Wilson Bull., 97(4), 1985, pp. 421–435

GENETIC EVIDENCE FOR RELATIONSHIPS AMONG THE RED-EYED, YELLOW-GREEN, AND CHIVI VIREOS

NED K. JOHNSON AND ROBERT M. ZINK

One of the most enduring puzzles in the systematics of New World birds concerns the relationships and taxonomic status of forms in the Red-eyed (*Vireo olivaceus*)-Yellow-green (*V. flavoviridis*)-Chivi Vireo (*V. chivi*) complex. In recent decades, the latter two taxa have been treated either as distinct species (A.O.U. 1957, Hamilton 1962) or as races of the Red-eyed Vireo (Hamilton 1958, Barlow 1980, A.O.U. 1983). Arguments for specific status of the Yellow-green Vireo have stated that this form has a wing formula (Eisenmann 1962) and advertising song (Borror 1972) distinctive from those of the Red-eyed Vireo and Chivi Vireo, and have emphasized the lack of definitely intermediate specimens between *flavoviridis* and either other form. No hybrids have been recorded despite the fact that the known breeding range of the Yellow-green Vireo approaches that of the Red-eyed Vireo in southern Texas (Eisenmann et al. 1968, A.O.U. 1983) and is contiguous with that of the Chivi Vireo near the border of Panama and Colombia (Eisenmann 1962). Reasons for subspecific treatment for both *flavoviridis* and *chivi* have stressed that (1) *olivaceus, flavoviridis,* and *chivi* are all allopatric during the breeding period; (2) plumage differences among them are either minor (*olivaceus* and *chivi* vs *flavoviridis*) or subtle (*olivaceus* vs *chivi*); and (3) their songs are generally similar (Slud 1964, Robbins et al. 1966, Barlow 1981). All forms of *olivaceus* and *flavoviridis* have red irides as adults and brown irides as immatures. In contrast, insofar as we have been able to determine from the literature and from specimen tags with eye color information, the forms of *chivi* have brown irides at all ages. Eye color of adults is thus the only obvious difference between *chivi* and *olivaceus.*

Oddly, the breeding ranges of the two most similar forms, *olivaceus* in North America and *chivi* in South America, are separated by all of Middle America, which is the nesting distribution of the more distinctive form, *flavoviridis* (Barlow 1980). Such a geographic-phenotypic pattern is reminiscent of the situation seen even more strikingly in a number of avian taxa in the Andes (Remsen 1984). In view of this peculiar geographic layout, systematists have been reluctant to take an intermediate stance in their classification of these vireos, namely, to combine two of the taxa as subspecies of one species while giving species status to the remaining

taxon. Nonetheless, the evidence on genetic differentiation presented here supports exactly such a position. We propose that the two geographically disjunct taxa, *olivaceus* and *chivi,* are conspecific but that the form that occurs between them, *flavoviridis,* deserves species rank. Although we studied only the subspecies *diversus* from Paraguay, all forms of the Chivi Vireo are considered here to be subspecies of the Red-eyed Vireo. Admittedly, eventual genetic study of the other geographic differentiates of *chivi* could provide results that might drastically alter this position.

The genic data, considered together with the current breeding distributions of the taxa, also enable a more objective discussion of phyletic history than heretofore has been possible. Unfortunately, we lack genetic information from several other forms of the genus, including the Black-whiskered Vireo (*V. altiloquus*), Yucatán Vireo (*V. magister*), and the Golden Vireo (*V. hypochryseus*). Although some or all of these species are probably closely related to the *V. olivaceus-flavoviridis-chivi* complex (Hamilton 1962, Barlow 1980), in the absence of data on genetic distances we do not consider them further in this paper.

MATERIALS AND METHODS

Our analysis is based on 17 specimens of the Red-eyed Vireo (*V. o. olivaceus*), one specimen of the Yellow-green Vireo (*V. f. flavoviridis*), 14 specimens of the "Chivi" Vireo (*V. o. diversus*), and one specimen of the Rufous-browed Peppershrike (*Cyclarhis gujanensis ochrocephala*). The latter form was used as an outgroup taxon.

Samples of heart, liver, kidney, and pectoral muscle were removed from specimens 1–4 h after collection in the field, preserved in liquid nitrogen, then stored in Berkeley, California, at −76°C (Johnson et al. 1984). Tissue extracts were prepared for electrophoresis according to methods described by Selander et al. (1971). Gel and buffer combinations used were essentially those outlined by Yang and Patton (1981). Thirty-eight presumptive genetic loci were scored. Although some loci were scored on more than one gel type, our basic approach was "one pass" (Aquadro and Avise 1982). Precise electrophoretic conditions are available from the authors. The most common allele at a locus was designated M; alleles with more anodal or cathodal migrations were coded as F or S, respectively. Further refinements in migratory position were coded with plus and minus signs.

