PHYLOGENETIC RELATIONSHIPS AMONG CRANES (GRUIFORMES: GRUIDAE) BASED ON DNA HYBRIDIZATION

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ABSTRACT.—I used a DNA-DNA hybridization method to generate more than 1,200 pairwise comparisons among species of the family Gruidae (cranes) and an outgroup Limpkin (Aramidae). A matrix of genetic distances included average delta T_m values for all cells, and reciprocals among cranes, based on 3–10 replicate experiments per cell. I chose delta T_m as the appropriate dissimilarity measure because virtually all homologous single-copy DNA sequences in crane genomes were sufficiently similar to form hybrid duplexes under standard experimental conditions. The normalized percent hybridization (NPH) values approached 100 for all crane species pairs. The delta T_m data departed slightly from metricity as a result of experimental variation associated with the measurement of small genetic distances. The DNA data, analyzed with a best-fit tree approach and checked for internal consistency by jackknifing, support the traditional view that crowned cranes (*Balearica*) are the most ancient lineage of extant gruids. The enigmatic Siberian Crane (*Grus leucogeranus*) appears as the sister group of the remaining species, which fall into four closely related groups. *Bugeranus* and *Anthropoides* are sister groups. The three species of Australasian *Grus* (*antigone, rubicunda,* and *vipio*) form a clade, as do five predominantly Palearctic *Grus* (*grus, monachus, nigricollis, japonensis,* and *americana*). The Sandhill Crane (*G. canadensis*), while clearly a member of the gruine clade, is an old lineage without close relatives. *Received 21 March 1989, accepted 18 May 1989.*

THE CLASSIFICATIONS of Peters (1934) and Wetmore (1934) established the traditional family-level distinction of cranes (Gruidae) within the Order Gruiformes. This arrangement, though not universally accepted (Cracraft 1973), has persisted in most recent revisions (e.g. Wetmore 1960, Storer 1971, Sibley et al. 1988). Opinions regarding species affinities within the Gruidae have varied greatly. Following Peters (1934), most workers have accepted the existence of 14 extant species in four genera, though Walkinshaw's (1964) designation of a 15th species has gained many adherents. The crowned cranes (*Balearica*) are commonly placed in the Balearicinae, apart from the remaining species (Gruinae; Brodkorb 1967) based on their lack of sternal excavation and tracheal convolution. Balearicines, apparently the more ancient lineage, are abundantly represented by Tertiary fossil materials from western Eurasia (Brodkorb 1967). Gruine fossils appear in the Miocene of Europe, but are best represented in North American Pliocene deposits and by a scattering of Pleistocene remains worldwide (Johnsgard 1983). Cracraft (1973) inferred from these materials that cranes diverged from a common ancestor with limpkins (Aramidae) in the late Paleocene.

Archibald (1975, 1976) performed the first comprehensive analysis of cranes based on a coherent set of characters. His study of crane unison calls (stereotyped behavior patterns involved in pair-bonding) led him to identify clusters of similar species (Fig. 1). Archibald's work verified the distinctness of crowned cranes (*Balearica*) and suggested an unexpected relationship between *Bugeranus carunculatus* (Wattled Crane) and *Grus leucogeranus* (Siberian Crane). Archibald included *leucogeranus* as a member of *Bugeranus*, and recommended "Species Group" status for the remaining *Grus* and *Anthropoides* clusters. Wood (1979), like Archibald, found a high level of similarity between Wattled and Siberian cranes, which, aside from complicated multivariate resemblances, lack the exaggerated tracheal convolutions present in other gruines.

Ingold et al. (1987) attempted to resolve crane relationships with allele-frequency analysis. Their species arrangement was a major departure from traditional views, although *Balearica* appears distinct from gruines. Their work involved small sample sizes, and only approxi-

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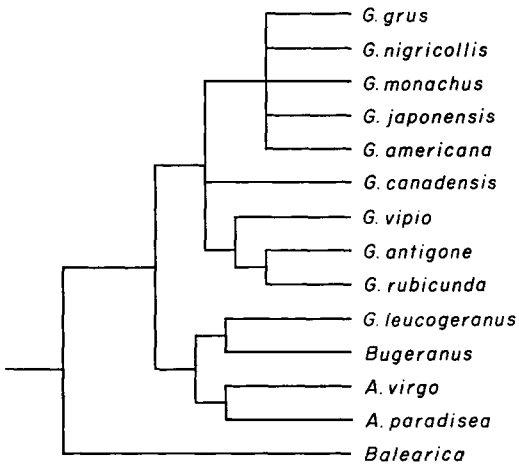

Fig. 1. Crane relationships as indicated by overall similarity in their unison calls (Archibald 1975; figure redrawn from Wood 1979). The horizontal axis is arbitrary, showing only Archibald's view of nested similarity levels.

mately two birds per species were assayed for allele-frequencies.

Based on a very limited DNA hybridization study of the cranes, Ingold (1984) concluded that substantial genetic divergence exists between putative *Balearica* species, and that *G. leucogeranus* was not particularly "close" to *Bugeranus*, contra Archibald (1975, 1976) and Wood (1979). The latter conclusion seems difficult to defend in light of Cracraft's (1987) criticisms regarding the inadequacies of partial genetic distance data for resolving relationships.

I derive a phylogeny for the cranes based on a complete matrix of pairwise DNA hybridization comparisons. Previous avian work with this technique (see references in Sibley et al. 1987) has focused on high-level taxonomy, though Sheldon (1987a) used it to resolve intrafamilial relationships among herons (Ardeidae). Crane relationships are predominantly those of congeneric species, and previous work has left some doubt as to the resolving power of DNA hybridization at this level. I will show that the method provides limited resolution among cranes, yet, with numerous replicate comparisons, it is capable of defining clusters of phylogenetically related species.

METHODS

DNA hybridization.—The biochemical protocol for DNA hybridization was essentially that described by Sibley and Ahlquist (1981, 1983). Detailed descriptions are given in Krajewski (1988) and Springer and Kirsch (1989). DNA was recovered from erythrocytes lysed with sodium lauryl sulfate in TNE buffer, purified by repeated phenol/chloroform extractions, and treated with protease and RNase (Maniatis et al. 1982). Native DNA was fragmented into short strands (100–2,000 bp) using high-frequency sound. Fragment size distributions were monitored for each sample by agarose gel electrophoresis and comparison with commercial size markers.

Single-copy sequences (scDNA) were recovered from each species using the reassociation-kinetic and hydroxyapatite column-chromatography methods of Kohne and Britten (1971). Sheared DNA was boiled and incubated at 60°C in 0.48 M phosphate buffer (PB) to a C_0t of 200, diluted to 0.12 M PB, applied to hydroxyapatite (HAP) columns at 60°C, and single-copy sequences eluted in 20 ml of 0.12 M PB. Samples were dialyzed for 24–48 h, then frozen and lyophilized.

The method employed to produce radioactive tracer DNA was derived from the general iodination protocols of Commorford (1971), Davis (1973), Tereba and McCarthy (1973), Orosz and Wetmur (1974), Altenberg et al. (1975), Scherberg and Refetoff (1975), Chan et al. (1976), Anderson and Folk (1976), Prensky (1976), and Sibley and Ahlquist (1981). Modifications of these procedures are noted below.

Lyophilized, single-copy DNAs were rehydrated in a small volume (25–100 μl) of 0.2 M NaAc (pH 5.7), transferred to a 1.5 ml microfuge tube, vortexed for 30 s, and centrifuged at 3,000 g for 30 s. Reaction mixtures were prepared by combining 100 μg of scDNA with enough 0.2 M NaAc (pH 5.7) to make a solution at 0.77 μg/μl, as well as 5 μl of 0.002 M KI and 10.4 μl bromcresol green dye (BCG), in 1 ml stoppered septum vials. Samples were adjusted to pH 4.7–4.8 with 0.2 M NaAc at pH 4.0.

Iodinations were usually carried out for a set of eight DNA samples. The isotope (^{125}I, Amersham IMS-300) was obtained in 5 mCi amounts (ca. 10 μl), diluted with 340 μl of 0.2 M NaAc and 10 μl of 0.001 M KI, and allowed to equilibrate for 1 h. Each tracer sample received 40 μl of isotope solution (0.625 mCi), followed by 60 μl of 0.0018 M thallium (III) chloride ($TlCl_3$). Samples were incubated at 60°C for 15 min, then cooled in an ice bath for 5 min. The pH of the reaction mixtures was raised above neutrality by addition of 30 μl 1.0 M Tris, samples were heated again at 60°C for 10 min, and chilled on ice for 5 min. After cooling, samples were dialyzed against 4 l of "iodination buffer" (Sibley and Ahlquist 1981).

DNA hybrids were prepared with 0.5 μg tracer DNA and 250 μg sheared, native DNA ("driver") in 0.48 M PB. Hybrids were boiled and incubated at 60°C to a C_0t of at least 5,600, diluted to 0.12 M PB, and applied to HAP columns in an automated Thermal Elution Device. Column temperatures were raised to 60°C and three 8-ml fractions of 0.12 M PB were collected. Additional fractions were collected at 2°C intervals from 64°C to 94°C, and a final fraction was collected at 98°C.

