Genetic Differentiation Between North American Kinglets and Comparisons with Three Allied Passerines

JAMES L. INGOLD,[1] LEE A. WEIGT, AND SHELDON I. GUTTMAN
Department of Zoology, Miami University, Oxford, Ohio 45056 USA

The genus *Regulus* is composed of five species, two of which are native to the Western Hemisphere (Clements 1978). Mayr and Short (1970) discussed the possible relationships between the Ruby-crowned Kinglet (*R. calendula*) and the Golden-crowned Kinglet (*R. satrapa*). They suggested that the Golden-crowned Kinglet is most closely related to the Goldcrest (*R. regulus*) of the Palearctic faunal region and that the Ruby-crowned Kinglet is not closely related to any of the other species of kinglet, even though it has hybridized with the Golden-crowned Kinglet (Gray 1958). We present genetic evidence that the two North American kinglets are not closely related.

The birds used in this study were mist-netted near Oxford, Butler Co., Ohio, and were collected for part of a larger study on the history of the North American avifauna. Yellow-breasted Chats (*Icteria virens*; $n = 7$) and Common Yellowthroats (*Geothlypis trichas*; $n = 22$) were collected during late summer and autumn 1980. Ruby-crowned Kinglets ($n = 10$), Golden-crowned Kinglets ($n = 14$), and House Wrens (*Troglodytes aedon*; $n = 7$) were collected during autumn 1981. The birds were brought to the laboratory alive, were killed, and then were kept frozen whole at −70°C until used.

Heart, liver, muscle, and kidney samples were removed from all individuals, homogenized separately, centrifuged, and the supernatant stored at −70°C overnight. Standard electrophoretic techniques (Ingold et al. 1984) were used to resolve 19 presumptive genetic loci for the House Wren, Common Yellowthroat, and Yellow-breasted Chat and 16 loci for the two kinglets. Proteins studied (Table 1) were: aspartate aminotransferase (AAT), aconitase (ACON), esterase (EST), glucokinase (GK), glucose-3-phosphate dehydrogenase (G-3-PDH), isocitrate dehydrogenase (ICD), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), malic enzyme (ME), nonspecific protein (NSP), peptidase (PEP), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (6-PGD). A matrix of Nei's (1972) genetic distance and modified Rogers' genetic distance (Wright 1978) values was obtained for the 16 shared loci using the BIOSYS-1 program (Swofford and Selander 1981). Both distance values were calculated to facilitate comparisons with other electrophoretic studies. A phenogram using the unweighted pair-group algorithm with arithmetic means (UPGMA) was prepared from the modified Rogers' genetic distance (Wright 1978) values (Fig. 1). We also analyzed the allozymes as characters to avoid the problems and loss of information associated with reducing electrophoretic data sets to distance coefficients (Farris 1981, Felsenstein 1984). Branch lengths of cladograms derived in this manner have biological meaning. There are several ways to code and order allozyme character states, however, and no general concensus exists on the most appropriate approach (reviewed by Buth 1984). We used the alleles as characters with the character states being "presence" or "absence"; character coding in this manner acknowledges the presence (or absence) of alleles rather than particular suites of alleles. The character-state data were analyzed with the Phylogenetic Analysis Using Parsimony (PAUP) provided by Swofford (1984). Character states were weighted such that each locus provided equal information; the tree (Fig. 2) was rooted to the House Wren as the designated ancestor.

Only one locus, nonspecific protein (NSP), was monomorphic across all species (Table 1). The Yellow-breasted Chat was fixed for a different allele for AAT-1 and AAT-2 than the other four species. The House Wren was fixed for a different allele at MDH-2, while the chat and yellowthroat shared an allele at this locus. We could not score MDH-2 in the kinglets. The chat and yellowthroat were fixed for a shared allele at MDH-1 that differed from the other three species. Heterozygosity values (Table 2) ranged from 0.045 (House Wren) to 0.106 (Ruby-crowned Kinglet).

Nei's (1972) genetic distance and modified Rogers' genetic distance (Wright 1978) data are presented in Table 3. The largest genetic distance was between the House Wren and the Yellow-breasted Chat, with a modified Rogers' genetic distance of 0.878. The two kinglets showed the lowest modified Rogers' genetic distance of 0.597.

The conservatism of the supernatant (S-MDH, or MDH-1 of this study) and mitochondrial (M-MDH, or MDH-2 of this study) forms of malate dehydrogenase in birds has been well studied (Kitto and Wilson 1966, Kakizawa et al. 1982, Kuroda et al. 1982). Most passerine birds possess alleles in common at both MDH loci. In our hands, the two alleles of MDH-1 migrated to just slightly different places on the gel. Because the difference was consistent for different individuals of the same species, we are confident that different alleles are present. The two alleles for MDH-2 had distinct relative mobilities. Kitto and Wilson (1966) also found a slower allele for MDH-1 in the Cedar Waxwing (*Bombycilla cedrorum*). The studies on MDH-2 (Kakizawa et al. 1982, Kuroda et al. 1982) did not include North American passerines, and Kitto and

[1] Present address: Department of Biology, Shippensburg University, Shippensburg, Pennsylvania 17257 USA.