The distribution of observed and expected numbers of heterozygotes (Table 1), over all loci in a sample, was tested for departure from Hardy-Weinberg expectation (Hartl 1981) with a χ^2 test (Barrowclough 1980). Individual heterozygosity was calculated by dividing the number of loci at which an individual was heterozygous by the total number of loci scored (38 for all birds). Two measures of average individual heterozygosity per population sample ($H_{obs.}$ and $H_{exp.}$) were calculated as described in Johnson and Zink (1983:873). Individual genotypes were converted to allelic frequencies (Table 2) for each sample. Genetic distances between samples (Table 3) were computed using the methods of Nei (1978) and Rogers (1972). Branching diagrams depicting patterns of genic similarity among samples (Fig. 1) were constructed using the methods of Sneath and Sokal (1973; UPGMA and WPGMA phenograms), Farris (1972; distance Wagner tree, optimized according to Swofford [1981]), and Fitch and Margoliash (1967; "F-M" trees).

TABLE 1

GENETIC VARIABILITY MEASURES FOR FOUR TAXA OF VIREONIDAE

Taxon	N	Sample area[a]	$H_{obs.} \pm SE$	$H_{exp.} \pm SE$	Percent polymorphic loci[b]	Average number of alleles[c]
V. o. olivaceus	17	Minnesota (15) Oklahoma (2)	0.059 ± 0.014	0.054 ± 0.017	39.47	1.53
V. o. diversus	14	SE Paraguay	0.060 ± 0.016	0.065 ± 0.025	26.32	1.47
V. f. flavoviridis	1	Costa Rica	0.0	0.0	0.0	1.00
C. g. ochrocephala	1	SE Paraguay	0.053	0.026	5.26	1.03
Total	33					
Mean[d]			0.057	0.036	17.76	1.26

[a] Breeding specimens only. Exact localities can be obtained from the authors.
[b] Frequency of most common allele ≤0.99.
[c] Per locus.
[d] Unweighted by sample size.

RESULTS

Variation at loci and heterozygosity.—Of the 38 loci scored, 19 (50%) showed at least a single heterozygote. At five other loci, the species were fixed at alternative alleles. Therefore, 24 (63.2%) of the total loci were variable among the taxa examined. Allelic frequencies at the polymorphic loci are listed by taxon in Table 2. The 14 monomorphic loci, within and between taxa, were: LDH-1, AB-1, AB-2, AB-3, LAP, SOD-1, EAP, GOT-1, GOT-2, MDH-1, MDH-2, GLUD (=GDH), ADH, and EST-1. Acronyms for enzymes follow Harris and Hopkinson (1976).

The observed and expected heterozygosities, the percentage of polymorphic loci and the mean number of alleles per locus for the four taxa of vireonids treated are given in Table 1. The fact that we examined only a single individual each of *V. f. flavoviridis* and *C. g. ochrocephala* presents no problem in the estimation of either mean levels of heterozygosity or genetic distance. According to theoretical (Nei 1978) and empirical (Gorman and Renzi 1979) rationales, good estimates of these parameters can be obtained from single specimens, given that a relatively large number of loci is analyzed and that heterozygosity is relatively low.

Values for observed heterozygosity for *V. o. olivaceus* and *V. o. diversus* were 0.059 and 0.060, respectively (Table 1). These values are somewhat higher than 0.048, the figure reported by Avise et al. (1982:97) for a large sample of *V. olivaceus* (presumably *V. o. olivaceus*), and 0.053, the average value reported for birds in general by Barrowclough (1983). In average

TABLE 2

ALLELIC FREQUENCIES FOR POLYMORPHIC LOCI IN THREE TAXA OF *VIREO* AND IN *CYCLARHIS GUJANENSIS OCHROCEPHALA*

Locus	*V. o. olivaceus* (N = 17)	*V. o. diversus* (N = 14)	*V. f. flavoviridis* (N = 1)	*C. g. ochrocephala* (N = 1)
GPI	A = 0.03, B = 0.97	A = 0.04, B = 0.96	B = 1.00	B = 1.00
NP	B = 0.79, C = 0.21	A = 0.18, B = 0.46, C = 0.29, D = 0.04, F = 0.04	B = 1.00	E = 1.00
CK	A = 1.00	A = 1.00	A = 1.00	B = 1.00
LGG	B = 0.77, C = 0.24	A = 0.25, B = 0.68, C = 0.07	B = 1.00	B = 1.00
LA-1	C = 0.94, D = 0.06	B = 0.04, C = 0.96	C = 1.00	A = 0.50, C = 0.50
LA-2	B = 0.94, C = 0.03, D = 0.03	A = 0.11, B = 0.86, D = 0.04	B = 1.00	B = 1.00
GDA	A = 0.94, C = 0.06	A = 1.00	B = 1.00	A = 1.00
EST-4	B = 0.03, C = 0.97	C = 0.93, D = 0.07	B = 1.00	A = 1.00
αGPD	B = 1.00	B = 1.00	C = 1.00	A = 1.00
ALD	A = 1.00	A = 1.00	A = 1.00	B = 1.00
6-PGD	A = 0.09, B = 0.88, C = 0.03	B = 1.00	B = 1.00	B = 1.00
ADA	C = 0.97, D = 0.03	C = 1.00	C = 1.00	A = 0.50, B = 0.50
MPI	B = 0.03, D = 0.97	B = 0.04, C = 0.04, D = 0.93	D = 1.00	A = 1.00
SDH	A = 1.00	A = 0.96, B = 0.04	A = 1.00	C = 1.00
GPT	A = 1.00	A = 1.00	A = 1.00	B = 1.00
ME	A = 0.94, B = 0.03, D = 0.03	A = 1.00	A = 1.00	C = 1.00
GSR	B = 1.00	B = 1.00	A = 1.00	A = 1.00
PGM-1	A = 0.79, B = 0.21	A = 0.96, B = 0.04	A = 1.00	B = 1.00
PGM-2	C = 0.06, E = 0.94	A = 0.04, B = 0.04, C = 0.61, E = 0.32	D = 1.00	D = 1.00
ICD-1	A = 0.03, B = 0.15, C = 0.03, D = 0.79	D = 1.00	D = 1.00	D = 1.00
ICD-2	A = 0.97, B = 0.03	A = 1.00	A = 1.00	A = 1.00
ACON	B = 1.00	B = 1.00	B = 1.00	A = 1.00
LDH-h	A = 1.00	A = 1.00	A = 1.00	B = 1.00
CK-h	A = 1.00	A = 1.00	B = 1.00	C = 1.00