Elution fractions were assayed for radioactivity in a Packard Auto-Gamma 5000 Series gamma counter.

Two tracer DNA preparations were employed for every crane species, with replicate interspecies hybrids arranged in experimental sets of 25. Each experimental set was defined by one or two reference ("homologous") hybrids (i.e. tracer and driver DNAs from the same extraction of the same individual bird), with which all interspecies ("heterologous") hybrids were compared. Three to 10 replicate heterologous hybrids were examined for each pairwise combination of species (reciprocals treated separately).

The end product of each thermal elution experiment is a list of radioactivities of eluate for each of the 20 temperature intervals. Of these 20, the first three 60°C fractions represent counts from isotope atoms which either did not bind to tracer DNA or bound atoms on tracer fragments which did not form stable duplexes during reassociation. Such unreassociated strands in homologous hybrids likely represent very small DNA fragments (<100 bp), produced during sonication and iodination, which are prohibited by their size from forming stable duplexes under standard conditions. In heterologous preparations, this fraction will also contain tracer sequences which are too divergent from their driver homologs to form stable duplexes at 60°C. Thus, the "percent hybridization" of a particular hybrid preparation is the ratio of counts eluted at and above 64°C divided by total counts multiplied by 100. For heterologous hybrids, this value is standardized for comparison (i.e. expressed as "normalized percent hybridization" or NPH) by dividing the heterologous percent hybridization by that for its corresponding homolog.

Because the normalized percent hybridization (NPH) values among cranes were essentially 100% (see below), I used T_m to measure hybrid thermal stability (Sheldon 1987a, b). T_m is the median melting point of all DNA sequences which have hybridized in a particular experiment, and is given by the temperature at which 50% of total counts above 60°C were eluted (values between specific elution temperatures were estimated by linear interpolation).

Genetic distance measurements were calculated as delta T_m values between homologous and heterologous hybrids within the same experimental set. Final distance estimates were the averages of all replicate delta T_m values for each pairwise comparison of species (reciprocals treated separately). In three cases, a single value was "trimmed" from the sample of replicate measurements (Krajewski 1988; see below) to obtain a more robust estimate of actual distance.

Outgroup comparisons were provided by DNA hybrids between the 14 cranes and the Limpkin (*Aramus guarauna;* Gruiformes: Aramidae). Reciprocal comparisons involving *Aramus* were not performed, because DNA from the Limpkin did not produce adequate tracer preparations. The final matrix of genetic distances contained 15 rows (drivers) and 14 columns (tracers).

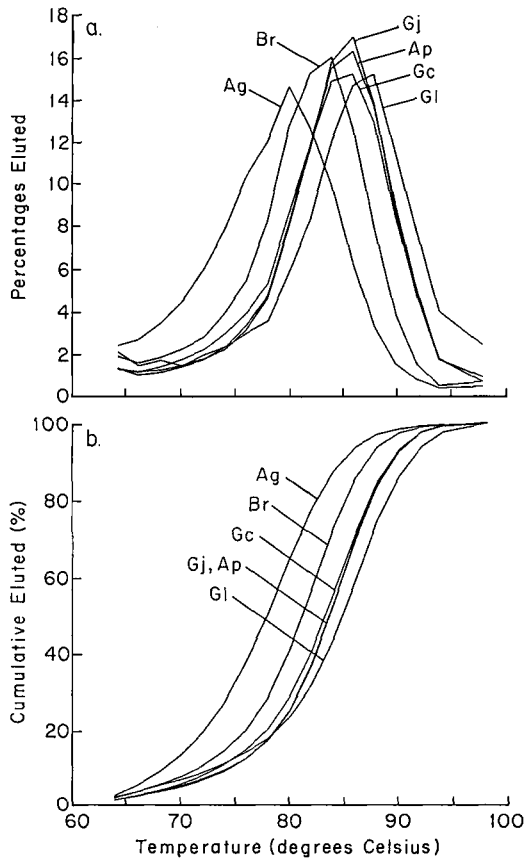

Fig. 2. Thermal elution profiles for DNA hybrids among cranes and outgroups. **Top**: Stepwise plot of percent counts eluted at indicated temperatures, illustrating modal divergence. **Bottom**: Cumulative plot of percent counts eluted at indicated temperatures, illustrating divergence in T_m. Tracer species is *Grus leucogeranus* (Gl). Other species abbreviations are Ap = *Anthropoides paradisea*, Gc = *Grus canadensis*, Gj = *Grus japonensis*, Br = *Balearica regulorum*, Ag = *Aramus guarauna*.

Tree construction.—I generated a phenogram for a folded matrix of distances with trimmed means (see below) using the unweighted pair-group method with arithmetic averages (UPGMA) implemented in Rohlf's NTSYS software package for the IBM-PC.

Estimates of phylogenetic branching order were made with the FITCH routine of Felsenstein's PHYLIP software package (version 2.8; for a justification see Felsenstein 1982, 1986; Springer and Krajewski 1989). Relevant parameter values for this algorithm were set at $P = 0.0$ (least-squares criterion of fit) and $n = 1$ (default). Negative distances in the input matrix (see below) were set equal to 0.01 and negative branch-lengths were disallowed in the output trees. To increase the likelihood of globally optimum results, I employed six alternate input orders of taxa, and I took

TABLE 1. Average and standard deviation values for normalized percentage hybridization (NPH) of DNA among cranes.

	B. reg.	*A. vir.*	*A. par.*	*B. car.*	*G. leuc.*	*G. can.*	*G. ant.*	*G. rub.*	*G. vip.*	*G. grus*	*G. mon.*	*G. amer.*	*G. nig.*	*G. jap.*
B. regulorum	100.7	101.5 (6.9)	102.5 (10.9)	106.4 (19.1)	118.1 (27.5)	102.5 (16.1)	98.2 (10.3)	96.2 (1.7)	97.4 (14.9)	89.3 (3.2)	93.8 (7.6)	94.5 (3.5)	85.5 (4.9)	100.6 (12.0)
A. virgo	105.1 (14.7)	105.4	110.6 (7.5)	99.2 (0.9)	84.0 (53.9)	110.9 (4.0)	105.3 (6.4)	109.9 (8.2)	99.5 (11.5)	95.9 (5.2)	99.7 (6.1)	99.5 (3.3)	92.7 (7.9)	99.4 (11.6)
A. paradisea	105.9 (17.6)	99.8 (16.9)	89.4 (4.5)	100.2 (15.0)	97.3 (34.3)	102.9 (13.2)	106.8 (10.4)	98.0 (7.0)	97.2 (16.7)	92.5 (6.1)	91.3 (9.6)	98.6 (4.4)	91.0 (5.3)	107.1 (22.0)
B. carunculatus	103.8 (5.0)	91.5 (13.7)	100.8 (8.2)	102.5 (38.2)	101.5 (31.8)	105.0 (7.4)	100.7 (12.5)	95.7 (10.0)	108.7 (8.2)	97.4 (3.3)	91.2 (3.4)	99.5 (4.6)	94.7 (3.4)	97.9 (5.5)
G. leucogeranus	101.4 (20.5)	89.2 (10.3)	94.2 (12.6)	96.8 (19.0)	108.0 (0.9)	96.2 (7.1)	102.5 (4.6)	87.0 (18.8)	99.5 (19.4)	98.9 (14.1)	99.6 (11.3)	103.1 (11.1)	84.6 (5.6)	103.5 (7.6)
G. canadensis	100.6 (8.8)	101.9 (14.3)	103.8 (8.6)	103.3 (14.4)	128.5 (27.8)	99.4 (0.1)	101.2 (11.3)	103.2 (6.2)	105.0 (11.2)	98.2 (14.2)	100.9 (9.5)	105.6 (10.7)	96.2 (7.1)	105.3 (10.1)
G. antigone	108.3 (16.2)	102.0 (12.9)	114.0 (11.6)	99.4 (9.1)	107.0 (7.0)	100.8 (5.1)	99.2 (14.0)	96.8 (7.6)	114.5 (17.4)	101.1 (11.9)	98.8 (10.9)	106.7 (9.4)	94.3 (3.3)	109.1 (16.5)
G. rubicunda	108.1 (6.8)	112.6 (8.3)	101.8 (6.2)	97.7 (3.5)	101.1 (14.9)	103.8 (9.6)	103.5 (9.3)	100.6 (0.1)	100.1 (7.6)	102.1 (3.3)	104.0 (7.4)	104.7 (2.6)	92.0 (2.3)	103.6 (10.9)
G. vipio	107.1 (6.8)	96.8 (13.1)	102.9 (8.6)	95.7 (10.8)	133.4 (28.0)	106.2 (8.0)	112.8 (3.0)	96.2 (14.9)	111.7 (6.7)	95.7 (6.9)	101.9 (8.2)	102.6 (8.8)	96.2 (5.7)	102.4 (9.0)
G. grus	105.5 (20.0)	104.9 (13.8)	107.7 (6.5)	103.0 (17.8)	138.6 (24.0)	106.1 (8.0)	106.4 (8.3)	103.1 (5.7)	109.1 (14.8)	103.8 (5.4)	96.5 (2.4)	105.5 (6.5)	98.2 (8.5)	103.2 (8.0)
G. monachus	103.0 (4.5)	106.8 (15.3)	104.6 (7.6)	95.7 (7.6)	101.9 (16.6)	103.9 (8.4)	103.9 (7.3)	97.4 (2.8)	114.1 (13.0)	103.9 (1.2)	88.1	102.1 (8.4)	95.0 (4.8)	99.7 (9.4)
G. americana	116.3 (10.7)	97.2 (5.7)	113.1 (3.3)	98.5 (7.8)	93.5 (25.8)	104.7 (5.8)	100.8 (10.9)	92.0 (6.7)	108.5 (7.0)	101.8 (3.2)	94.2 (11.9)	111.5	95.0 (3.5)	102.8 (10.8)
G. nigricollis	106.4 (7.5)	100.0 (6.2)	105.9 (5.7)	95.8 (8.2)	93.4 (29.7)	102.4 (7.3)	109.7 (3.9)	95.9 (4.6)	110.5 (11.8)	104.2 (3.9)	95.5 (6.8)	95.0 (9.9)	95.1	105.8 (13.3)
G. japonensis	98.4 (21.8)	91.6 (16.7)	97.3 (15.0)	87.1 (12.7)	87.9 (18.8)	97.2 (19.5)	98.9 (14.7)	96.2 (3.2)	105.7 (13.2)	96.5 (11.2)	101.1 (13.5)	106.0 (9.1)	94.6 (6.1)	95.6 (0.1)
A. guarauna	89.6 (11.6)	101.3 (7.5)	89.7 (6.7)	87.7 (3.6)	82.2 (42.1)	94.3 (2.7)	93.8 (6.5)	93.2 (2.5)	85.6 (15.7)	90.4 (7.7)	96.6 (3.2)	97.9 (2.5)	85.9 (3.0)	100.2 (5.4)

