carolina), and the Freckled Duck (FD) (*Stictonetta naevosa*) from the Wildfowl Trust (Slimbridge, United Kingdom). Gamma-labeled ATP^{32} was obtained from ICN (Irvine, California).

Isolation of DNA.—DNA was isolated from blood cells by a procedure modified from Blin and Stafford (1976). Blood was obtained from a wing vein and collected in a heparinized syringe. After one-fold dilution with 0.15 M NaCl containing 0.05 M EDTA at pH 7.6 (buffer A), cells were collected by centrifugation, resuspended in 25 volumes of buffer A, and lysed by the addition of 0.3% sodium dodecyl sulfate. An equal volume (with respect to the total lysate) of redistilled phenol, equilibrated with 0.1 M Tris-HCL buffer pH 9.0, was added, and the mixture was shaken in a gyrotory waterbath overnight at room temperature (25°C). After the addition of one-half volume of chloroform, the phases were separated by centrifugation, the aqueous phase collected, and the crude DNA precipitated by the addition of an equal volume of absolute ethanol. The DNA was washed with 70% ethanol and dissolved in TE (0.01 M Tris-HCL buffer pH 7.6, 0.001 M sodium EDTA) (5 ml/ml original blood). After complete solution was obtained, 0.5 ml of 1.0 M Tris-HCL buffer pH 8.0 was added followed by 100 μg of pancreatic ribonuclease. The mixture was incubated for 30 min at 37°C, 5 mg of pronase was added, followed immediately by 0.2 ml of 10% sodium dodecyl sulfate, and the solution was incubated for 2 h at 60°C. The mixture was again deproteinized by shaking overnight with an equal volume of phenol saturated with 0.1 M Tris-HCL buffer pH 9.0, the phases were separated by centrifugation, and the DNA was precipitated with two volumes of cold ethanol after the addition of ammonium acetate to a final concentration of 2.5 M. After briefly washing with 70% ethanol, the DNA was dissolved in TE buffer and the concentration adjusted to 1.0 mg/ml.

Isolation of single-copy DNA.—A DNA fraction enriched for single-copy material was isolated as described by Sibley and Ahlquist (1983). DNA was sheared by sonication into fragments with an average length of 500 nucleotides. Fragment size was determined by electrophoretic comparison with calibrated markers. The DNA was denatured by immersion in boiling water for 10 min and reannealed at 50°C in 0.48 M sodium phosphate buffer (pH 7.0) to a Cot of 1,000. A single stranded fraction was isolated by hydroxyapatite chromatography at 50°C. The fraction was dialyzed against TE buffer, concentrated with isobutanol (Maniatis et al. 1982), and precipitated with ethanol. After washing with 70% ethanol the DNA was dissolved in TE buffer and the concentration adjusted to 1.0 mg/ml.

Radio-labeling of single-copy DNA.—Single-copy DNA was labeled with 32P at the 5′ terminus by incubation with polynucleotide kinase and gamma-labeled ATP^{32}. A 5′ terminus labeling kit was obtained from Bethesda Research Laboratories and used according to the instructions provided by the manufacturer. The specific activity of the radio-labeled DNA prepared by this procedure averaged 5×10^6 counts/μg of DNA. The labeled DNA was freed of unincorporated labeled ATP by the spin-column technique (Maniatis et al. 1982). The maximum amount of unincorporated labeled ATP contamination was less than 3%. Terminal labeling was substituted for iodination (Sibley and Ahlquist 1983) for safety reasons.

Hybridization of single-copy tracer DNA with driver DNA.—Hybrids were formed from a mixture composed of 1 part (=200 ng) radio-labeled single-copy DNA and 1,000 parts (=200 μg) sheared, whole DNA (Sibley and Ahlquist 1983). Hybrids were denatured by immersion in boiling water for 10 min, precipitated with ethanol, and redissolved in 0.5 M sodium phosphate (pH 7.0). Reannealing was carried out at 60°C until a Cot of 8,000 was reached. The formation of double-stranded hybrid DNA was analyzed by thermal elution from hydroxyapatite. Thermal elution columns were submerged within an insulated column box connected to a Haacke temperature-controlled (±0.1°C) circulating waterbath. After application of the samples to the column (200 μg of DNA/ml of hydroxyapatite) at 55°C in 0.03 M sodium phosphate, the column was washed with 40 ml of 0.12 M sodium phosphate (pH 7.0) to remove the unbound tracer. The last fraction of the wash (5 ml) was counted in a liquid-scintillation counter, and radioactivity was determined to be no higher than the normal background, indicating that the unbound tracer was eluted from the column. At each of the eight temperatures (60–95°C in 5°C increments) the single-stranded DNA was eluted with 10 ml of 0.12 M sodium phosphate