TABLE 3
MATRIX OF GENETIC DISTANCES BETWEEN FOUR TAXA OF VIREONIDAE[a]

	V. o. olivaceus	*V. o. diversus*	*V. f. flavoviridis*	*C. g. ochrocephala*
V. o. olivaceus	—	0.014	0.177	0.533
V. o. diversus	0.053	—	0.176	0.528
V. f. flavoviridis	0.188	0.182	—	0.495
C. g. ochrocephala	0.427	0.419	0.391	—

[a] Nei's (1978) *D*-values are above the diagonal and Rogers' (1972) *D*-values are below the diagonal.

number of alleles per locus, *V. o. olivaceus* and *V. o. diversus* were also very similar (1.53 vs 1.47, respectively). No genetic variation was detected in the single individual of *V. f. flavoviridis*. The specimen of Rufous-browed Peppershrike had only two heterozygous loci (LA-1 and ADA). The observed number of heterozygotes in both samples of the Red-eyed Vireo (sensu lato) did not differ from Hardy-Weinberg expectation: *V. o. olivaceus*, $\chi^2_9 = 5.1$; *V. o. diversus*, $\chi^2_{10} = 6.3$.

When the four taxa were compared at specific loci, several major differences were seen (Table 2). For example, *V. f. flavoviridis* is apparently fixed at an allele which is different from the predominant one shared by *V. o. olivaceus* and *V. o. diversus*, at GDA, EST-4, αGPD, GSR, PGM-2 and CK-h. *C. g. ochrocephala* is apparently fixed at a unique allele at 13 (54.2%) of the 24 variable loci: NP, CK, EST-4, αGPD, ALD, ADA, MPI, SDH, GPT, ME, ACON, LDH-h, and CK-h, indicating large differences between it and the forms of *Vireo*.

Some differences in relative variability at particular loci were also evident. At both 6-PGD and ME, *V. o. olivaceus* had three alleles whereas *V. o. diversus* was fixed. At PGM-2, *V. o. diversus* showed four alleles whereas *V. o. olivaceus* had two. Furthermore, at that locus, the predominant allele was different in the two taxa. Otherwise, these two taxa, considered to be conspecific in this paper, are very similar in allelic frequency distributions.

Genetic distances.—Rogers' (1972) and Nei's (1978) values for genetic distances among the four taxa treated are given in Table 3. *V. o. olivaceus* and *V. o. diversus* are genetically very similar (Nei's $D = 0.014$). On the other hand, both *V. o. olivaceus* and *V. o. diversus* are quite different from *V. f. flavoviridis*, and at a nearly identical level ($D = 0.177$ and 0.176, respectively). The outgroup, *C. g. ochrocephala*, differed substantially ($D = 0.519$) from the three taxa of *Vireo*.

Phenograms and phylogenetic trees.—Despite the continuing debate over the suitability of different methods of branching (see Farris 1981

contra Felsenstein 1984) for describing phylogenies, we find that they reveal structure in the genetic data and are helpful in "visualizing" genetic distances. Also, because of the differing assumptions of each technique (e.g., relative homogeneity of rates of allozymic change), different kinds of information are revealed. In the present study, great congruence of pattern was achieved. The UPGMA and WPGMA clustering procedures (Sneath and Sokal 1973), for example, yielded virtually identical results; therefore, only the former is illustrated (Fig. 1A). All diagrams clearly show the close alliance of *V. olivaceus* and *V. "chivi,"* the more distant attachment of *V. flavoviridis* as a sister group to the first pair of forms, and the great separation of *C. gujanensis* from the clade comprised of the three forms of *Vireo.* We feel confident, therefore, that the consistent pattern of branching achieved in these analyses was not a simple result of the methodology employed (Presch 1979). That is, the branching pattern was independent of assumptions regarding the nature of the data.