TABLE 2. Square matrix of average delta T_m values among cranes (SD, *n* indicated below averages). Asterisks (*) indicate trimmed means.

	B. reg.	*A. vir.*	*A. par.*	*B. car.*	*G. leuc.*	*G. can.*	*G. ant.*	*G. rub.*	*G. vip.*	*G. grus*	*G. mon.*	*G. amer.*	*G. nig.*	*G. jap.*
B. regulorum		3.7 0.1, 4	3.8 0.3, 6	4.0 0.4, 5	3.9 0.6, 7	3.9 0.2, 4	3.6 0.5, 4	3.6 0.2, 4	3.0 0.6, 5	3.7 0.5, 4	4.2 0.4, 4	3.5 0.6, 4	3.3 0.2, 4	4.0 0.4, 4
A. virgo	3.8 0.2, 4		0.4 0.6, 6	1.1 0.1, 3	2.0 1.0, 5	1.5 0.4, 7	1.8 0.8, 5	1.3 0.4, 5	0.7 0.6, 5	1.0 0.6, 4	1.1 0.3, 5	1.1 0.4, 5	1.6 0.7, 4	1.7 0.4, 4
A. paradisea	3.9 0.8, 5	0.6 0.3, 7		1.4 0.8, 7	2.2 1.0, 6	1.1* 0.2, 5	2.0 0.6, 5	0.9 0.2, 5	0.9 0.7, 6	1.0 0.4, 5	1.3 1.0, 4	1.2 0.5, 6	1.1 0.4, 6	1.8 0.4, 4
B. carunculatus	4.2 0.7, 8	1.1 0.4, 8	1.1* 0.7, 5		1.5 0.6, 8	1.2 0.2, 7	1.4 0.5, 6	1.3 0.3, 8	0.7 0.8, 6	1.2 0.4, 5	1.7 0.7, 6	1.1 0.5, 7	1.2 0.3, 7	1.5 0.3, 6
G. leucogeranus	3.2 0.5, 8	1.6 0.6, 8	1.6 0.4, 6	1.6 0.6, 8		1.4 0.4, 7	1.0 0.2, 6	1.3 0.5, 8	1.5 0.7, 6	1.3 0.4, 8	1.5 0.9, 10	1.7 0.5, 5	1.5 0.8, 8	2.0 0.8, 7
G. canadensis	3.9 0.4, 8	1.2 0.7, 8	1.5 0.7, 6	1.2 0.3, 8	1.5 0.4, 8		1.2 0.5, 6	1.2 0.4, 8	0.8 0.5, 6	1.0 0.6, 8	1.5 0.3, 8	1.5 0.6, 8	1.7 0.6, 7	1.8 0.3, 8
G. antigone	3.8 0.4, 8	1.2 0.5, 8	1.5 0.5, 6	1.6 0.4, 8	1.3 0.1, 8	1.6 0.2, 8		0.6 0.5, 7	−0.2 0.4, 6	1.1 0.6, 8	1.8 0.5, 10	1.0 0.6, 8	1.5 0.4, 8	1.5 0.2, 8
G. rubicunda	3.6 0.5, 5	1.0 0.4, 7	1.1 0.6, 5	1.5 0.4, 6	1.7 0.5, 8	1.2 0.5, 8	0.6 0.2, 6		0.2 0.6, 6	1.0 0.4, 6	1.5 0.2, 7	1.4 0.8, 8	2.0 0.6, 7	1.3 0.4, 6
G. vipio	4.2 0.6, 8	1.4 0.4, 7	1.4 0.4, 7	2.0 0.5, 8	1.7 0.5, 8	1.4 0.2, 8	1.0 0.4, 6	1.1 0.2, 8		1.4 0.5, 8	1.6 0.5, 9	1.7 0.6, 8	1.6 0.6, 7	1.4 0.4, 6
G. grus	3.4 0.3, 5	1.0 0.5, 6	1.1 0.3, 4	1.0 0.8, 4	1.2 0.3, 6	1.2 0.1, 6	1.2 0.2, 5	1.2 0.5, 5	0.8 0.5, 5		0.4 0.4, 6	0.2 0.6, 2	0.4 0.5, 6	0.5 0.1, 4
G. monachus	3.9 0.5, 4	1.0 0.8, 5	1.1 0.3, 3	1.5 0.4, 6	1.6 0.7, 6	1.4 0.5, 6	1.8 0.8, 5	1.3 0.4, 5	0.7 0.7, 5	−0.2 0.5, 5		0.4 0.6, 6	0.6 0.6, 6	0.8 0.3, 4
G. americana	3.8 0.2, 3	1.4 0.8, 4	1.4 0.3, 3	1.5 0.4, 4	1.6 0.8, 4	1.3 0.1, 5	1.9 1.0, 4	1.6 0.5, 4	1.0 0.5, 5	0.1 0.1, 4	0.3 0.6, 6		0.8 0.8, 5	0.8 0.2, 5
G. nigricollis	3.8 0.3, 4	1.4 0.7, 5	1.2 1.0, 4	1.3 0.1, 5	1.5 0.3, 5	1.8 0.8, 5	1.5 0.2, 5	1.5 0.7, 8	1.0 0.5, 5	0.8 0.5, 3	0.6* 0.7, 5	0.5 0.5, 6		0.6 0.2, 4
G. japonensis	3.6 0.5, 8	1.4 0.7, 8	1.7 0.2, 5	1.3 0.5, 8	1.3 0.4, 8	1.3 0.3, 8	1.3 0.2, 6	1.4 0.7, 8	0.7 0.5, 6	0.2 0.5, 8	0.3 0.5, 9	0.5 0.6, 8	0.7 0.6, 8	

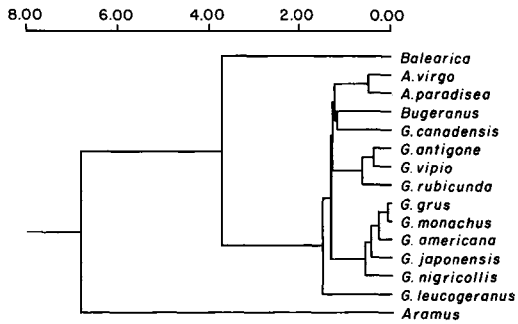

Fig. 3. UPGMA phenogram for DNA hybridization distances among cranes. Based on a folded distance matrix of delta T_m values.

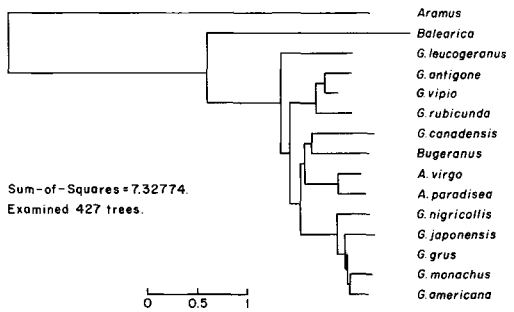

Fig. 4. Best-fit least-squares tree for a folded matrix of delta T_m values. Generated by the FITCH routine in PHYLIP ($P = 0.0$). *Aramus* was specified as the outgroup. Six alternate input orders were examined. The terminal branch to *G. grus* has length 0.

the solution of choice as that with lowest sum-of-squares value.