TABLE 1. Tissues, buffer systems, and enzyme loci for the 5 species examined.

Tissue	Buffer system[a]	Protein locus	E.C. no.	*Regulus calendula*	*Regulus satrapa*	*Geothlypis trichas*	*Icteria virens*	*Troglodytes aedon*
Heart	TC 8.0	AAT-1	2.6.1.1					
		A		1.00	1.00	1.00		1.00
		B					1.00	
		AAT-2	2.6.1.1					
		A					1.00	
		B		1.00	1.00	1.00		1.00
		G-3-PDH	1.2.1.12					
		A				0.98		
		B						1.00
		C				0.02		
		D					0.29	
		E					0.71	
		ICD	1.1.1.42					
		A			0.04			
		B			0.96			
		C		0.05				
		D				0.05		
		E		0.95				
		F				0.95		
		G						1.00
		H					1.00	
		LDH	1.1.1.27					
		A				1.00	1.00	
		B						1.00
		MDH-1	1.1.1.37					
		A		1.00	1.00			1.00
		B				1.00	1.00	
Liver	LiOH	EST-2	3.1.1.1					
		A		1.00	1.00	0.77		
		B					0.14	
		C				0.02		1.00
		D				0.21	0.14	
		E					0.72	
		EST-3	3.1.1.1					
		A		0.20				
		B		0.50		0.07		
		C		0.20	0.79	0.80	0.29	
		D						0.07
		E		0.10				0.14
		F					0.71	
		G				0.13		0.43
		H			0.21			
		I						0.36
		ME-1	1.1.1.40					
		A						1.00
		B		1.00		0.03		
		C				0.97		
		D					0.10	
		E					0.90	
		F			1.00			
		ME-2	1.1.1.40					
		A						1.00
		B				1.00		
		C					0.25	
		D					0.75	
		E			1.00			
		F		1.00				
	TC 8.0	GK	2.7.1.1					
		A			0.04			

TABLE 1. Continued.

Tissue	Buffer system[a]	Protein locus	E.C. no.	*Regulus calendula*	*Regulus satrapa*	*Geothlypis trichas*	*Icteria virens*	*Troglodytes aedon*
		B			0.96			
		C						0.14
		D						0.57
		E				0.05		
		F		1.00				
		G				0.95		0.29
		H					1.00	
		PEP-1	3.4.13.1					
		A				0.98		
		B						0.07
		C			0.07			
		D					0.29	
		E						0.93
		F					0.42	
		G		1.00	0.86			
		H				0.02		
		I					0.29	
		J			0.07			
		PEP-2	3.4.13.1					
		A					1.00	
		B		0.90	0.96			
		C				0.05		
		D				0.90		
		E		0.10	0.04			
		F						1.00
		G				0.05		
		MDH-2	1.1.1.37					
		A				1.00	1.00	
		B						1.00
		6-PGD-1	1.1.1.44					
		A		0.70		0.05		
		B		0.30	0.07	0.95		1.00
		C					0.29	
		D			0.93		0.71	
		6-PGD-2	1.1.1.44					
		A		1.00	0.50			0.20
		B			0.50	1.00	1.00	0.80
	RW	PGM	2.7.5.1					
		A			0.04		0.07	
		B				0.05		
		C		1.00	0.96	0.93	0.93	
		D				0.02		1.00
		NSP						
		A		1.00	1.00	1.00	1.00	1.00
Liver	LiOH	ACON	4.2.1.3					
		A						0.29
		B				0.05		
		C						0.71
		D				0.95		
		E		0.30	0.04			
		F					1.00	
		G		0.70				
		H			0.96			

[a] TC 8.0 and LiOH buffers from Selander et al. 1971. RW buffer from Ridgway et al. 1970.

TABLE 2. Intraspecific variation in 5 North American passerine species (standard error in parentheses).

	Mean alleles/ locus	Percentage of loci polymorphic[a]	Mean heterozygosity: Direct count	Mean heterozygosity: Hardy-Weinberg expected[b]
Regulus calendula	1.4 (0.2)	31.3	0.106 (0.048)	0.117 (0.054)
Regulus satrapa	1.6 (0.2)	50.0	0.076 (0.030)	0.069 (0.026)
Troglodytes aedon	1.4 (0.2)	26.3	0.045 (0.038)	0.119 (0.053)
Icteria virens	1.5 (0.2)	42.1	0.074 (0.039)	0.171 (0.053)
Geothlypis trichas	1.8 (0.2)	63.2	0.073 (0.023)	0.090 (0.026)

[a] A locus was considered polymorphic if more than one allele was detected.

[b] Unbiased estimate (Nei 1978).