DISCUSSION AND CONCLUSIONS

Age of the Taxa

Several researchers (e.g., Nei 1975, Sarich 1977, Yang and Patton 1981, Gutierrez et al. 1983) have attempted to determine the absolute timing of cladogenetic events by various calibrations applied to Nei's *D* values among existing species. Gutierrez et al. (1983), for example, used the mid-Miocene date of a fossil quail, *Cyrtonyx cooki* (Wetmore 1934), in conjunction with average genetic distances between modern *Cyrtonyx montezumae* and its Odontophorine sister taxa, to derive a calibration value of $t = 26.3 \times 10^6 D$, where t is the time since divergence and D is Nei's (1978) genetic distance. Such calibrations are dependent on the existence of a molecular clock (Wilson et al. 1977, Thorpe 1982), that is, a process by which nucleotide substitutions and, therefore, allelic differences among populations and species accumulate stochastically through mutation and drift in a uniform, time-dependent fashion. The recent finding of Barrowclough et al. (1985) that patterns of genetic divergence in diverse taxa of birds agree with the predictions of a neutral, mutation-drift process (Kimura 1979, 1982) lends credence both to the existence of a clock and to these dating attempts.

Nonetheless, several problems attend the use of such calibrations: (1) the relative imprecision of the dating of most fossils, including that of the quail mentioned above; (2) the fact that large standard errors are usually associated with estimates of genetic distance; and (3) the typical lack of independent means, beyond general stratigraphic date or degree of morphologic change, by which to judge the validity of divergence times

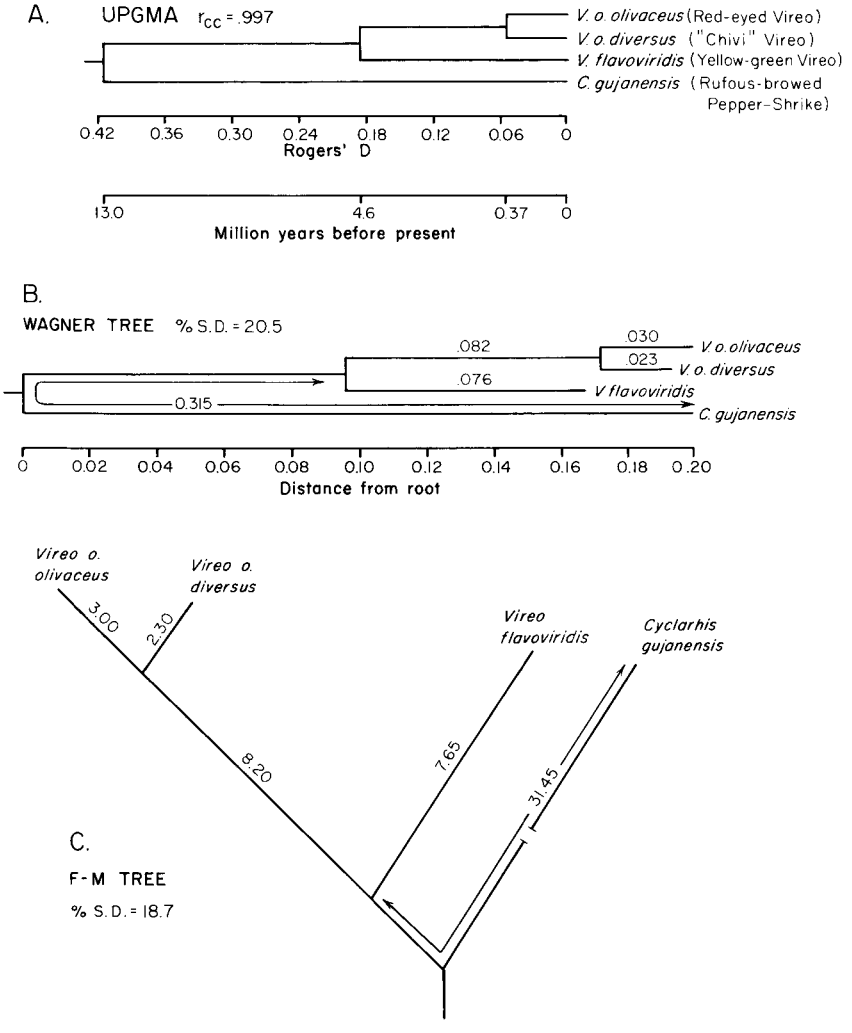

FIG. 1. (A) Phenogram based on Rogers' *D*-values (Table 3) and derived by the UPGMA method. The high cophenetic correlation coefficient (r_{cc} = 0.977) indicates very close agreement between the distances shown in the phenogram and the original distance matrix. See text for the derivation and interpretation of the time scale. (B) Distance Wagner Tree rooted at the outgroup, *Cyclarhis gujanensis*. The *F*-value (Farris 1972) is 0.002 and the cophenetic correlation is 1.00. This analysis produced no negative branches. (C) Branching diagram derived by the method of Fitch and Margoliash (1967). Branch lengths are in units of Rogers' *D* (×100). The tree is "rooted" (see Farris 1972) at *Cyclarhis gujanensis*. Of 6 F-M Trees examined, the one illustrated best summarized the original matrix based on the lack of negative branches and lowest percentage standard deviation.

calculated in this manner. Despite these caveats, gross approximations of cladogenetic dates are useful because they offer a hypothetical framework within which *possible* phyletic history can be addressed. Without such estimates, one is left with the alternative of blind speculation.