Because *Aramus* was not labeled, the genetic distance matrix was folded (i.e. reciprocal samples were pooled and averaged) to position the root of the crane tree. With *Aramus* specified as the outgroup, this root occurred as expected between the two traditional subfamilies (see below). All subsequent analyses were performed on the square matrix (reciprocals distinguished) with *Balearica* designated as the outgroup to other cranes. The best-fit tree obtained from analysis of the square matrix was tested for internal consistency using the Jackknife Strict Consensus Tree method of Lanyon (1985a, 1987).

RESULTS

Normalized percent hybridization (NPH).—Figure 2 illustrates typical elution profiles for crane DNA hybrids. The average NPH among crane species was 101.5 (SD = 20.0), and virtually all homologous sequences between species form duplexes in hybridization experiments (Table 1). Standard deviation values of the NPH statistic for individual cells in the matrix are characteristically high (range: 0.9–53.9) as described by Sheldon (1987a, b). The range of off-diagonal NPH values for tracer crane species is from 84.0 to 138.6, which implies considerable experimental error in this measurement. Nevertheless, no species departed consistently from the average value. The Limpkin (*Aramus*) also had a high degree of similarity to cranes (average NPH = 92.1, SD = 3.0, $n = 55$).

Genetic distance matrices.—DNA hybridization is subject to an inherent level of imprecision, and distance measurements must be replicated repeatedly to obtain high confidence in an average value or to assess accurately the level of variation imposed by the technique. Delta T_m is expected to be a consistent estimator of the actual percent nonidentity between the sequences compared when NPH is near 100 (this assumes that the error distribution of delta T_m is symmetrical about its expectation). For this reason, I report genetic distances between taxa as *average* delta T_m values based on a specified number of replicates (n). The replicate delta T_m values are tabulated in the Appendix. Average delta T_m values among cranes range from −0.2 to 4.2 (see below for a discussion of negative distances), n range from 3 to 10, and SD from 0.07 to 1.40 (average = 0.48 ± 0.66 SD) (Table 2). The average SD of 0.48 is slightly higher than that obtained for delta $T_{50}H$ (0.35) by Sibley et al. (1987) and for delta T_m (0.20) by Sheldon (1987a). Standard deviation values in this range probably reflect a lower limit on the precision of average delta T_m's obtained with the standard experimental protocol.

Phenetic relationships.—As clustered by UPGMA (Fig. 3), *Aramus* appears, as expected, as an outgroup to the cranes and links to the gruid cluster between *Balearica* and the other (gruine) species. The gruines form a relatively tight cluster, from which *Grus leucogeranus* departs first. The remaining species fall into three clusters. One contains five members of *Grus* (*grus, monachus, americana, japonensis,* and *nigricollis*); one contains *Anthropoides, Bugeranus* and *G. canadensis;* and the third contains *G. antigone, G. vipio,* and *G. rubicunda.* Although internodal lengths are extremely short, it is significant to note that this phenogram represents the distances in the folded matrix with very little distortion: the cophenetic correlation is 0.99364.

Best-fit tree for the folded matrix.—A FITCH

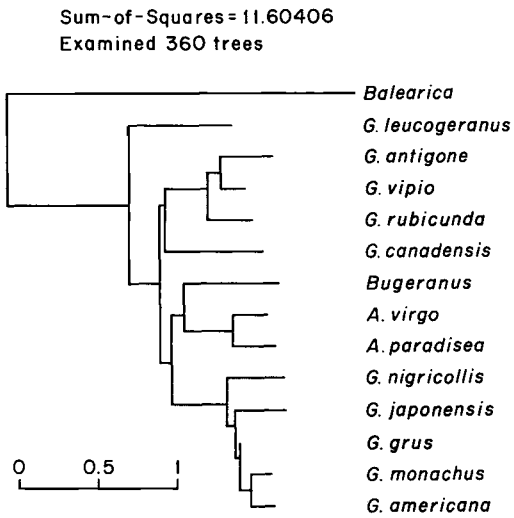

Fig. 5. Best-fit least-squares tree for the square matrix of distances (Table 2). *Balearica* was specified as the outgroup, and the root positioned (Fig. 4). Generated by the FITCH routine of PHYLIP ($P = 0.0$). Six alternate input orders were examined. The terminal branch to *G. grus* has length 0.

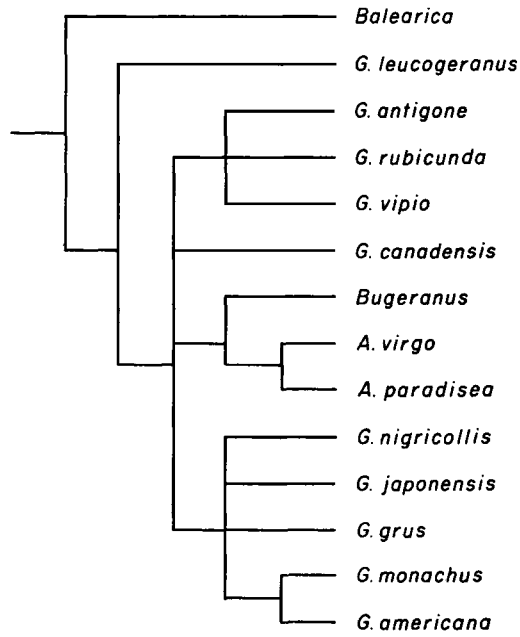

Fig. 6. Jackknife strict consensus tree for the square matrix of distances (Table 2). Pseudoreplicate trees generated by the FITCH routine of PHYLIP ($P = 0.0$).

best-fit tree for the folded matrix of genetic distances (Fig. 4) placed *Aramus* outside the crane group ($d' = 5.2$ from *Aramus* to the node linking *Balearica*). *Balearica* is isolated on a long branch ($d' = 2.2$) and a relatively long internode ($d' = 0.7$), supporting the traditional view that these birds lack an extant close relative. The most important conclusion from the folded-matrix analysis is that *Balearica* is suitable as a designated outgroup with which to root square matrix trees. The position of *Balearica* in the folded-matrix tree remained consistent when the data were challenged by jackknifing.

The folded-matrix topology is virtually identical to the DNA phenogram (Fig. 3) with respect to the composition of species groups, though the suggested relationships among them are altered. The short internodes which separated most of the species clusters in the phenogram are found again in best-fit trees (Fig. 4). This is the first hint of lowered confidence in some of the branchings above *G. leucogeranus*.

Best-fit tree for the square matrix.—The optimum tree solution was found for the distance data (Table 2) with *Balearica* designated as the outgroup (Fig. 5). Three alternate input orders gave identical topological results and differed from the branching order in Figure 6 only by interchanging the positions of *G. rubicunda* and *G. vipio*. Another input order moved *Bugeranus* adjacent to the *G. grus* group, but this tree had a noticeably poorer fit than the others. A final shuffling of the matrix gave a tree which separated *G. canadensis* from the *G. antigone* group, but produced a much poorer fit than the optimal solution.

The species groups indicated in Figure 5 are almost identical to those in Figures 3 and 4. *Balearica* roots the tree between *G. leucogeranus* and the remaining species. Among the latter taxa, *G. grus, G. monachus, G. americana, G. nigricollis,* and *G. japonensis* form a clade whose sister group is composed of *Bugeranus* and *Anthropoides. G. canadensis* appears as an early branch from the *G. antigone, G. vipio,* and *G. rubicunda* lineage. The only substantive difference between this tree and that for the folded matrix is in the position of *G. canadensis.* Relationships among groups shown are based on relatively small fitted branch lengths (Fig. 5). While the internodes which separate *Balearica* and *G. leucogeranus* from the main tree appear substantial ($d' = 0.74$ and 0.20, respectively), that which places *canadensis* in the *antigone* group is only $d' = 0.03$, that which unites *Bugeranus* with *Anthropoides* is only $d' = 0.08$, and that which unites the *G. grus* and *Bugeranus/Anthropoides* groups is only

$d' = 0.07$. With the exception of the *Bugeranus/Anthropoides* clade, most internodes within species groups are less than 0.10.

Jackknife strict consensus tree.—Lanyon (1985a) united jackknifing with systematic consensus-tree methods to deal with problems of estimating the confidence that can be placed in a tree derived from distance data. Lanyon (1985a) presented the details of his algorithm, the product of which is a "jackknife strict consensus tree" (JSC tree). Jackknifing has been applied to DNA hybridization studies by Lanyon (1985b) and Sheldon (1987a).

The JSC tree (Fig. 6) for the square matrix of crane distances (Table 2) agrees with the best-fit tree (Fig. 5) in that *Balearica* and *G. leucogeranus* are the earliest branches, but resolution breaks down at the next interior node. The composition of terminal groups is preserved, but their internal structure is mostly lost. Once again, *G. antigone*, *G. vipio*, and *G. rubicunda* form a clade, but the association between the first two is obscured. *G. canadensis* loses its association with the *antigone* group, but the branching order of the *Bugeranus/Anthropoides* clade is retained. Resolution virtually disappears for the *G. grus* cluster, except an unexpectedly stable association between *G. americana* and *G. monachus*.