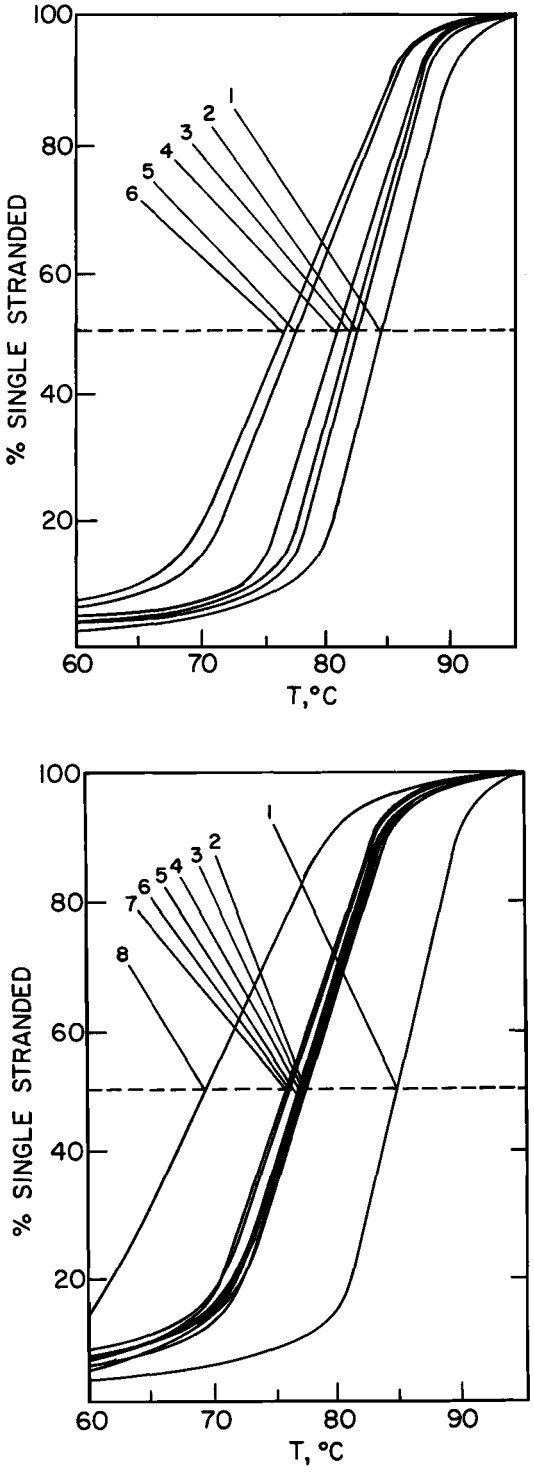

Fig. 1. Top: Thermal dissociation curves in which the tracer DNA of the Hooded Merganser (*Lophodytes cucullatus*) was hybridized with the driver DNAs of Hooded Merganser (1), Orinoco Goose (*Neochen jubata*; 2), Mallard (*Anas platyrhynchos*; 3), Wood Duck (*Aix sponsa*; 4), Ruddy Duck (*Oxyura jamaicensis*; 5), and White-faced Whistling-Duck (*Dendrocygna viduata*; 6). Bottom: Thermal dissociation curves in which the tracer DNA of the White-faced Whistling-Duck was hybridized with the driver DNAs of White-faced Whistling-Duck (1), Mallard (2), Hooded Merganser (3), Orinoco Goose (4), White-eyed Pochard (*Aythya nyroca*; 5), Wood Duck (6), Ruddy Duck (7), and Spur-winged Plover (*Vanellus spinosus*; 8).

(pH 7.0) and counted in a liquid-scintillation counter. The average number of samples chromatographed simultaneously was 6, with a maximum number of 10. We used T_m to contrast homoduplex thermal dissociation distribution with those of heteroduplexes (Sibley and Ahlquist 1983, Sheldon 1987). Figure 1 gives examples of thermal elution curves from which ΔT_m values were extrapolated. Each 1° difference for ΔT_m values indicates a 1% divergence in nucleotide sequences (Bonner et al. 1973). All other statistics (percentage hybridization [%H], normalized percentage hybridization [NPH], and $T_{50}H$) were calculated as described by Sheldon (1987).