According to the calibration of Gutierrez et al. (1983), the Nei's genetic distance of 0.495 between *V. f. flavoviridis* and *C. g. ochrocephala* indicates that the ancestors of these two taxa split approximately 13 million years before the present (MYBP), or in the late Miocene (Fig. 1A). Subsequent separation of the lineage leading to *V. olivaceus*-"*chivi*" from that leading to *flavoviridis,* which differ by a Nei's *D* of 0.176, occurred at approximately 4.6 MYBP, in the late Pliocene. Finally, the small Nei's *D* (0.014) recorded between *V. o. olivaceus* and *V. o. diversus* (="*chivi*") suggests that these taxa diverged comparatively recently, on the order of 370,000 years ago, during the Pleistocene. The substantial, formally recognized geographic variation of *V.* "*chivi*" in South America (10 subspecies, Blake 1968) is in keeping with such a date.

Avise et al. (1982) reported that congeneric vireos are separated by genetic distances that average considerably higher than those between congeneric species in other passerine families. On these grounds they suggested that speciation events in *Vireo* may have been older compared with those of other groups. Our finding of a relatively large genetic distance between *flavoviridis* and both *olivaceus* and *chivi* provides corroboration of their result. We are currently examining the issue of increased genetic distances among vireos through the electrophoretic analysis of additional forms, including several species not studied by Avise et al. (1982).

The substantial genetic distance between *Cyclarhis* and the members of *Vireo* is perhaps not surprising in view of the fact that the pepper-shrikes have often been considered on other grounds to represent either a distinct subfamily or a distinct family (Cyclarhidae) related to the Vireonidae. Based on evidence from DNA-DNA hybridization, however, Sibley and Ahlquist (1982) concluded that *Vireo* and *Cyclarhis* were similar enough to be placed in the same subfamily. This apparent disagreement between the data from electrophoresis and those from DNA-DNA hybridization cannot be resolved at present. But it is clear that any explanation proposed for the difference must take into account the unusually large genetic distances now known among vireos and their close relatives.

Historical Biogeography of the Taxa

The unambiguous genetic results permit evaluation of possible historical biogeographic events that led to the observed geographic pattern. From many conceivable speculative scenarios, we depict four diagrammatically (Fig. 2). In these diagrams, the breeding range of the ancestral