DISCUSSION

Distance measures and normalized percent hybridization (NPH).—Melting points of DNA hybrids are often expressed as the temperature at which 50% of duplexed tracer DNA has dissociated (Kohne 1970; Sibley and Ahlquist 1981, 1983). This value is the one which Kohne (1970) and others related to sequence divergence. Sibley and Ahlquist (1981, 1983) also calculated modal and $T_{50}H$ values for thermal elution profiles after fitting those profiles to one of four distribution functions by nonlinear least-squares regression. In some cases (e.g. for hybrids which display a "low temperature component"), the mode may be a more precise measure of thermal stability than T_m (Sarich et al. 1989). The crane profiles, however, are nearly symmetrical and one would expect modes and T_m's to be roughly equivalent. Analysis of a subset of the crane data revealed that delta-mode and delta T_m values are almost identical, with average values of the former within a single standard deviation of the latter. Both measures had approximately the same level of repeatability and implied virtually identical rank-orderings of drivers within tracer-sets. I emphasize the T_m values simply to avoid the necessity of computer curve-fitting, which may introduce an additional level of error into distance calculations. A reassociation-kinetic survey of crane DNAs (Krajewski 1989) revealed no gross differences in the structure of crane genomes which would complicate interpretation of either distance measure.

The $T_{50}H$ measure has received much critical attention (Sarich et al. 1989). This index takes "unhybridized sequences" into account in the calculation of a median melting point (Sibley and Ahlquist 1983). Given that such sequences are rare in crane hybrids (as indicated by NPH values near 100), one would expect $T_{50}H$ and T_m values to be equivalent. However, the inherent variability of NPH measurement is carried over into the estimation of $T_{50}H$, and delta $T_{50}H$ is a very imprecise measure of genetic distance (Sheldon 1987a, b). This problem is especially acute for closely related species such as cranes, where high variance in distance measures will result in low phylogenetic resolving power unless a very large number of replicate experiments are performed. In any case, NPH levels among cranes are such that calculation of $T_{50}H$ values adds nothing to the robustness of the distances.

Trimming means.—If the level of experimental error described here and in Sibley and Ahlquist's work (1983) represents a lower threshold of precision with this experimental protocol, few analytical options are available which will further improve the "signal to noise" ratio of DNA hybridization data. Sibley and Ahlquist (1981, 1983) describe a simple statistical procedure to minimize the effect of a few aberrant data points on the average value of a larger set of replicates. Such aberrant values may exert a significant influence when the technique is required to measure distances at the low end of its precision range. Sibley and Ahlquist omitted the largest and smallest distances from each set of replicate measures, and averaged the remaining values to obtain a "trimmed mean." Sibley and Ahlquist claimed that this procedure improved the robustness of their distance measures. Indeed, this follows from the assumption of a symmetric error distribution.

The omission of both extremes of a range of distances has the disadvantage of reducing the sample sizes for each cell and assumes that the

sample of scores, however small, is symmetric about its average. Both these difficulties can be mitigated by omitting only a single extreme score (i.e. that with the greatest magnitude of departure from the sample mean). A problem related to trimming is the assumption that aberrant values occur in every sample (i.e. no criteria are employed to identify such values, and the effects of trimming may vary from sample to sample). An obvious method to screen samples for aberrant scores is suggested by our prior knowledge of the variation expected of DNA hybridization measurements. From this information and the conclusions of Sibley et al. (1987), we expect each sample of scores for which $n > 5$ to have a standard deviation of about 0.5 (the sample size constraint is significant, because smaller samples will generally have higher variance). Samples with substantially higher SDs are likely to contain at least one aberrant score. I chose a conservatively high SD value of 1.0 to insure that only aberrant values (not merely marginal ones) were omitted.

This modified trimming procedure was used for the matrix (Table 2) where only three cells were found to meet the trimming criteria. These were d(*A. paradisea, Bugeranus*), d(*G. canadensis, A. paradisea*), and d(*G. monachus, G. nigricollis*). In all three cases, the aberrant value was a large delta T_m, consistent with the notion that poorly reassociated hybrid preparations were responsible for the error.

Levels of genetic and experimental variation.—I used species as the "operational taxonomic unit," but it is of interest to examine intraspecific variation to attempt to estimate levels of experimental error and genetic differentiation. One may consider, for example, variation among DNA extracts from the same individual. For multiple hybrids formed between crane tracer and driver DNAs of the same extract (i.e. homologous hybrid preparations), the average SD among T_m values is 0.47 (SD = 0.51, $n = 25$; see Appendix). The average difference in homologous T_m values between different extracts of the same individual crane is 0.38 (SD = 0.86, $n = 7$; see Appendix), which approximated the magnitude of experimental error (though the sample size is relatively small). The DNA hybridization data for cranes had almost no measurable variation, on average, between conspecific individuals (average delta $T_m = 0.07$, SD = 0.62, $n = 19$; see Appendix). I believe that experimental error is of sufficient magnitude to hamper measurements of such small intraspecific divergences.

Intraspecific variation and the potential problems it may cause for phylogenetic reconstruction are discussed by Springer and Krajewski (1989) and, briefly, by Sheldon (1987a). Aside from the effects of unusual bottlenecks in the speciation history of a clade, genetic distances among species will be phylogenetically informative when the amount of detectable intraspecific differentiation is less than the amount of detectable interspecific differentiation. In cranes, the precise amount of intraspecific distance may not be detectable over experimental "noise" and, in any case, appears comparable in magnitude to only the smallest distances between species.

Reciprocity.—Some reciprocal delta T_m's (Table 2) showed substantial departures from the symmetry expected of metric distances. Although some level of asymmetry is expected from experimental "noise" (imprecision), this phenomenon may also signal a bias in the measurements. Groups of delta T_m values derived from different tracer preparations within each column covary (Felsenstein 1987). The most obvious source of such bias is the "compression effect" on distances, introduced when a particular tracer preparation gives a low (e.g. <82°C) homologous T_m as a result of biochemical degradation. Several such tracers were of necessity employed here (see Appendix), and it is likely that their influence accounts for much of the "internal inconsistency" detected by jackknifing.

Negative distances.—Two cells in the crane matrix contain average delta T_m values of less than 0: d(*G. vipio, G. antigone*) = d(*G. grus, G. monachus*) = −0.2. Whether these average values are significantly less than zero is irrelevant to the nature of measurement error. Both values are associated with species for which one tracer preparation gave low-melting (T_m < 82°C) homologous hybrids and with driver species that were extremely similar to the tracers. This combination of biased experimental error and increased demand for precision is the cause of the negative averages. The negative average delta T_m does not imply a negative genetic distance (this makes no sense), but rather indicates measurement variation in delta T_m around a small actual distance. These negative distances occur within species groups whose internal structure cannot be fully resolved (Figs. 5 and 6).

Triangle inequality.—Although Sibley and Ahlquist (1983) have claimed that DNA hybridization distances usually satisfy the triangle inequality, it is unclear if measurement error associated with small distances causes violations of the inequality. Such violations do occur: the square matrix (including *Aramus* in the lower half) contains 85 triplets which fail the triangle test, <4% of the total (there are 2,302 nonredundant triplets in all: 1,080 in the upper half, 1,222 in the lower half). Most of the triangle violations involve one or more of the species *G. grus, G. monachus,* and, in the upper half-matrix, *G. vipio.* Many of the violations that involve *grus* and *vipio* also involve the two negative distances discussed above. These two species also gave one low-melting tracer and, consequently, may show somewhat compressed column values. As noted above, all three species belong to closely related clusters which cannot be resolved consistently by the DNA data.

Jackknifing and internal inconsistency.—The jackknifing procedure does not constitute a statistical test of the best-fit tree or assign levels of confidence to particular nodes. Rather, it represents an attempt to evaluate conservatively the effect of "internal inconsistency" in the original data. The relationship between the best-fit tree and the jackknife strict consensus (JSC) tree is not, in fact, perfectly clear. Given the distance matrix and the tree-building algorithm, our method of estimation directs us to choose the best-fit solution. This solution may correctly report the evidential meaning of the data, even though the data set contains some internal inconsistency. Jackknifing does not answer this question about estimation, it merely detects the points on which the data are equivocal. To this extent, a JSC tree represents a conservative interpretation of the distance data.

Crane phylogeny.—The phylogenetic hypotheses (Figs. 5 and 6) are self-explanatory, but the associations of species merit some additional comments. The degree to which the DNA phylogeny concurs with previous opinions of relationships is striking. The limited DNA hybridization data (Sibley and Ahlquist 1985) produce a phenetic branching order for four species consistent with Figures 6 and 7. The fitted distances in Sibley and Ahlquist (1985, fig. 3) are only slightly different from those I found, although their measurements are in units of delta $T_{50}H$.

With one notable exception, the compositions of species-groups I detected (Fig. 6) is precisely that suggested by Archibald (1976). Perhaps the most basic agreement among all systematic treatments of cranes, including this one, is that the *Balearica* represent a distinct clade.