To calibrate ΔT_m and $T_{50}H$ values with time, one must assume that DNA evolves at a constant rate (Sibley and Ahlquist 1983). In view of recent evidence suggesting a nonconstant rate (Britten 1986, Sheldon 1987), we use ΔT_m values only to estimate the branching order.

Construction of the data matrix and phylogenetic tree.— A 13 × 13 matrix was constructed (Table 1) from the average ΔT_m values for each pairwise comparison. The 23 missing ΔT_m values required for constructing a complete matrix were estimated using the following rationale. First, a distance function must satisfy the triangle inequality:

$$d_{ij} < d_{ik} + d_{jk}.$$

Second, some distance functions satisfy a stronger rule called the "ultrametric inequality" (Hartigan 1975):

$$d_{ij} < \max(d_{ik}, d_{jk}).$$

If a distance function satisfies the ultrametric inequality, then a matrix of such pairwise distances has an exact representation as a tree.

Conversely, any tree has an exact representation in terms of a matrix of distances that satisfy the ultrametric inequality (Hartigan 1975). We estimated missing values from the ultrametric inequality. For example, if d_{23} was missing, then $d_{2k} < \max(d_{2k}, d_{3k})$ for

TABLE 1. Mean ± SD ΔT_m values.[a] See text for the method of estimation of missing values. Sample sizes are given in parentheses.[b]

	ML	WC	WP	WD	OG	HM	BG	TS	FD	RD	WW	MG
WC	1.1 ± 0.2 (4, 2)											
WP	1.7 ± 0.5 (4, 2)	1.7 (est)										
WD	3.0 ± 0.3 (4, 2)	2.8 ± 0.4 (2, 2)	3.2 ± 0.4 (3, 2)									
OG	2.8 ± 0.3 (4, 2)	2.6 (est)	3.0 ± 0.4 (2, 2)	2.9 ± 0.3 (2, 2)								
HM	2.7 ± 0.4 (4, 3)	2.6 ± 0.4 (2, 2)	3.1 ± 0.4 (2, 2)	2.6 ± 0.4 (2, 3)	2.0 ± 0.4 (2, 2)							
BG	6.0 ± 0.5 (4, 3)	5.8 (est)	5.8 (est)	5.8 ± 0.4 (2, 2)	6.2 ± 0.5 (2, 2)	5.8 (est)						
TS	6.2 ± 1.0 (3, 2)	5.8 (est)	5.8 (est)	5.8 (est)	6.5 ± 1.0 (2, 1)	5.8 (est)	4.0 ± 0.4 (2, 2)					
FD	6.5 ± 0.5 (1, 1)	6.1 (est)	6.1 (est)	6.3 (0, 1)	6.1 (est)	6.7 ± 0.6 (1, 1)	6.1 (0, 1)	6.7 ± 0.7 (1, 1)				
RD	7.5 ± 0.5 (3, 3)	7.3 (est)	7.3 ± 0.4 (2, 3)	7.7 ± 0.6 (2, 3)	7.7 ± 0.5 (2, 3)	7.7 ± 0.5 (2, 2)	7.5 ± 0.9 (2, 3)	7.3 ± 0.6 (1, 1)	7.3 (est)			
WW	7.8 ± 0.9 (6, 5)	8.1 ± 0.7 (2, 2)	8.3 ± 0.7 (2, 4)	8.1 ± 0.5 (2, 2)	8.0 ± 0.7 (2, 4)	7.6 ± 0.5 (2, 4)	6.8 ± 1.0 (3, 3)	7.9 ± 0.6 (1, 1)	7.5 ± 0.9 (1, 1)	7.6 ± 0.6 (2, 3)		
MG	9.5 ± 0.8 (4, 3)	8.5 (est)	8.5 ± 0.9 (2, 1)	8.5 (est)	8.5 (est)	8.5 (est)	10.2 ± 1.6) (1, 2)	9.5 (1, 0)	9.0 (1, 0)	9.4 ± 0.8 (2, 2)	9.0 ± 0.9 (2, 2)	
SC	12.0 ± 1.0 (3,2)	10.5 (est)	10.5 (est)	11.2 ± 1.5 (1, 2)	12.2 ± 1.2 (2, 2)	10.5 (est)	10.5 ± 2.1 (1, 1)	10.5 (est)	11.4 ± 0.9 (1, 1)	10.5 (est)	10.5 (est)	10.5 ± 0.9 (1, 1)