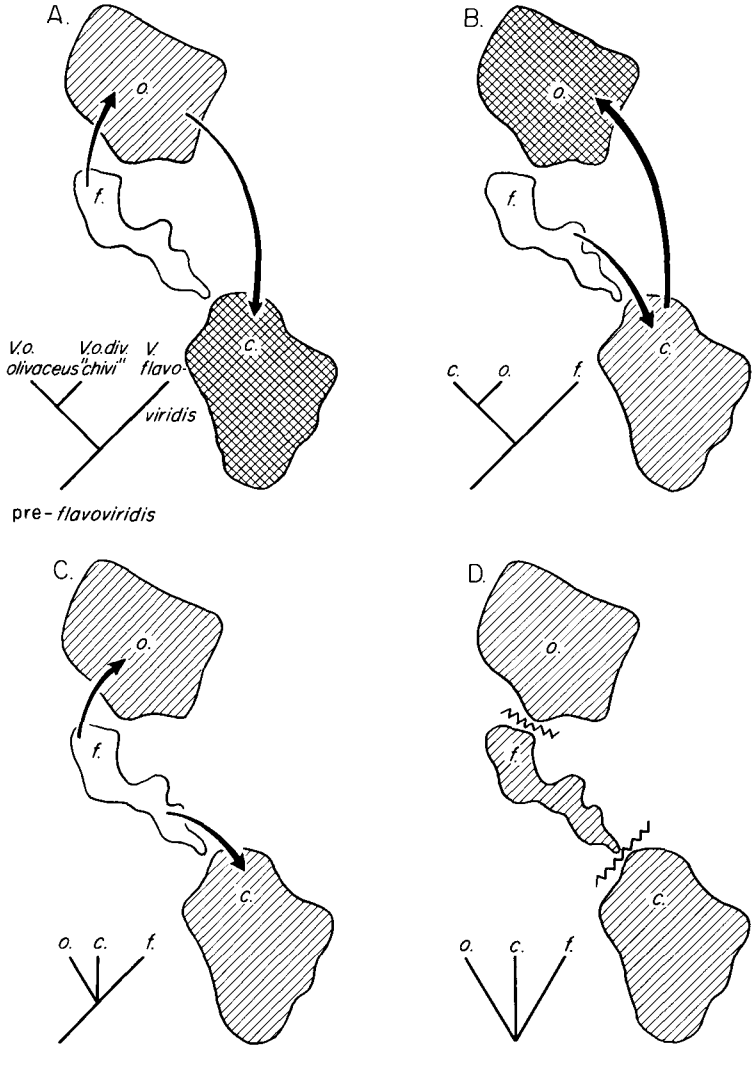

FIG. 2. Schematic representations of four hypothetical scenarios of the biogeographic history of *Vireo olivaceus*, *V. flavoviridis*, and *V.* "*chivi*" in North, Middle, and South America, respectively. See text for additional explanation.

taxon is unpatterned, that of the first derivative taxon is single-hatched, and that of the most recently derived taxon is crosshatched. The first three scenarios (A–C) are dispersal models. These assume that the ancestral taxon occupied part or all of one of the three current ranges and

dispersed to and differentiated in the remaining two distributions after simple colonization followed by range expansion. The fourth scenario (D), a vicariance model, assumes that much or all of the present combined range of the three taxa was occupied by a common ancestor that occurred through North, Middle, and South America. Subsequent fragmentation of all or part of this ancestral distribution provided for the isolation necessary for the differentiation of *olivaceus, flavoviridis,* and *chivi.*

Many other more complex models, which involve either colonization or vicariance processes, admittedly are possible. Models that conceive of the origin of *V. flavoviridis* in South America, with subsequent budding of *V. olivaceus* and *V. chivi,* are also plausible. None of these more intricate models is considered here in detail because in our view one of the simple models presented in Fig. 2 adequately explains the observed data.

Scenario A.—This phylogenetic hypothesis proposes that pre-*flavoviridis* or *flavoviridis* was ancestral and originated in Middle America. It gave rise to *olivaceus,* which, in turn, spawned *chivi* through leapfrog fall migration over the centrally located taxon. Presumably, *chivi* arose through colonization of South America by wintering individuals of *olivaceus* that failed to return to their breeding grounds. This scenario (1) is in agreement with the morphologic and genetic distinctiveness of *flavoviridis* compared with the similarity of *olivaceus* and *chivi*; (2) implicates the present migratory patterns as possible clues to phyletic events; (3) permits the ancestral taxon, *flavoviridis,* to occupy an intermediate "refuge-position" during late Pliocene-Pleistocene climatic refrigeration; (4) allows colonists from pre-*flavoviridis* to establish *olivaceus* as habitats became available to the north after glaciation; and (5) is in keeping with a Middle American center of diversification for the Vireonidae, as suggested by Hamilton (1962, but see Cracraft 1973:518). Furthermore, this hypothesis does not require us to invoke rapid evolutionary rates in *flavoviridis,* versus more moderate rates of change in *olivaceus* and *chivi,* to account for the greater phenetic-genetic differences of the former taxon.

Vireo flavoviridis is also migratory, with populations moving between the breeding grounds in Mexico and Middle America and the wintering regions in Amazonia. Morton (1977) has suggested that both the present scheduling of annual movement and the ultimate selective reason for the evolution of migration in this vireo are related to the seasonal scarcity of fruit in the nesting region. Whatever the reason, the fact of migration in this species in no way reduces the likelihood of Scenario A; a *nesting* population of either pre-*V. flavoviridis* or of *V. flavoviridis* presumably would have provided the stock that eventually budded off the ancestors of *V. olivaceus* to the north.

Scenario B.—This scenario resembles the one depicted in Fig. 2A in

that it suggests a stepwise origin of the two derivative taxa, *olivaceus* and *chivi,* from ancestral *flavoviridis.* In this scenario, however, colonists leading to *chivi* budded off first; these then gave rise to *olivaceus* by a northward leapfrog movement over *flavoviridis.* The fact that southern populations of *chivi* are presently migratory and move north in the late austral summer perhaps provided in the past the possibility for migratory overshoot by groups that became established north of *flavoviridis* and then evolved into *olivaceus.* Several tropical or subtropical species with migratory populations in the Southern Hemisphere (e.g., Fork-tailed Flycatcher [*Muscivora tyrannus*] and Tropical Kingbird [*Tyrannus melancholicus*]) currently are vagrant to North America (National Geographic Society 1983) and provide examples of such migratory overshoot.

Scenario C.—This scenario assumes that both *olivaceus* and *chivi* arose independently, and in essentially the same span of time, from ancestral *flavoviridis.* The improbability that separate groups of colonists from *flavoviridis,* moving northward and southward, respectively, would by chance be nearly identical genetically, however, renders this suggestion unlikely.