The sole discrepancy between Archibald's (1976) arrangement and the DNA hybridization consensus tree lies in the placement of *Grus leucogeranus.* My results do not support a close relationship between *G. leucogeranus* and *Bugeranus,* but rather they indicate that *leucogeranus* is the first branch from the gruine line. The DNA data agree with Archibald's linkage of *Bugeranus* and *Anthropoides.* It appears that the highly variable and, in many respects, unique features of the *leucogeranus* unison call render its similarity to *Bugeranus* phylogenetically misleading. The primary aspects of unison-call resemblance between the two species are more likely due to convergent evolution than to the retention of primitive characters. These features are not found in other cranes (the tracheal/sternal similarities are more problematic, in light of their absence in balearicines).

Though most often associated with *Grus grus* (Johnsgard 1983), *G. canadensis* appears as a relatively isolated species, perhaps an ancient branch from the *antigone* line. The *Anthropoides* species are sister groups (Figs. 5 and 6), which, though now expected, has not always been recognized. The *antigone* and *grus* clusters are essentially those recognized by earlier systematists (Krajewski 1988).

Within the *antigone* and *grus* clusters, the DNA results provide little resolution. In the *antigone* cluster, the best-fit tree associates *antigone* with *vipio* rather than *rubicunda.* Taxonomic tradition has favored a sister-group relationship for *antigone* and *rubicunda,* since Sharpe (1894) united the two in the genus "*Antigone*" on the basis of plumage similarities. The behavioral (Archibald 1975, 1976) and anatomical-phenetic (Wood 1979) studies support this association based on similarity. Moreover, natural hybridization has been documented between *antigone sharpei* and *rubicunda* where the two are sympatric in northeastern Australia (Johnsgard 1983).

The *G. grus* cluster (Fig. 6) is even more enigmatic. The proposal Archibald (pers. comm.) developed on the basis of behavior and anatomy is noncladistic but presents a hypothesis for subsequent investigation. Archibald considers *G. grus* as the earliest derivative within the group, a position now reflected by its wide-

spread distribution (the eastern end of its Palearctic range brings it into close geographic proximity to all of the other species within the cluster). *G. japonensis, G. monachus,* and *G. nigricollis* each display relatively specialized behavioral repertoires and habitat requirements, and they have restricted ranges in the eastern Palearctic. The relationships among them are unclear (Johnsgard [1983] speculates that *monachus* is the "nearest living relative" of *grus,* but presents no argument to support the claim). *G. americana,* the only Nearctic representative of the *grus* cluster, most closely resembles *japonensis* in anatomy, and *grus* in behavior. Resolution of relationships at this level will require further study.

Lanyon (1985a) warned that a breakdown of resolution among groups upon jackknifing should not be viewed to imply that they originated simultaneously. In general, this caution is well-taken. However, the small internodal distances on the best-fit crane tree suggest that the ancestors of the *antigone, Bugeranus/Anthropoides,* and *grus* clusters became isolated over a short span of time. This assumes that genetic distances assayed by DNA hybridization behave more or less like a "molecular clock," a notion endorsed cautiously by Sibley et al. (1987), and which is a reasonably accurate interpretation of the crane data (Krajewski 1988, in prep.).

Rates of molecular evolution.—ANOVA and Felsenstein's (1986) *F*-ratio tests revealed that rate variation implied by DNA hybridization distances among the cranes is significant (Krajewski 1988, in prep.). The rate disparity seems to be localized in the *Balearica* lineage, which evolves some 1.3–1.6 times faster than gruines. Although *G. leucogeranus* appears to have a relatively short root-to-tip pathlength on the best-fit tree (Fig. 6), rate variation among gruines is not statistically significant.

Nomenclatural and Classificatory Recommendations

Because the jackknife strict consensus tree (Fig. 6) leaves relationships among species clusters unresolved, it is not possible, on the basis of DNA hybridization data alone, to propose a formal revision of the Gruidae in which sister-groups are given coordinate rank. The genus *Grus,* as currently conceived, is probably polyphyletic (Figs. 5 and 6). In light of the very small genetic divergence among gruine species, it seems most reasonable to endorse Ingold's (1984) recommendation that *Anthropoides* and *Bugeranus* be merged with *Grus* (see Sibley et al. 1988, for typical genetic divergences at various levels among avian taxa). Until further phylogenetic resolution within *Grus* can be obtained, I suggest that the informal designation of "Species Group" be retained for the terminal clusters (Fig. 6).

The DNA data support the traditional subfamilial distinction of balearicines from gruines, though they do not address the correctness of current partitions of extinct taxa among the subfamilies. A classification of extant species, consistent with the DNA hybridization results and entailing minimal departures from traditional views, is given below.

Family Gruidae
- Subfamily Balearicinae
  - Genus *Balearica*
    - *B. pavonina*
    - *B. regulorum*
- Subfamily Gruinae
  - Genus *Grus*
    - Species Group Leucogeranus
      - *G. leucogeranus*
    - Species Group Antigone
      - *G. antigone*
      - *G. rubicunda*
      - *G. vipio*
    - Species Group Canadensis
      - *G. canadensis*
    - Species Group Anthropoides
      - *G.* [="*Anthropoides*"] *virgo*
      - *G.* [="*Anthropoides*"] *paradisea*
      - *G.* [="*Bugeranus*"] *carunculatus*
    - Species Group Grus
      - *G. grus*
      - *G. monachus*
      - *G. americana*
      - *G. nigricollis*
      - *G. japonensis.*

The compositions of species groups within *Grus* are similar to those given by Archibald (1976), except that group names are chosen by priority (i.e. Group Grus replaces Archibald's Group Americana). Authorities are easily recovered from Peters (1934) or Sharpe (1894).

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APPENDIX. **Tabulation of experimental results.** These data represent the results of individual pairwise DNA hybridization comparisons and are recorded as the delta T_m values for each experiment. Raw data (i.e. lists of radioactive counts) for the construction of melting profiles or for the calculation of distance statistics are available from the author and are filed permanently with J. A. W. Kirsch at the Department of Zoology, University of Wisconsin–Madison.

What follows is a summary, by tracer species, of all measured distances among cranes. For each combination of tracer/driver species, I listed calculated delta T_m values. Averages, standard deviations, and sample sizes are given in Table 2. Two tracer preparations were employed for each species. Each is identified by an extraction number at the top of the record (in parentheses), along with its average homologous T_m (in degrees Centigrade). For each driver species, results involving different tracer preparations are offset by colons (:). Results of replicate drivers involving the same DNA extraction are offset by commas (,); replicates involving different driver DNA extractions are offset by semicolons (;). Trimmed values are shown in parentheses. Asterisks (*) denote distances measured between different conspecific individuals; other conspecific distances involve different driver preparations from the individual used as tracer. Delta T_m values between cranes and the American Coot (*Fulica americana*) are given to establish a frame of reference, but they were not included in the phylogenetic analyses discussed in the text.

Each DNA extraction may be traced to an individual bird. Detailed information on individuals, including pedigrees, is on file at the International Crane Foundation in Baraboo, Wisconsin. Inquiries should be addressed to the author.

Tracer *Balearica regulorum* (281:84.13, 290:83.28)

Driver spp.	Delta T_m
B. pavonina	1.09, 0.63
B. regulorum	0.08
A. virgo	3.61: 3.82, 3.86; 4.11
A. paradisea	2.98: 3.48, 3.60; 4.55, 4.75
Bugeranus	4.73, 5.71; 3.75, 3.46: 4.08, 3.91; 4.08, 3.90
G. leucogeranus	3.80, 2.31: 3.01; 2.98, 3.57, 2.97; 3.53, 3.63
G. canadensis	3.34, 4.39: 3.61, 4.33; 3.96, 3.86; 3.98, 3.86
G. antigone	3.11, 3.12: 3.91, 4.19; 4.21, 3.88; 3.87, 3.73
G. rubicunda	3.04, 3.21: 4.08, 4.18; 3.54
G. vipio	3.37, 3.16: 4.77, 4.55; 4.54, 4.20; 4.22, 4.42
G. grus	3.22, 3.71: 3.30, 2.93; 3.69
G. monachus	3.72: 3.92, 4.52; 3.27
G. americana	3.83, 3.89: 3.61
G. nigricollis	3.28: 3.99, 3.84; 3.92
G. japonensis	2.61, 3.31: 3.62, 3.83; 3.58, 3.90; 4.13, 3.43
Aramus	7.33: 7.56, 7.33
F. americana	12.22, 12.62

Tracer *Anthropoides virgo* (390:81.65, 534:84.41)