[a] ML = Mallard, WC = White-cheeked Pintail, WP = White-eyed Pochard, WD = Wood Duck, OG = Orinoco Goose, HM = Hooded Merganser, BG = Bar-headed Goose, TS = Trumpeter Swan, FD = Freckled Duck, RD = Ruddy Duck, WW = White-faced Whistling-Duck, MG = Magpie Goose, SC = Northern Screamer.

[b] First value is the number of hybrids made with the vertical taxon labeled; second value is the number of hybrids made with the horizontal taxon labeled. Est = estimated value.

TABLE 2. Reciprocal ΔT_m values.

	Mean[a]	n[b]	Range
Anas platyrhynchos	0.7	12	1.1
Anas bahamensis	0.1	4	0.3
Aythya nyroca	0.5	7	0.8
Aix sponsa	0.3	9	0.7
Neochen jubata	0.4	8	1.7
Lophodytes cucullatus	0.3	8	0.8
Anser indicus	1.3	7	2.7
Cygnus buccinator	1.0	6	1.6
Stictonetta naevosa	1.0	5	0.6
Oxyura jamaicensis	0.5	9	0.9
Dendrocygna viduata	0.7	11	1.7
Anseranas semipalmata	1.0	6	2.4
Chauna chavaria	1.1	6	2.8

[a] Average distance between average of a labeled *Anas platyrhynchos* distance from another species and average of the reciprocal test.

[b] Number of species for which reciprocal tests were made with *Anas platyrhynchos*.

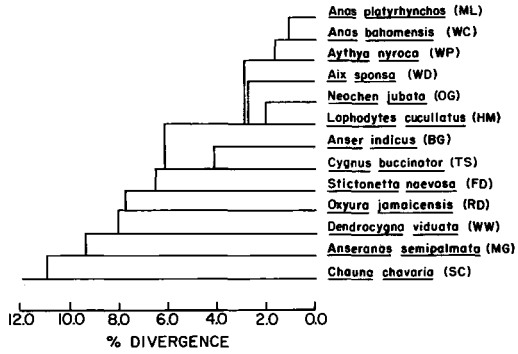

Fig. 2. Dendrogram generated by UPGMA based on DNA-DNA hybridization (ΔT_m) of 13 species of waterfowl representing 10 tribes in 3 subfamilies. See Table 1 for the distance matrix and abbreviations.

all k and hence is bounded above by the minimum of the maxima (Meeter pers. comm.). This estimate, the largest distance consistent with the ultrametric inequality, was used for the missing distances. In the estimate of the ΔT_m for WC and WP (Table 1), 1.7 was the smallest maximum value of all distances between other taxa compared individually with WC and WP. To verify the accuracy of this method, a data matrix was constructed using only species for which complete data were available (*Anas platyrhynchos, Aythya nyroca, Aix sponsa, Neochen jubata, Lophodytes cucullatus, Oxyura jamaicensis,* and *Dendrocygna viduata*). Five cells within this 7 × 7 complete matrix were chosen randomly, and estimated values were compared with actual values. The mean difference between measured and estimated values was 0.26 (SD = 0.11). The difference between measured and estimated values ranged from 0.1 to 0.4.

The distance matrix was clustered by the unweighted pair-group method using arithmetic averages (UPGMA; Sneath and Sokal 1973).

RESULTS

Delta T_m values for a pair of taxa varied from 10% to 30% of the average value (Table 1). In almost all individual experiments, however, the order of the ΔT_m was the same. Discrepancies occurred in some reciprocal values (Table 2). Many of the higher reciprocal ΔT_m values were influenced by the one or two aberrant values relating to a particular species or an abnormally low homoduplex T_m value (see below and Discussion). The NPH values ranged from 90% for species with a ΔT_m of less than 3.0, to 75–90% for those with a ΔT_m of less than 8.0 and more than 3.0, to about 70% for the hybrids of single-copy DNA between the Mallard and the Magpie Goose (*Anseranas*) or the screamer (*Chauna*). Hybrids between single-copy DNA of the Mallard and the total DNA of the chicken or the Spur-winged Plover (*Vanellus*) had NPH values of 40–50%. For unknown reasons NPH values among closely related species often exhibit a high degree of variance (Sheldon 1987). We found a high degree of NPH variance (10–20%) for both distantly related and closely related species. For this reason we used the T_m statistic in lieu of $T_{50}H$. The T_m statistic does not incorporate NPH values when calculated; therefore, any variance in NPH will not obscure the branching pattern.