Scenario D.—This scheme derives modern *flavoviridis, olivaceus,* and *chivi* from an extensive and continuous range that underwent fragmentation. In this model, migration evolved in the various isolated taxa after their initial split. This scenario also requires more rapid evolution in the component that led to *flavoviridis,* than in the sections that evolved into *olivaceus* and *chivi,* to account for the greater genetic and phenetic differences of the former taxon. The degree to which the invocation of rapid evolution presents a problem is debatable. On the one hand, it is well-documented that differences in morphology and coloration in birds can evolve quickly (Johnston and Selander 1964, 1971). On the other hand, rapid genetic change seems less likely, especially in view of the findings of Barrowclough et al. (1985).

Although any of these schemes is plausible, we prefer Scenario A. It is simple because it does not require the added assumption of differing evolutionary rates of genes among the three taxa, and, most importantly, is realistic. Modern populations of *olivaceus* migrate annually to Amazonia to spend the winter. These wintering hordes of *olivaceus* in South America could have provided abundant opportunity in the past for the establishment of breeding colonies that evolved into *chivi.* Members of the *chivi* group in South America have brown irides, a feature shared by immatures of *V. o. olivaceus.* This fact suggests that the developmental pathway leading to red irides in adult *V. o. olivaceus* was switched off during the differentiation of the stock that led to *chivi.* Brown eyes in adults of the latter taxon may be thought of, therefore, as a paedomorphic character. Welty (1982:554) cites examples of colonization and establish-

ment by wintering individuals of other species, thus our suggestion of this phenomenon in vireos is not novel.

Barlow (1980:103) speculated on the origin and phyletic history of "proto-*Vireosylva*" and one of its branches, the "*V. olivaceus* subspecies complex." He proposed that the proto-*Vireosylva* lineage originated in Middle America and subsequently sent a segment into South America in the late Tertiary. There it diversified and eventually gave rise to progenitors of the *V. olivaceus* group. Early representatives of the *V. olivaceus* complex then spread northward in waves, into the Caribbean through the Lesser Antilles and from the mainland of Middle America, leaving proto-*V. magister* and *V. altiloquus,* and continued into North America. Although Barlow does not hypothesize details of branching of forms in the *V. olivaceus* subspecies complex, we interpret his scenario to imply that the South American representative (*V. "chivi"*) is oldest, the Middle American form (*V. flavoviridis*) is of intermediate age, and the North American component (*V. olivaceus* sensu stricto) is youngest.

The new genetic data offered here, however, do not support Barlow's suggested phyletic history of the *V. olivaceus* group. Instead of being intermediate in age, *V. flavoviridis,* based on its significant genetic differences, is probably the oldest of the three taxa. We feel that the steady, time-dependent accumulation of more allelic changes in *V. flavoviridis* than in *V. "chivi"* and *V. olivaceus* verifies the greater age of the former taxon. We emphasize, however, that despite our reliance on a genetic explanation, we still are presenting only a partial analysis of branching patterns in this complex of *Vireo.* A complete understanding of phylogenesis in *V. olivaceus* and its relatives will be possible only when electrophoretic data for *V. altiloquus, V. magister,* and perhaps other forms are available for comparison.

SUMMARY

Using starch gel electrophoresis, we assessed genetic distances among samples of the Red-eyed Vireo (*Vireo olivaceus*), the Yellow-green Vireo (*V. flavoviridis*), and the "Chivi" Vireo (*V. "chivi"*). Tissues from a Rufous-browed Peppershrike (*Cyclarhis gujanensis ochrocephala*) provided an outgroup comparison. The three complexes of *Vireo* have been treated in the past either as a single species or as three separate species.

Thirty-eight genetic loci were scored. The Red-eyed and "Chivi" vireos are very similar genetically (Nei's $D = 0.014$). In contrast, these two forms differ strikingly from the Yellow-green Vireo (Nei's $D = 0.177$ and 0.176, respectively). Several branching methods (UPGMA, WPGMA, Wagner Tree and F-M Tree) gave congruent results on relationships. These data support the position that the Red-eyed and "Chivi" vireos are conspecific. The Yellow-green Vireo, however, clearly deserves full species status.

The genic data suggest that the clade leading to *Vireo olivaceus-flavoviridis-"chivi"* split from *Cyclarhis* at approximately 13 MYBP. Ensuing separation of *V. flavoviridis* from *V. olivaceus-"chivi"* occurred at about 4.6 MYBP. The division of North American *V. olivaceus* from its South American relatives in the *"chivi"* complex occurred approximately 370,000 years ago, during the Pleistocene. We speculate that *"chivi"* arose from wintering individuals of *V. olivaceus* that failed to return to North America.

