Condor 84:40–44

THE RELATIONSHIPS OF THE WRENTIT AS INDICATED BY DNA-DNA HYBRIDIZATION

CHARLES G. SIBLEY
AND
JON E. AHLQUIST

ABSTRACT.—The relationships of the Wrentit (*Chamaea fasciata*) have been debated since its discovery in 1845. The titmice (*Parus*), babblers (Timaliini), Old World warblers (Sylviini), bushtits (*Psaltriparus*), wrens (Troglodytidae), and mockingbirds (Mimini) have been suggested as its closest relatives. Many authors have placed the Wrentit in a monotypic family, Chamaeidae. DNA-DNA hybridization comparisons between the homologous nucleotide sequences of the single-copy DNAs of the Wrentit and other passerines indicate that *Chamaea* is most closely related to the babblers and the Old World warblers, and that the latter two groups are ecotypes of a single, varied, monophyletic assemblage. The Wrentit is approximately equidistant from the sylviines and the timaliines, but ecologically it is a babbler.

The Wrentit (*Chamaea fasciata*) occurs in chaparral and other dense, brushy, plant formations in western Oregon, California, and northwestern Baja California. The question of its affinities to other passerines has been debated since the 1850's. Sibley (1970:57–60) reviewed the taxonomic history of the Wrentit and presented electrophoretic comparisons between its egg white proteins and those of many other oscine taxa. He concluded that the total evidence did not offer a clear choice between the sylviine warblers and the timaliine babblers as the closest relatives of *Chamaea* and that the question of the "relationships of the wren-tit remains open and further evidence should be presented before the affinities of the wren-tit are considered to have been proved beyond doubt" (p. 60). More recently, Sibley and Ahlquist (1980) concluded that *Chamaea* is a babbler. In the present paper we offer additional data showing that the sylviine warblers and timaliine babblers are actually ecotypes of a single varied assemblage and that the Wrentit probably was derived from an Asiatic babbler.

METHODS

We have used the DNA-DNA hybridization technique to examine the taxonomic relationships between the Wrentit and other passerines. The genetic material, deoxyribonucleic acid (DNA), is a double-stranded molecule composed of linear sequences of four "nucleotides" which differ in the chemical structures of their nitrogenous bases, namely, adenine (A), thymine (T), guanine (G), and cytosine (C). In double-stranded DNA the bases occur as complementary pairs: an A in one strand pairs only with a T in the other strand, a G pairs only with a C. Genetic information is encoded in the *sequences* of the bases. The two strands of native DNA molecules will separate if heated in solution to ca. 100°C, which dissociates ("melts") the hydrogen bonds between base pairs. Upon cooling, the double-stranded molecules reform because the complementary bases on the two strands reassociate. If the temperature is maintained at or near 60°C, base pairing will occur only between *long* homologous sequences of nucleotides. This is because only long sequences of complementary bases have sufficient bonding strength to maintain stable duplexes at that temperature, and only homologous sequences possess the necessary degree of complementarity. Thus, under appropriate conditions of temperature and salt concentration, the dissociated single strands of conspecific DNA will reassociate only with their homologous partners and the matching of complementary base pairs will be essentially perfect.

Similarly, if the single-stranded DNA molecules of two different species are combined under conditions favoring reassociation, "hybrid" double-stranded molecules will form between homologous sequences. However, these hybrid molecules will contain mismatched base pairs because of the differences in their nucleotide sequences (i.e., nucleotide substitutions) that have evolved since the two species diverged from their most recent common ancestor. The mismatched bases reduce the bonding strength holding the two strands together and cause them to dissociate at a temperature lower than that required to melt conspecific double-stranded DNA molecules. The

TABLE 1. DNA-DNA hybridization values between the radioiodine-labeled single-copy DNA of the Wrentit and the DNAs of other members of the suborder Passeres.