Species in the subfamily Anatinae (Mallard [Anatini], White-eyed Pochard [Aythyini], Hooded Merganser [Mergini], Wood Duck [Cairini], and Orinoco Goose [Tadornini]) apparently diverged more recently than the other taxa (Fig. 2). These birds exhibited ΔT_m values smaller than 3.2. In addition, the White-eyed Pochard was more closely related to the Mallard (ΔT_m = 1.7) than to the Wood Duck, Hooded Merganser, or Orinoco Goose. The latter two species were consistently more closely related (ΔT_m = 2.0) to each other than to other species. The White-cheeked Pintail (Anatini) was also more closely related to the Mallard (ΔT_m = 1.1) than to the other species tested.

The remaining species showed large ΔT_m values with respect to each other and to the five Anatinae species. The Magpie Goose (Anseranatinae) was the most distant species, with an average ΔT_m value of 9.5 with respect to all other

waterfowl. With respect to the Mallard, the White-faced Whistling-Duck (Dendrocygnini) showed a very early divergence (ΔT_m = 7.8), followed by the Ruddy Duck (Oxyurini; ΔT_m = 7.5), Freckled Duck (Stictonettini; ΔT_m = 6.5), and Bar-headed Goose (Anserini; ΔT_m = 6.0).

Discrepancies among reciprocal values were higher when the number of hybridizations for each comparison was limited. Yet, when the Bar-headed Goose and White-faced Whistling-Duck were compared (n = 6), a large discrepancy in reciprocal values remained. When the Bar-headed Goose tracer DNA was hybridized to the White-faced Whistling-Duck driver DNA, the average ΔT_m was 5.9 (n = 2, SD = 0.14). The reciprocal experiment was repeated twice and had an average ΔT_m of 7.7 (SD = 0.49). The experiment was repeated with a new tracer DNA for each species. The ΔT_m values were within 0.3° of the previous values. The overall ΔT_m averages for the tracer DNAs of the Bar-headed Goose and White-faced Whistling-Duck were 5.9 (n = 3, SD = 0.10) and 7.7 (n = 3, SD = 0.38). A similar condition existed in the Red-breasted Goose and the Plumed Whistling-Duck. The Trumpeter Swan was more closely related to the Bar-headed Goose and the Red-breasted Goose (ΔT_m = 4.0) than any of the waterfowl tested (ΔT_ms > 5.8). The ΔT_m value (4.0) indicates that this swan and goose lineage diverged relatively long ago.

Species from the family Anatidae were compared with the screamers (Anhimidae), Spur-winged Plover (Charadriidae), and domestic chicken (Phasianidae) (data not shown). These data (including reciprocal values) indicated that ducks, geese, and *Anseranas semipalmata* are more closely related to screamers (average ΔT_m = 11.3) than to Spur-winged Plovers (ΔT_m = 14.4) or chickens (average ΔT_m = 14.1).

Discussion

We found that a single-copy DNA similarity based on relationships among different taxa of waterfowl was in good agreement with results based on other criteria. The tribes in the subfamily Anatinae apparently radiated more recently than the Anserinae, Oxyurini, Dendrocygnini, Stictonettini, and Anseranatinae, which diverged much earlier. Our analysis generally agreed with the schemes advanced by Delacour and Mayr (1945), Johnsgard (1961), Brush (1976), and others (Frith 1955, 1964; Kear 1967; Sibley and Ahlquist 1972; Jacob and Glaser 1975; Bottjer 1983; Kessler and Avise 1984; Livezey 1986) who used anatomical, behavioral, and other chemical or immunological data. The branching pattern (Fig. 2) among certain species (e.g. *Lophodytes cucullatus, Neochen jubata,* and *Aix sponsa*) may not represent the true phylogenetic pattern because of the high degree of variance and the closeness of ΔT_m values. Yet, for the species tested two main periods of radiation have occurred. We believe that the Magpie Goose (*Anseranas*) lineage diverged very early (von Boetticher 1940) and the whistling-ducks (Dendrocygnini) and the stifftails (Oxyurini) diverged somewhat more recently from the main lineage. Specific results with respect to the early divergence of the Ruddy Duck (*Oxyura*) are interesting because in virtually all schemes for the classification of waterfowl this tribe has been included in the subfamily Anatinae. Delacour and Mayr (1945) considered the Oxyurini a predabbler; Johnsgard (1978) considered the Oxyurini also to be in the Anatinae and to have diverged after the shelducks. Feather-protein data (Brush 1976) and morphological characteristics (Livezey 1986) have supported a possible link between the Oxyurini and the seaducks (Mergini). The large ΔT_m value between members of these taxa is inconsistent with this interpretation. Feather protein and morphological similarities may be the result of convergent evolution due to diving in both tribes.