Driver spp.	Delta T_m
B. pavonina	4.81
B. regulorum	3.63, 3.62: 3.67, 3.79
A. virgo	−0.44*: −0.70*
A. paradisea	0.92, 0.18; 0.54, 1.17: 0.45, 0.60; 0.47
Bugeranus	0.85, 0.79; 0.45, 0.90: 1.45, 1.35; 1.21, 1.45
G. leucogeranus	1.63, 1.43; 1.92, 2.51: 1.88, 1.69; 1.10, 0.71
G. canadensis	1.86, 1.25; 0.28, 0.43: 1.13, 1.09; 2.06, 1.79
G. antigone	0.64, 1.34; 0.82, 0.95: 1.13, 0.98; 2.07, 2.00
G. rubicunda	1.69, 0.54; 0.55: 1.31, 1.09; 1.07, 1.11
G. vipio	1.59, 1.89; 0.82, 0.95: 1.64, 1.46; 1.35
G. grus	1.30, 0.10; 0.81: 1.14, 1.20; 1.65
G. monachus	0.16, 0.16: 1.24, 1.16; 2.03
G. americana	0.81, 1.11: 2.56, 1.31
G. nigricollis	0.74, 0.59: 2.15, 1.74; 1.79
G. japonensis	0.85, 0.74; 0.59, 0.81: 1.94, 1.93; 2.08, 2.06
Aramus	7.01, 6.21: 6.70, 6.58
F. americana	11.46: 11.49

Tracer *Anthropoides paradisea* (282:84.50, 391:84.50)

Driver spp.	Delta T_m
B. pavonina	
B. regulorum	3.98, 4.28; 3.52, 3.65: 3.35, 3.94
A. virgo	0.64, 1.54; −0.08, −0.04: −0.05, 0.18
A. paradisea	0.48*; −0.56*
Bugeranus	2.20, (4.24); 1.15, 1.12: 0.75, 0.46
G. leucogeranus	1.18, 1.40; 1.61, 1.25: 1.72, 2.39
G. canadensis	1.56, 2.46; 0.94, 0.66: 1.63, 1.89
G. antigone	1.56, 1.61; 2.27, 0.79: 1.06, 1.76
G. rubicunda	1.88, 1.68; 0.49: 1.10, 0.56
G. vipio	0.90, 1.00; 1.72, 1.21, 1.29: 1.92, 1.70
G. grus	1.22, 0.93; 0.80: 1.57
G. monachus	1.38, 1.09: 0.85
G. americana	1.62, 1.66; 1.06
G. nigricollis	2.34, 1.23; 0.64: 0.27
G. japonensis	1.56, 1.64; 1.60: 1.99, 1.71
Aramus	6.52, 6.98, 5.90
F. americana	12.46: 12.61

Tracer *Bugeranus carunculatus* (392:85.20, 392:83.90)

Driver spp.	Delta T_m
B. pavonina	
B. regulorum	4.42, 4.03: 3.83, 3.39; 4.12
A. virgo	0.99, 1.23: 1.01
A. paradisea	1.45, 1.36; 1.04: 0.60, 1.07; 1.34, 3.23
Bugeranus	1.93: 0.67
G. leucogeranus	1.92, 1.29; 1.35, 2.44: 1.24; 1.60, 1.98, 0.56
G. canadensis	1.68, 1.54; 1.14, 1.22: 1.09, 0.78; 1.38, 1.06
G. antigone	2.33, 1.56; 1.19, 0.93: 1.84, 1.55; 1.90, 1.53
G. rubicunda	1.26, 1.81; 0.89: 1.96, 1.57; 1.59
G. vipio	2.07, 2.02; 1.56, 1.37: 1.27, 2.64; 2.52, 2.19
G. grus	1.40, 1.57; 1.29, −0.13
G. monachus	1.10, 1.47; 1.16: 2.05, 1.69; 1.29
G. americana	1.69, 1.65; 0.93, 1.72
G. nigricollis	1.24, 1.41; 1.30: 1.26, 1.23
G. japonensis	1.31, 1.17; 0.94, 1.23: 0.19, 0.28; 1.11, 1.45
Aramus	6.94, 6.94: 7.20, 6.09
F. americana	12.36

Tracer *Grus leucogeranus* (136:85.57, 137:85.06)

Driver spp.	Delta T_m
B. pavonina	
B. regulorum	4.97; 3.95, 4.18: 3.66, 3.53; 3.54, 3.35
A. virgo	3.02, 3.01: 1.12, 1.08; 1.85
A. paradisea	3.15, 3.45: 1.17, 1.18; 2.04, 2.50
Bugeranus	2.19, 2.66: 1.12, 0.91; 1.38, 1.40; 1.04, 1.05
G. leucogeranus	0.41*; 0.31*
G. canadensis	1.03, 1.26: 2.00, 1.15; 1.27, 1.26; 1.78, 1.79
G. antigone	1.18, 1.09: 1.39, 1.49; 1.38, 1.29; 1.32, 1.40
G. rubicunda	1.35, 1.25: 1.43, 1.48; 1.55, 1.62; 1.88, 2.88
G. vipio	1.50, 1.37: 1.68, 1.54; 1.74, 1.95; 1.99, 2.10
G. grus	1.56, 1.36: 0.82, 0.81; 1.43, 1.03
G. monachus	1.22, 2.92: 1.45, 1.41; 1.43, 1.14
G. americana	1.71, 2.16: 1.36; 0.79
G. nigricollis	1.57, 1.68: 1.30, 1.79; 1.11
G. japonensis	1.11, 1.23: 0.61, 1.19; 1.48, 1.60; 1.29, 2.09
Aramus	6.43, 7.21: 6.78, 6.90
F. americana	12.40, 12.67

APPENDIX. Continued.

Tracer *Grus canadensis* (143:84.75, 393:84.20)

Driver spp.	Delta T_m
B. pavonina	
B. regulorum	3.94, 3.85: 3.81, 4.12
A. virgo	1.62, 1.86: 1.19, 1.55; 1.22
A. paradisea	1.09, 1.29; (3.64): 1.19, 1.21; 0.79
Bugeranus	1.21, 1.26; 1.32: 1.39, 1.12; 1.05, 0.70
G. leucogeranus	1.82, 1.42; 1.34, 1.41: 1.94; 1.36, 0.86
G. canadensis	0.03*: 0.13*
G. antigone	1.44, 1.90; 1.64, 1.28: 1.71, 1.22; 1.51, 1.76
G. rubicunda	0.69, 1.62; 1.62, 0.60: 0.96, 1.25; 2.08, 1.15
G. vipio	1.61, 1.37; 1.10, 1.48: 1.69, 1.33; 1.66, 1.02
G. grus	1.28, 1.16; 1.42: 1.34, 1.09; 1.08
G. monachus	1.18, 1.14; 1.14: 2.46, 1.30; 1.11
G. americana	1.32, 1.26; 1.28: 1.46, 1.13
G. nigricollis	2.22, 2.96; 0.82: 1.43, 1.81
G. japonensis	1.24, 1.72; 1.46, 1.28; 0.74, 1.07; 1.55, 1.23
Aramus	7.24, 7.29: 7.46, 7.11
F. americana	12.69: 12.50

Tracer *Grus antigone* (138:84.98, 395:85.02)

Driver spp.	Delta T_m
B. pavonina	
B. regulorum	3.08, 3.31: 4.12, 4.03
A. virgo	2.97, 2.27: 1.40, 0.93; 1.38
A. paradisea	2.22, 2.91: 1.44, 1.58; 1.73
Bugeranus	2.07, 1.55: 1.49, 0.79; 1.53, 0.87
G. leucogeranus	0.78, 0.64: 1.10, 1.05; 1.04, 1.20
G. canadensis	0.58, 0.66: 1.25, 1.23; 1.34, 1.95
G. antigone	0.43*, 1.13*; 0.89*
G. rubicunda	0.50, 0.20: 0.67, 0.77; 0.65, 0.57
G. vipio	0.48, 0.46: 0.99, 1.22; 1.43, 0.97
G. grus	1.39, 0.91: 1.19, 1.12; 1.40
G. monachus	3.07, 1.07: 1.78, 1.34; 1.73
G. americana	1.36, 3.34: 1.74, 1.22
G. nigricollis	1.29, 2.79: 1.42, 1.61; 1.52
G. japonensis	1.28, 1.52: 0.90, 1.37; 1.41, 1.33
Aramus	5.67, 6.11: 6.79, 6.92
F. americana	12.57

Tracer *Grus rubicunda* (396:84.25, 397:84.82)

Driver spp.	Delta T_m
B. pavonina	4.42, 4.39
B. regulorum	3.56, 3.33: 3.78, 3.79
A. virgo	1.00, 1.02: 1.83, 1.14; 1.48
A. paradisea	0.70, 0.75: 1.28, 0.88; 1.11
Bugeranus	1.23, 1.51; 1.14, 1.32: 1.07, 0.86; 1.93, 1.49
G. leucogeranus	1.03, 1.53; 1.10, 1.21: 1.99, 1.85; 0.74, 0.72
G. canadensis	0.91, 0.99; 1.19, 0.88: 1.81, 1.70; 1.34, 1.02
G. antigone	0.60, 1.57; 0.94, 0.78: 0.28, 0.18; −0.20
G. rubicunda	1.07*, −0.06*
G. vipio	1.46, 1.18; 1.45, 1.11: 1.07, 1.03; 0.93, 0.85
G. grus	0.68, 1.95: 1.14, 1.13; 1.14
G. monachus	1.28, 0.81: 1.30, 1.08; 1.99
G. americana	2.13, 1.75: 1.25, 1.12
G. nigricollis	2.07: 1.16, 1.19; 1.42
G. japonensis	1.05, 0.97; 2.83, 2.11: 0.89, 0.93; 1.19, 1.29
Aramus	7.41, 7.01: 6.98, 6.54
F. americana	11.72, 12.66: 12.75