Species	Delta mode
Wrentit (*Chamaea fasciata*)	0.0
Short-tailed Jungle-Babbler (*Trichastoma malaccense*)	6.8
Blackcap (*Sylvia atricapilla*)	7.0
Rufous-winged Fulvetta (*Alcippe castaneceps*)	7.1
Lesser Whitethroat (*Sylvia curruca*)	7.3
Striped Wren-Babbler (*Kenopia striata*)	7.4
Black-capped Babbler (*Pellorneum capistratum*)	7.8
Common Babbler (*Turdoides caudatus*)	7.9
Chiffchaff (*Phylloscopus collybita*)	7.9
White-crested Laughingthrush (*Garrulax leucolophus*)	8.1
Black-throated Babbler (*Stachyris nigriceps*)	8.1
Tufted Titmouse (*Parus bicolor*)	9.1
Black-tailed Gnatcatcher (*Polioptila melanura*)	9.2
Eurasian Robin (*Erithacus rubecula*)	9.4
Ruby-crowned Kinglet (*Regulus calendula*)	9.7
Red-eyed Vireo (*Vireo olivaceus*)	9.7
Common Bushtit (*Psaltriparus minimus*)	10.0
Brown Creeper (*Certhia familiaris*)	10.4
Verdin (*Auriparus flaviceps*)	11.8

TABLE 2. DNA-DNA hybridization values between the radioiodine-labeled single-copy DNA of the Short-tailed Babbler and the DNAs of other passerine taxa. All are members of the suborder Passeres, except the suboscine tyrannid, *Elaenia flavogaster*.

Species	Delta mode
Short-tailed Jungle-Babbler	0.0
Ferruginous Jungle-Babbler (*Trichastoma bicolor*)	1.2
Scaly-crowned Babbler (*Malacopteron cinereum*)	3.3
Striped Wren-Babbler	3.9
Wrentit	6.2
Rufous-winged Fulvetta	6.4
Rufous-winged Fulvetta*	6.5
Garden Warbler (*Sylvia borin*)	6.9
Garden Warbler*	7.1
White-crested Laughingthrush	7.3
White-crested Laughingthrush*	7.5
Great Reed-Warbler (*Acrocephalus arundinaceus*)	8.2
Ruby-crowned Kinglet	9.4
American Robin (*Turdus migratorius*)	10.2
White Wagtail (*Motacilla alba*)	10.6
Mimic Honeyeater (*Meliphaga analoga*)	11.1
Red-backed Shrike (*Lanius collurio*)	11.7
Yellow-bellied Elaenia (*Elaenia flavogaster*)	17.2

* Duplicate DNA hybrid.

reassociation of homologous sequences, and the decreased thermal stability of partly mismatched hybrid sequences, form the basis of the DNA-DNA hybridization technique.

The extent to which the bases in the homologous nucleotide sequences of any two single strands of DNA form complementary A–T and G–C pairs can be determined by measuring (1) the percentage of hybridization and (2) the thermal stability of the reassociated double-stranded molecules. Following is a synopsis of the technique, which is described in more detail by Sibley and Ahlquist (1981), and based upon procedures described by Kohne (1970) and Britten et al. (1974).

DNAs of the species in Tables 1–4 were obtained from the nuclei of avian erythrocytes, purified according to the procedures of Marmur (1961) and Shields and Straus (1975), and "sheared" into fragments with an average length of ca. 500 nucleotides by sonication. Fragment size was determined by electrophoretic comparisons with DNA fragments of known size produced by the digestion of bacteriophage DNA with bacterial restriction endonucleases (Nathans and Smith 1975).

The single-stranded DNA's of the Wrentit, and the other three species to be "labeled" with radioiodine, were allowed to reassociate to a C_0t of 1,000 at 50°C in 0.48 M sodium phosphate buffer (C_0t = the concentration of DNA in moles/l times the duration of incubation in seconds. Kohne 1970:334). This period of reassociation permitted most of the repeated sequences to form double-stranded molecules while the slowly reassociating single-copy sequences remained single-stranded. The latter were recovered by chromatography on a hydroxyapatite column. This process produced a single-copy DNA preparation consisting of one copy per genome of each original single-copy sequence and *at least* one copy per genome of each different repeated sequence. Such a single-copy preparation contains at least 98%, and probably 100%, of the "sequence complexity" of the genome, i.e., the total length of *different* DNA sequences (Britten 1971, pers. comm.). Kohne (1970:334–347) has discussed the reasons for using only single-copy DNA in studies designed to determine "the extent of nucleotide change since the divergence of two species."

