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Comparative DNA Reassociation Kinetics of Cranes

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INTRODUCTION

Although quantitative methods for the study of genome structure in eukaryotic organisms have been available for at least two decades, these methods have seldom been applied in a comparative survey. This is especially true for birds, the published data on which have come either from a scatter of species within high-level taxa or from isolated representatives intended as exemplars of the entire class. These data have been valuable in establishing a baseline of comparison between birds and other groups (e.g., mammals) but reveal little about systematic variations within Aves.

I have employed the reassociation-kinetic methods of Britten *et al.* (1974) in a survey of genome structure among the species of cranes (family Gruidae). Gruidae is comprised of 15 species in four genera distributed on all continents except South America and Antarctica. Though many species of cranes are now endangered, a fortunate correlate of conservation efforts is that raw material for molecular systematic research has been made available from captive birds.

METHODS

DNA from 14 of the 15 species of cranes was obtained from whole-blood samples provided by the International Crane Foundation in Baraboo, Wis. DNA was isolated according to the procedure of Marmur (1961), as modified by Sibley and Ahlquist (1983) and Maniatis *et al.* (1982). Native DNA in 0.02 M sodium acetate solution was sheared by high-frequency sound, and the

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resulting DNA fragments were sized by agarose gel electrophoresis via comparison with standard commercial size markers (*Eco-RI- and Hind*III-digested lambda phage DNA). Fragment size distributions centered on about 750 base pairs (bp), with a range of 50 to 1500 bp.

One to three replicate reassociation-kinetic profiles (i.e., "C_0t plots") were constructed for each crane species, using the technical approach of Sibley and Ahlquist (1983). Approximately 300 μl of sheared DNA at 0.1 mg/ml in 0.48 M phosphate buffer (PB) was loaded into microcuvettes, which were immediately sealed with silicone to prevent evaporation. DNA concentrations were obtained by reading sample absorbances at 260 nm in a Gilford Response II UV–VIS spectrophotometer equipped with a thermocuvette block.

The temperature of the thermal block was raised to 99°C to dissociate duplex DNA fragments. Absorbances were read after 10 min at 99°C, and the temperature was immediately reset to 60°C to allow reassociation to commence. Percentages of single-stranded DNAs were calculated according to the formulas of Britten *et al.* (1974) from absorbances read at regular logarithmic intervals of C_0t. Incubation times ranged from several days to several months, covering C_0t values from 0.01 to 1000. Cuvettes were transferred to a convection oven for long-term incubation. C_0t plots were constructed by graphing the percentage of single-stranded DNA (ssDNA) as a function of C_0t on five-cycle semilog paper, and interpolation was used to estimate specific points of reaction.

C_0t plots were analyzed using the formulas of Hood *et al.* (1975), in which three parameters are estimated: (i) the genomic fraction (f) of each kinetic component, (ii) the kinetic complexity (X) of each component, and (iii) the relative frequency, or repetition number (R), of sequences in each component. I have assumed, for the sake of comparison, that sequences are distributed among the three traditional frequency classes of Britten and Kohne (1968) and Britten *et al.* (1974), namely, highly repetitive DNA (hrDNA), middle-repetitive DNA (mrDNA), and single-copy DNA (scDNA). Moreover, the formulas of Hood *et al.* (1976) were modified to account for the specific fragment sizes and cation concentrations employed.

RESULTS

Estimates of f for the three kinetic classes in each crane species are given in Table I. It was not possible to estimate the complexity of highly repetitive sequences, since these had completely reassociated before the first absorbance reading at C_0t 0.01. Values of X for mrDNA and scDNA are given in Table I, along with $C_0t_{1/2}$ values for each component. The latter estimates are subject to experimental variation which limits precise, quantitative comparisons among species.

Table I. Frequency Class Proportions (f) and Estimated Complexities (X) of Crane DNA[a]

	Fraction						
	hrDNA	mrDNA[b]			scDNA[b]		
Species	f	f	$C_0t^1/_2$	X	f	$C_0t^1/_2$	X^c
Balearica regulorum ($N = 3$)	0.21	0.09	2.1	7.4×10^5	0.70	318	0.9×10^9
Anthropoides virgo ($N = 2$)	0.24	0.10	1.0	3.8×10^5	0.66	250	0.6×10^9
A. paradisea ($N = 2$)	0.16	0.13	0.2	1.1×10^5	0.71	1261	3.5×10^9
Bugeranus ($N = 3$)	0.18	0.15	0.8	4.7×10^5	0.67	350	0.9×10^9
Grus leucogeranus ($N = 3$)	0.22	0.08	4.0	12.3×10^5	0.70	470	1.3×10^9
G. canadensis ($N = 2$)	0.22	0.08	2.2	7.0×10^5	0.70	200	0.6×10^9
G. antigone ($N = 3$)	0.22	0.10	6.2	24.0×10^5	0.67	160	0.4×10^9
G. rubicunda ($N = 2$)	0.20	0.04	0.7	1.1×10^5	0.76	—	—
G. vipio ($N = 2$)	0.22	0.08	0.8	2.3×10^5	0.70	(700)	(1.9×10^9)
G. grus ($N = 2$)	0.22	0.10	5.1	19.7×10^5	0.68	440	1.2×10^9
G. monachus ($N = 2$)	0.22	0.09	5.3	22.5×10^5	0.67	520	1.3×10^9
G. americana ($N = 1$)	0.21	0.09	5.0	17.4×10^5	0.70	600	1.6×10^9
G. nigricollis ($N = 1$)	0.22	0.08	1.3	3.8×10^5	0.70	(900)	(2.4×10^9)
G. japonensis ($N = 3$)	0.22	0.07	0.6	1.5×10^5	0.71	390	1.1×10^9
Average	0.21	0.09		9.2×10^5	0.70		1.4×10^9

[a] Values of N indicate the number of replicate experiments performed for each species.
[b] $C_0t^1/_2$ values for mixed components.
[c] X_{sc} values in parentheses are based on extrapolation.