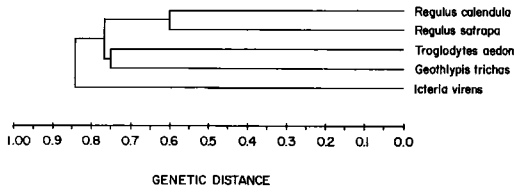

Fig. 1. Phenogram based on modified Rogers' genetic distance (Wright 1978) values. The *F*-value (Farris 1972) is 0.153, the percentage standard deviation (Fitch and Margoliash 1967) is 2.88, and the cophenetic correlation is 0.953.

Wilson (1966) suggested that the conservatism in MDH-2 is not as severe as it is in MDH-1.

We obtained heterozygosity values consistent with other studies, although the Ruby-crowned Kinglet value (0.106) was higher than that obtained by Avise et al. (1980a; $H = 0.048$, $n = 10$). Sample sizes were the same in both studies, but few of the loci examined were shared.

The electrophoretic data strongly support the contention of Mayr and Short (1970) that the Ruby-crowned and Golden-crowned kinglets are not closely related. The Nei (1972) genetic distance of 0.50 is larger than genetic distance values for species in other passerine genera such as *Dendroica* (0.00–0.17; Avise et al. 1980b), *Catharus* (0.01–0.03; Avise et al. 1980a), *Toxostoma* (0.09; Avise et al. 1982), *Vireo* (0.03–0.51; Avise et al. 1982), and *Parus* (0.00–0.07; Braun and Robbins 1986). The Nei distance falls within the range of 0.40–0.80, similar to those reported for comparisons between very closely related avian families or between highly divergent, confamilial genera (Avise et al. 1982). The electrophoretic data are supported by DNA-DNA hybridization studies (Sibley and Ahlquist 1985) that showed the Ruby-crowned Kinglet was more closely related to the Firecrest (*R. ignicapillus*; $\Delta T_{50}H = \sim 2.2$) than either was to the Golden-crowned Kinglet ($\Delta T_{50}H = \sim 2.5$). Although these DNA values are fairly high, they are within the range determined for other passerine congeners (Sibley and Ahlquist 1985). We propose that the two kinglets are the result of independent invasions from the Old World. The DNA data suggest that the Ruby-crowned Kinglet is the most recent arrival. A comprehensive understanding of relationships among the kinglets, however, would require a complete study of all five *Regulus*.

The UPGMA tree (Fig. 1) shows that the yellowthroat and wren are equally similar to the kinglets. The cladogram (Fig. 2), by providing systematic inferences based on branch length, permits resolution of the equalities apparent in Fig. 1. The sum of the branch lengths from the kinglet basal node to the wren (93) is shorter than to the yellowthroat (136) and implies that the wrens are more similar to the kinglets than are the wood warblers, as represented by the Common Yellowthroat or Yellow-breasted Chat. Sibley and Ahlquist (1982a) suggested, based on unpublished DNA-DNA hybridization data, that the kinglets and wren are most closely related because of a close relationship of both groups to the Old World warblers and babblers.

Both figures demonstrate that the Yellow-breasted Chat, considered to be a wood warbler (Sibley and Ahlquist 1982b), is the most divergent member of this specific group of birds. The correct systematic position of the Yellow-breasted Chat has been widely debated (see Sibley and Ahlquist 1982b for review). Avise et al. (1980b) showed that the chat was more similar to the wood warblers than to either the Red-

TABLE 3. Nei's (1972) genetic distance (above diagonal) and modified Rogers' genetic distance (Wright 1978; below diagonal) estimates among 5 species.

	1	2	3	4	5
1. *Regulus calendula*	—	0.495	1.136	1.939	1.008
2. *Regulus satrapa*	0.597	—	1.216	1.625	1.011
3. *Troglodytes aedon*	0.773	0.796	—	2.212	1.210
4. *Icteria virens*	0.860	0.843	0.878	—	1.005
5. *Geothlypis trichas*	0.756	0.765	0.797	0.748	—

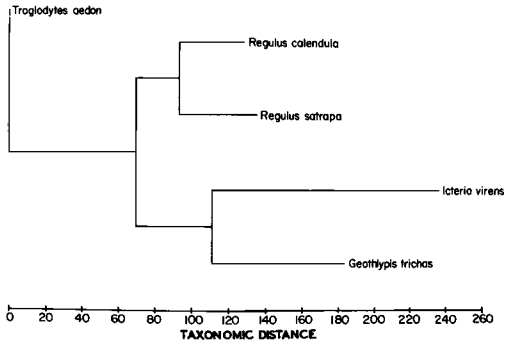

Fig. 2. The most parsimonious tree for 5 taxa of passerines from PAUP package using alleles as characters and presence/absence as character states. Consistency index = 0.877; tree length = 407.

eyed Vireo (*Vireo olivaceus*) or Swainson's Thrush (*Catharus ustulatus*), two groups for which affinities have been suggested. Sibley and Ahlquist (1982b) also showed that the chat is a wood warbler, although probably a very early branch in the phylogeny of the family. The branch length from the yellowthroat to either kinglet is shorter than that to the chat; if the chat is a wood warbler, it is quite distinct from the yellowthroat.

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