The single-copy DNA sequences of the Wrentit, the Short-tailed Jungle Babbler (*Trichastoma malaccense*), the Garden Warbler (*Sylvia borin*), and the Lesser Whitethroat (*S. curruca*) were labeled with radioactive iodine (^{125}I) according to the procedures of Commorford (1971) and Prensky (1976). DNA-DNA hybrids were formed from a mixture composed of one part (=250 ng) radioiodine-labeled single-copy DNA and 1,000 parts (=250 μg) sheared, whole DNA. The hybrid combinations were heated to 100°C for 10 min to dissociate the double-stranded molecules into single strands, then incubated for 120 h (=C_0t

TABLE 3. DNA-DNA hybridization values between the radioiodine-labeled single-copy DNA of the Garden Warbler and the DNAs of other members of the suborder Passeres.

Species	Delta mode
Garden Warbler	0.0
Black-throated Babbler	5.5
Striped Wren-Babbler	6.9
Scaly-crowned Babbler	7.0
Wrentit	7.1
Chiffchaff	7.5
Ferruginous Jungle-Babbler	7.5
Great Reed-Warbler	7.7
Red-eyed Vireo	10.1
House Sparrow (*Passer domesticus*)	10.4
Gray Catbird (*Dumetella carolinensis*)	10.5
Yellow-breasted Sunbird (*Nectarinia jugularis*)	11.2

TABLE 4. DNA-DNA hybridization values between the radioiodine-labeled single-copy DNA of the Lesser Whitethroat and the DNA's of other members of the suborder Passeres.

Species	Delta mode
Lesser Whitethroat	0.0
Striped Tit-Babbler (*Macronous gularis*)	7.1
Common Babbler	7.5
White-crested Laughingthrush	7.7
Chiffchaff	8.3
Black-tailed Gnatcatcher	9.3
Spot-winged Monarch (*Monarcha guttula*)	10.2
Red-eyed Vireo	10.4
Spotted Flycatcher (*Muscicapa striata*)	10.8
Eurasian Robin	10.9
White-throated Sparrow (*Zonotrichia albicollis*)	11.3

16,000) at 60°C to permit the single strands to form double-stranded hybrid molecules.

The DNA-DNA hybrids were bound to hydroxyapatite columns immersed in a temperature-controlled water bath at 55°C and the temperature was then raised in 2.5°C increments from 55°C to 95°C. At each of the 17 temperatures, the single-stranded DNA produced by the melting of double-stranded hybrids was eluted in 20 ml of 0.12 M sodium phosphate buffer.

The radioactivity in each eluted sample was counted in a Packard Model 5220 Auto-Gamma Scintillation Spectrometer, optimized for ^{125}I. A teletype unit connected to the gamma counter printed out the data and punched a paper tape, which was read by the computer program. The computer program calculated the modal values of the frequency distributions of radioactive counts for each DNA-DNA hybrid by fitting the data to a modified form of the Fermi-Dirac equation. The difference in °C between the modal temperature of the homoduplex hybrid (e.g., *Chamaea* × *Chamaea*) and each of the heteroduplex hybrids (e.g., *Chamaea* × *Trichastoma*, etc.) is called the "delta mode," which is used as a single number statistic to compare the nucleotide sequences of the radioiodine-labeled species with each of the other species in a set of DNA hybrids. We have found that delta mode values up to ca. 3 are usual for congeneric species; up to ca. 9 for members of the same family; and between ca. 10 and 14 for members of different families in the same order. These values are approximate and subject to an error of ±1.0.