The Freckled Duck gave large ΔT_m values (>6.0) compared with all other waterfowl. This species was thought to be an aberrant member of the tribe Anatini (Delacour and Mayr 1945). Our data support the hypothesis advanced by Frith (1964) and Brush (1976) that *Stictonetta* is only distantly related to the other Anatidae and that its lineage must have diverged early from the main lineage leading to other waterfowl.

We found that different values for the ΔT_ms between a pair of species were generally stable, although never as stable as reported by other investigators (Sibley and Ahlquist 1983, Sheldon 1987). A number of factors could produce variance in ΔT_m values. One is the effect of homoduplex T_m variance on calibrations of heteroduplex ΔT_m values. The majority of homoduplex T_m values fell close to the ideal 85°C proposed by Sheldon (1987), but a few were 1–2° below; none were higher. A correction factor was not

employed (as done by Sheldon 1987) because the causes of variance (e.g. tracer DNA impurity or improper labeling) in the homoduplex T_m might equally affect the heteroduplex T_m. Decisions about which values, if any, should be corrected were difficult because the number of hybridizations was low. The small number of experiments for each comparison and mechanical error may contribute to the increased variance. Producing exact elution rates, column temperatures, and fraction volumes is difficult when performing DNA-DNA hybridization experiments. This was evident from the observed higher degree of interexperimental vs. intraexperimental variance.

Variance in reciprocal values was usually no greater than the degree of variance exhibited for interexperimental ΔT_m values. The most serious discrepancies were between the geese and the whistling-ducks. We have no explanation for this anomalous result. Nonreciprocity in ΔT_m values has been considered by other investigators as an indication of "experimental quality" (Sheldon 1987). The consistent difference in reciprocal values (1.8) for these two species, however, indicates that some factor other than experimental error was involved. The rather large variance precludes the resolution of inequalities of DNA evolution rates.

The DNA of several species of waterfowl, including the Bar-headed Goose, contains many "sequence families" that consist of considerable amounts of repetitive DNA (McHugh, Madsen, and de Kloet in prep.). The reiteration frequencies of the sequences in these families can differ considerably even among closely related species. Certain sequences that are barely detectable in one species are found in multiple copies in others (sometimes accounting for 5–10% of the total DNA). This phenomenon of sequence differences among closely related species also occurs in *Drosophila* (Zweibel et al. 1982). We have not determined the actual degree of sequence divergence among the members of such families of reiterated DNA in any species, but in some cases such sequences may have diverged farther than in others. This would create a disparity in the actual sequence complexity for species' tracer DNA. The conditions (0.5 M sodium phosphate at 60°C) for hybridization in general usage for taxonomic studies are considered to be of low stringency. Especially for heterologous hybrids, reciprocal tracer and driver combinations may give different results. The reiterated sequences in the driver may be partly responsible for the discrepancies. In experiments where single-copy instead of total DNA is used as the driver, the difference between the reciprocal values is reduced by approximately 50% (Madsen and de Kloet in prep.).

Despite the inconsistencies, the Anserini lineage apparently diverged relatively early in waterfowl evolution, although probably later than the Dendrocygnini. Although these tribes are classified in the same subfamily (Anserinae), they are not as closely related to one another as geese are to swans. We believe that the Orinoco Goose (Tadornini) and the Bar-headed Goose are only remotely related, and that their morphological and behavioral similarity is the result of convergent evolution (Delacour and Mayr 1945, Johnsgard 1961).

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