Tracer *Grus vipio* (41:82.94, 284:81.20)

Driver spp.	Delta T_m
B. pavonina	
B. regulorum	2.35, 2.17, 3.83: 3.52
A. virgo	0.53, 0.13, 1.64: 0.70, 0.70
A. paradisea	0.24, 0.37, 1.20: 0.78, 0.80; 2.10
Bugeranus	0.80, 0.79, 1.79: −0.46, 0.08; 1.00
G. leucogeranus	1.04, 0.76, 1.48: 2.09, 1.00, 2.68
G. canadensis	0.98, 0.82, 1.47: 0.10, 0.37; 0.80
G. antigone	−0.20, −0.68, −0.56: −0.09, 0.01; 0.54
G. rubicunda	0.40, 0.02, −0.89; 0.54, 0.58, 0.36
G. vipio	−0.36*, −0.60*: −0.78*
G. grus	0.32, 0.29, 1.55: 0.98, 0.76
G. monachus	0.13, 0.01, 1.72: 0.70, 0.88
G. americana	0.66, 0.47, 1.77: 1.22, 0.72
G. nigricollis	1.69, 0.40, 0.80: 0.92, 1.22
G. japonensis	1.38, 1.10, 0.35: 0.21, 0.33, 0.82
Aramus	6.14, 5.62, 6.63: 5.90, 5.53
F. americana	10.94

Tracer *Grus grus* (285:83.16, 293:84.36)

Driver spp.	Delta T_m
B. pavonina	2.63, 2.78
B. regulorum	3.39, 3.34: 3.85, 4.34
A. virgo	0.56, 0.37: 1.56, 1.34
A. paradisea	0.72, 0.40: 1.04, 1.20; 1.49
Bugeranus	1.10, 1.17: 1.15, 1.94; 0.88
G. leucogeranus	1.78, 1.06; 0.65, 1.54: 1.43, 1.46; 1.10, 1.11
G. canadensis	0.67, 1.49; 0.28, 0.09: 1.55, 1.82; 0.97, 1.31
G. antigone	1.25, 0.64; 0.36, 0.41: 2.40, 1.21; 1.07, 1.19
G. rubicunda	0.60, 1.06; 0.39, 1.27: 1.17; 1.33
G. vipio	1.31; 0.65, 0.43; 1.85: 1.63, 1.79; 1.36, 1.76
G. grus	−0.77*, −0.24
G. monachus	−0.95, −0.42: −0.08; −0.04, 0.38
G. americana	−0.03, 0.18: 0.26, 0.12
G. nigricollis	0.29: 1.08, 1.13
G. japonensis	1.11, −0.38; −0.45, 0.57: 0.09, 0.23; 0.13, 0.38
Aramus	5.58, 5.83: 6.96, 8.42
F. americana	10.64, 10.40: 13.04

Tracer *Grus monachus* (286:84.29, 401:85.08)

Driver spp.	Delta T_m
B. pavonina	
B. regulorum	4.39, 3.64: 4.48, 4.47
A. virgo	1.04, 1.07: 1.56; 1.11, 0.78
A. paradisea	0.61, 0.22: 2.07, 2.12
Bugeranus	2.80, 0.87: 1.33, 1.17; 1.90, 1.98
G. leucogeranus	1.17, 3.70; 1.58, 1.59; 0.81, 1.43: 1.30, 1.84; 0.95, 0.59
G. canadensis	1.64, 1.50; 1.13, 1.16: 1.94, 1.81; 1.43, 1.20
G. antigone	2.71, 2.07; 2.32, 1.20; 1.30, 1.67: 1.72, 1.71; 1.58, 1.30
G. rubicunda	1.60, 1.75; 1.40: 1.47, 1.57; 1.35, 1.26
G. vipio	0.70, 0.90; 1.60, 1.38; 1.06: 1.89, 2.00, 1.90; 1.55
G. grus	−0.07, 1.02; 0.37: 0.30, 0.16; 0.48
G. monachus	−0.01*: 0.65*
G. americana	−0.52, 0.27; 1.20: 0.54; 0.32. 0.42
G. nigricollis	(3.34), 1.81; 0.18: 0.23, 0.21; 0.35
G. japonensis	−0.42, 0.66; 0.57: 1.11, −0.23: 0.05, 0.40; 0.35, 0.59
Aramus	6.81, 7.36: 7.41, 7.23
F. americana	14.89: 12.76

Tracer *Grus americana* (287:83.58, 402:84.62)

Driver spp.	Delta T_m
B. pavonina	4.35
B. regulorum	3.10, 2.88: 4.23, 3.80
A. virgo	0.70, 0.85: 1.64, 1.47; 1.06
A. paradisea	0.70, 0.76; 0.69: 1.66, 1.74; 1.42
Bugeranus	0.41, 0.67; 0.52: 1.60, 1.68; 1.27, 1.27
G. leucogeranus	1.84; 1.33, 1.03: 2.38; 1.96
G. canadensis	1.52, 1.48; 0.62, 0.70: 1.96, 2.01; 1.86, 1.79
G. antigone	0.44, 0.34; 0.57, 0.71: 1.59, 1.20; 1.30, 1.91
G. rubicunda	0.66, 0.60; 0.47, 0.81: 2.05, 2.33; 2.16, 1.90
G. vipio	1.08, 1.06; 1.97, 1.28: 2.84, 2.16; 1.37, 1.84
G. grus	−0.44, −0.52; 0.32: 0.60, 1.03; 0.27
G. monachus	−0.52, 0.20; 0.39: 0.64, 0.78; 1.14
G. americana	0.54

APPENDIX. Continued.

G. nigricollis	−0.50; 0.77, 0.74: 0.63, 0.45; 0.69
G. japonensis	−0.72, −0.06; 1.19, 0.78: 0.89, 0.89; 0.66, 0.52
Aramus	6.48, 5.94: 8.12, 7.52
F. americana	11.78: 13.16

Tracer *Grus nigricollis* (403:81.56, 403:81.58)

Driver spp.	Delta T_m
B. pavonina	
B. regulorum	3.33, 3.34: 3.36, 3.01
A. virgo	1.53, 2.41: 1.79, 0.68
A. paradisea	0.80, 0.98; 1.88: 1.19, 0.85; 0.94
Bugeranus	1.10, 1.70; 1.40: 1.30, 0.68; 1.23, 0.97
G. leucogeranus	3.14, 1.50; 2.02, 1.00: 0.88; 0.94, 0.77
G. canadensis	1.28, 2.41; 1.35, 2.53: 1.56, 0.97; 1.74
G. antigone	2.11, 0.84; 1.35, 1.53: 1.42, 1.39; 1.58, 1.53
G. rubicunda	1.52, 1.41; 1.51: 2.54, 1.62; 2.79, 2.60
G. vipio	1.43, 1.84; 1.45, 1.98: 1.65, 1.39; 1.70, 1.29
G. grus	0.79, 1.04; 0.13: 0.19, −0.42; 0.35
G. monachus	0.13, 1.47; 0.01: 0.24, 0.98; 0.71
G. americana	0.63, 0.34; 0.51: 2.23, 0.47
G. nigricollis	0.50: −0.82
G. japonensis	1.46, 0.34; 0.74, 0.47: 0.11, 0.07; 1.51, 1.01
Aramus	6.22, 6.27: 7.11, 6.03
F. americana	10.78: 11.84

Tracer *Grus japonensis* (139:85.44, 404:84.73)

Driver spp.	Delta T_m
B. pavonina	4.63
B. regulorum	4.21, 3.34: 4.19, 4.09
A. virgo	2.21; 1.74: 1.64, 1.17
A. paradisea	2.37: 1.80, 1.76; 1.42
Bugeranus	1.92, 1.81: 1.18, 1.50; 1.39, 1.12
G. leucogeranus	1.00, 2.79; 2.63, 2.80: 1.78, 1.57; 1.16
G. canadensis	2.32, 1.21; 1.63, 1.96: 1.68, 1.53; 1.76, 1.88
G. antigone	1.74, 1.09; 1.11, 1.54: 1.59, 1.37; 1.63, 1.63
G. rubicunda	0.58, 1.15: 1.44, 1.60; 1.31, 1.54
G. vipio	0.65, 0.85; 2.36, 1.64: 1.39, 1.43, 1.70
G. grus	0.33: 0.53, 0.51; 0.54
G. monachus	0.97: 0.51, 0.62; 1.22
G. americana	0.89, 0.88: 0.45, 0.72; 1.08
G. nigricollis	0.57: 0.41, 0.65; 0.80
G. japonensis	0.65: 0.11
Aramus	7.10, 1.15: 7.17, 7.09
F. americana	13.24, 12.27