The delta mode values in Tables 1–4 are measurements between the labeled species and the other species in each table, but not among the latter. Two species that have the same delta mode values are equidistant phylogenetically from the labeled taxon, but may be any distance from one another which is equal to, or less than, their common distance from the labeled species. Only a complete matrix of DNA-DNA measurements provides the basis for a complete phylogeny. The delta mode values are subject to an experimental error of ±0.5°C.

RESULTS

In Tables 2–4 there are nine delta mode values for hybrids between the genus *Sylvia* and eight species of babblers. The average delta mode ± SD is 7.0 ± 0.6. This relatively low value indicates that *Sylvia* and the babblers are closely enough related to be included in the same family. When *Sylvia* is compared with the other genera traditionally included with it in the Sylviidae (*Phylloscopus, Acrocephalus*) the average delta mode for the three DNA-DNA hybrids is 7.8 ± 0.4. The average for all 12 values is 7.2 ± 0.7.

The eight DNA hybrids between the Wrentit and the seven species of babblers in the tables have an average delta mode of 7.4 ± 0.7. The average delta mode of the four DNA hybrids between *Chamaea* and the Old World warblers (*Sylvia, Phylloscopus, Acrocephalus*) in Tables 1 and 3 is 7.3 ± 0.4, and for the three delta modes between *Chamaea* and *Sylvia*, 7.1 ± 0.1. Thus, *Chamaea* is equidistant from the babblers and the Old World warblers and as far from each of them as they are from one another.

DISCUSSION

These data indicate that the Old World warblers, the babblers, and the Wrentit are closely enough related to be included within a single family; they also show that the boundary between the babblers and warblers is not clearly

defined. In Table 1 the delta mode values for babblers and warblers are interspersed in the sequence of taxa progressively more distant from *Chamaea*. The values in Table 2 suggest that the babbler genera *Trichastoma, Malacopteron* and *Kenopia* are especially closely related. The data in Tables 3 and 4 are similar to those in Table 1 in showing that several babblers are more closely related to *Sylvia* than are *Phylloscopus* and *Acrocephalus*.

The interspersed pattern of delta mode values indicates that the babblers, Old World warblers, and *Chamaea* are members of the same monophyletic group in which the various genera have branched from one another at different times. If the babblers and Old World warblers were separate monophyletic groups, they would represent the two branches resulting from a single ancestral divergence event. The members of each descendant sister group would be equidistant from all members of the other group, but at various distances from other members of their own group. Since we do not see such a pattern among the delta mode values, we conclude that the babblers and Old World warblers represent a single adaptive radiation in which the "babblers" and "warblers" are different ecotypes, rather than separate monophyletic taxa. Within this cluster the Wrentit is a "babbler" and, judging from the data in Table 2, it was probably derived from an Asiatic babbler. Our data are not extensive enough to identify its closest living relative among the babblers.

The lack of a clear boundary between babblers and Old World warblers is also demonstrated by the morphological and ecological similarities between the Wrentit and the Dartford Warbler (*Sylvia undata*) of western Europe and North Africa. Like the Wrentit, the Dartford Warbler inhabits dense thickets of woody shrubs. Their habitats, called "chaparral" in California, and "maquis" in the Mediterranean region, are the products of similar semi-arid climates in the two areas. Both species reach their northern limits in more humid climates (Oregon and southern England), but even there they inhabit dense thickets. They are similar in plumage coloration and both carry their relatively long tails cocked up over the back. In addition, both have bright irides, eat insects and small fruits, build similar nests, and are highly vocal. The two species thus appear to be convergently similar ecological counterparts. If the Dartford Warbler occurred in southeast Asia, instead of Europe, it seems likely that it would be considered a "babbler," not a "warbler." Several other species of *Sylvia*, including *S. sarda* and *S. deserticola*, are morphologically similar to *S. undata* and also occur in southwestern Europe and/or North Africa. Others, e.g., *S. cantillans* and *S. conspicillata*, are rather intermediate between *S. undata* and the more typical members of the genus such as *S. borin* and *S. curruca*.

A similar situation exists between the muscicapine flycatchers and the erithacine thrushes, which are more closely related to one another than either is to any other group. They too are ecotypes of a single monophyletic unit (Sibley and Ahlquist 1980).