Values for f_{hr} range from 0.16 (*Anthropoides paradisea*) to 0.24 (*A. virgo*), with an average of 0.21. Ingold (1984) estimated that f_{hr} was approximately 0.25 in *Balearica pavonina* and *Grus leucogeranus*. These values are similar to, but slightly higher than, the 18% reported by Sibley and Ahlquist (1983) for the herring gull (*Larus argentatus*) and the 15% given by Epplen *et al.* (1978) for three domestic birds. Wagenmann *et al.* (1981) found f_{hr}'s of about 14 and 17%, respectively, for pheasant (*Phasianus*) and pelican (*Pelecanus*) genomes. Arthur and Strauss (1978) reported an even lower value (7–8%) for the chicken hr fraction, while Stefos and Arrighi (1974) came in with an estimate of 17%. Shields and Strauss (1975) examined eight

passerines and determined that highly repeated sequences constituted about 20% of their genomes. The crane f_{hr}'s are approximately double the average value (11.7%) reported for eight rodents by Santiago and Rake (1973).

Crane single-copy fractions are also quite similar to one another. They range from 66% (*A. virgo*) to 76% (*G. rubicunda*), with an average of 70%. Ingold's (1984) plots suggest a single-copy fraction comprising 70–75% of genomic DNA for *B. pavonina* and *G. leucogeranus*. The crane average is larger than Sibley and Ahlquist's (1983) herring gull estimate (60%) and Shields and Strauss' (1975) passerine value (60–65%). It is comparable to the chicken/duck/pigeon mean (70%) of Epplen *et al.* (1978), to the pheasant and pelican values (71 and 73%, respectively) of Wagenmann *et al.* (1981), and to the chicken range (70–80%) of Sanchez de Jimenez *et al.* (1974). Crane f_{sc}'s are also similar to those of rodents [which average 70% according to Santiago and Rake (1973)] but are somewhat larger than the 65% reported for the cow (*Bos*) by Britten and Smith (1970).

The average fraction of crane genomes composed of middle-repetitive sequences is 9%, with a range of 4% (*G. rubicunda*) to 15% (*Bugeranus*). The average crane value is less than the f_{mr}'s for the herring gull [22% (Sibley and Ahlquist, 1983)] and passerines [16% (Shields and Strauss, 1975)] but comparable to those of fowl, duck, and pigeon [11–13% (Arthur and Strauss, 1978; Epplen *et al.*, 1978)] and pheasant and pelican [13% (Wagenmann *et al.*, 1981)]. Rodents appear to have a slightly larger mr fraction (15%) than cranes (Santiago and Rake, 1973).

Complexities of crane middle-repetitive fractions range from 1.1×10^5 to 24.0×10^5 nucleotide pairs (NTP), with an average of 9.2×10^5 NTP. The latter value is about an order of magnitude larger than those for various mrDNAs of mammalian species given by Britten and Davidson (1971). The average complexity of crane single-copy genomes is 1.4×10^9 NTP, somewhat less than the average bird value [2×10^9 (Sibley and Ahlquist, 1983)] and the value for *Bos* [1.6×10^9 (Britten and Davidson, 1971)].

Table II. Haploid Genome Size (N) and Repetition Number (R) of Middle-Repetitive and "Single-Copy" DNAs for Several Crane Species

Species	N (pg)	R_{mr}[a]	R_{sc}[a]
Anthropoides virgo	1.6[b]	384	1.6
Grus canadensis	1.6[b]	167	1.7
G. grus	1.5[b]	70	0.8
G. americana	1.4[c]	66	0.6

[a] $R = fN/X$, with N expressed as NTP; 1.0 pg DNA = 9.13×10^8 NTP (Britten and Davidson, 1971).
[b] Source: Rasch and Kurtin (1975).
[c] Source: Biederman *et al.* (1982).

Estimates of the relative frequency of repetition in middle-repetitive and single-copy fractions are given in Table II for those cranes with known genome sizes. R_{mr} values range from 66 to 384, near the low end of Britten and Kohne's (1968) range for salmon sperm (100–100,000) and substantially lower than most mammalian values given by Britten and Davidson (1971). The crane estimates are remarkably similar, however, to those for rodents [$R = 92$ to 380 (Santiago and Rake, 1973). R_{sc} estimates are close to the expected value of 1.0.

DISCUSSION

The general structure of reassociation-kinetic profiles of cranes is quite similar to that of other birds and mammals studied to date, but cranes have several unusual features. The complexity of mrDNA is quite high, with a correspondingly low value for repetition number. This suggests that cranes have few sequences with copy numbers in the upper portion of the mrDNA range (e.g., 10,000–100,000) and a higher diversity of sequences at the lower end of this range (e.g., 100–1000). Single-copy fractions of cranes are somewhat less complex than in most birds studied. Moreover, the almost sixfold differences in X_{sc} estimates for *A. virgo* and *A. paradisea* is striking in that these taxa are sister species (Krajewski, 1988). Disparities are also found among other closely related species pairs (e.g., *G. antigone* and *G. vipio*) and may suggest the independent accumulation of paralogous sequences ("gene families") of low copy numbers in several lineages. On the other hand, X_{sc}'s are quite similar for species in the clade composed of *G. grus, G. monachus, G. americana, G. nigricollis,* and *G. japonensis,* suggesting a more conservative pattern of genomic evolution. This general pattern is further supported by the similarity observed between crowned cranes (*Balearica,* the most ancient gruid lineage) and most typical (gruine) cranes.

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