The delta modes for the other taxa that have been proposed as possible close relatives of *Chamaea* are greater than those between *Chamaea* and the babbler-Old World warbler group. *Parus*, separated from *Chamaea* by a delta mode of 9.1, may also be included in the same family with the babblers and Old World warblers, but its DNA should be radiolabeled and compared with DNAs from an array of taxa to determine its relationships within the warbler-babbler assemblage.

The kinglets (*Regulus*) are sometimes included in the Sylviidae and sometimes placed in a separate family, Regulidae. The delta modes of 9.4 for the *Trichastoma-Regulus* hybrid (Table 2) and 9.7 for the *Chamaea-Regulus* hybrid (Table 1) do not lend support to either alternative. The DNA of *Regulus*, like that of *Parus*, must be labeled and compared with DNAs from additional taxa to determine its relationships.

The sylviine warblers, muscicapine flycatchers, and turdine thrushes have often been included in a single family, but the data in Tables 2 and 4 indicate that *Turdus, Muscicapa*, and *Erithacus* are as distant from the babbler-sylviine cluster as are many other passerine families. Other DNA hybridization data indicate that the erithacine thrushes, muscicapine flycatchers, dippers, mockingbirds and thrashers, starlings, and turdine thrushes are members of a single family, the Muscicapidae (Sibley and Ahlquist 1980).

We conclude that the sylviine warblers and timaliine babblers, including *Chamaea*, are closely related to one another and are ecotypes resulting from adaptive radiation within a single monophyletic group, rather than representatives of separate sister groups. Their next closest relatives are probably the titmice (Paridae) but we prefer to defer proposals for the restructuring of the classification of these forms until the completion of DNA comparisons among other oscine taxa.

ACKNOWLEDGMENTS

We thank C. Barkan, M. Pitcher, N. Snow, and F. C. Sibley for assistance in the laboratory. For help in the field we

thank H. L. Achilles, M. Bull, J. duPont, S. Ervin, P. Garayalde, L. Kiff, R. Liversidge, J. O'Neill, S. Rothstein, J. Schmitt, R. Semba, F. Sheldon, F. C. Sibley, J. Spendelow, N. and E. Wheelwright, and D. Wysham. The computer program was written by T. F. Smith. We are indebted to R. Britten, D. Kohne, G. Shields, and W. Thompson for advice. The laboratory work was supported by Yale University and National Science Foundation Grant DEB-77-02594.

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Peabody Museum of Natural History and Department of Biology, Yale University, New Haven, Connecticut 06511.
Accepted for publication 20 June 1981.

Condor 84:44

RECENT PUBLICATION

The Birds of Cameroon. An Annotated Check-list.—M. Louette. 1981. Verhandelingen van de Koninklijke Academie voor Wetenschappen, Letteren en schone Kunsten van Belgie, Klasse der Wetenschappen 43, No. 163. Brussels. 295 p. Price: BF 900,—. Source: Brepols I. G. P., Baron du Fourstraat 8, B 2300 Turnhout, Belgium. The Royal Belgian Academy of Sciences and Arts sponsored nine expeditions to Cameroon during 1970–1977 to study and collect the vertebrate fauna. Louette has already published extensively about the ornithological results of this undertaking, most recently (1977) a three-volume work in Flemish. He subsequently visited most of the leading museums of Europe and North America where material from the Cameroon is to be found, and reviewed the literature up to 1978. We now have a check-list (848 species) with 66 distribution maps pertaining to some 80 species. Dates and localities of the Belgian collection are given and a useful gazetteer with geographic coordinates helps to locate these places on any good map of western Africa. An important chapter following the check-list proper discusses several zoogeographical conclusions derived from the taxonomic and faunistic details found in the specimen survey. For the biogeographer these conclusions seem to be important, but are tantalizingly general, mostly lacking comprehensive documentation. It may be hoped that these will be forthcoming in a detailed study of the Cameroon ornithogeography promised by the author.—M. D. F. Udvardy.