ACKNOWLEDGMENTS

We are especially grateful to J. A. Marten, who performed the electrophoretic analysis and assisted with computations. M. Frelow ran the program for the construction of the Fitch-Margoliash ("F-M") tree. G. F. Barrowclough sponsored and led the field trip to Costa Rica on which the specimen of Yellow-green Vireo was taken. Specimens from Paraguay were taken under a permit kindly issued by Ing. H. Bertoni, Minister of Agriculture. A. and M. Reynaers and Sr. and Sra. H. Beckers graciously allowed us to work on their property in Paraguay. M. S. Foster and R. E. Jones assisted in the collection of specimens. V. Remsen and the students in the Avian Biology ("Bird Bunch") Seminar, University of California, Berkeley, provided helpful discussion of phylogenetic-distributional aspects of the problem. Two reviewers, J. C. Avise and J. C. Barlow, offered valuable comments. G. M. Christman prepared the final version of the figures. Ms. B. Resnick typed the manuscript. The work was funded by National Science Foundation Grant DEB 79-09807 and an Annie M. Alexander Grant from the Museum of Vertebrate Zoology to the first author.

LITERATURE CITED

AMERICAN ORNITHOLOGISTS' UNION. 1957. Check-list of North American birds, 5th ed. Am. Ornithol. Union, Baltimore, Maryland.

———. 1983. Check-list of North American birds, 6th ed. Am. Ornithol. Union, Washington, D.C.

AQUADRO, C. F. AND J. C. AVISE. 1982. Evolutionary genetics of birds. VI. A reexamination of protein divergence using varied electrophoretic conditions. Evolution 36:1003–1019.

AVISE, J. C., C. F. AQUADRO, AND J. C. PATTON. 1982. Evolutionary genetics of birds. V. Genetic distances within Mimidae (Mimic Thrushes) and Vireonidae (Vireos). Biochem. Genet. 20:95–104.

BARLOW, J. C. 1980. Patterns of ecological interactions among migrant and resident vireos on the wintering grounds. Pp. 79–107 *in* Migrant birds in the Neotropics: ecology, behavior, distribution, and conservation (A. Keast and E. S. Morton, eds). Smithson. Inst. Press, Washington, D.C.

———. 1981. Songs of the vireos and their allies. Ara Records, No. 7, Gainesville, Florida.

BARROWCLOUGH, G. F. 1980. Genetic and phenotypic differentiation in a wood warbler (Genus *Dendroica*) hybrid zone. Auk 97:655–668.

———. 1983. Biochemical studies of microevolutionary processes. Pp. 223–261 *in* Perspectives in ornithology (A. H. Brush and G. A. Clark, Jr., eds). Cambridge Univ. Press, Cambridge, England.

———, N. K. JOHNSON, AND R. M. ZINK. 1985. On the nature of genic variation in birds. Pp. 135–154 *in* Current ornithology, Vol. 2 (R. F. Johnston, ed.). Plenum Publ. Corp., New York, New York.

BLAKE, E. R. 1968. Family Vireonidae. Pp. 103–138 *in* Check-list of birds of the World (R. A. Paynter, Jr., ed.). Museum of Comparative Zoology, Cambridge, Massachusetts.

BORROR, D. J. 1972. Yellow-green Vireo in Arizona, with notes on vireo songs. Condor 74:80–86.

CRACRAFT, J. 1973. Continental drift, paleoclimatology, and the evolution and biogeography of birds. J. Zool. Lond. 169:455–545.

EISENMANN, E. 1962. Notes on some Neotropical vireos in Panama. Condor 64:505–508.

———, J. I. RICHARDSON, AND G. I. CHILD. 1968. Yellow-green Vireo collected in Texas. Wilson Bull. 80:235.

FARRIS, J. S. 1972. Estimating phylogenetic trees from distance matrices. Am. Nat. 106: 645–668.

———. 1981. Distance data in phylogenetic analysis. Pp. 3–23 *in* Advances in cladistics, Vol. 1 (V. A. Funk and D. R. Brooks, eds.). Ann. N.Y. Bot. Gardens, New York, New York.

FELSENSTEIN, J. 1984. Distance methods for inferring phylogenies: A justification. Evolution 38:16–24.

FITCH, W. M. AND E. MARGOLIASH. 1967. Construction of phylogenetic trees. Science 155: 279–284.

GORMAN, G. C. AND J. RENZI, JR. 1979. Genetic distance and heterozygosity estimates in electrophoretic studies: effects of sample size. Copeia 1979:242–249.

GUTIERREZ, R. J., R. M. ZINK, AND S. Y. YANG. 1983. Genic variation, systematic, and biogeographic relationships of some galliform birds. Auk 100:33–47.

HAMILTON, T. H., 1958. Adaptive variation in the genus *Vireo.* Wilson Bull. 70:307–346.

———. 1962. Species relationships and adaptations for sympatry in the avian genus *Vireo.* Condor 64:40–68.

HARRIS, H. AND D. A. HOPKINSON. 1976. Handbook of enzyme electrophoresis in human genetics. North-Holland Publ. Co., Amsterdam, Netherlands.

HARTL, D. L. 1981. A primer of population genetics. Sinauer Assoc., Inc., Sunderland, Massachusetts.

JOHNSON, N. K. AND R. M. ZINK. 1983. Speciation in sapsuckers (*Sphyrapicus*): I. Genetic differentiation. Auk 100:871–884.

———, R. M. ZINK, G. F. BARROWCLOUGH, AND J. A. MARTEN. 1984. Suggested techniques for modern avian systematics. Wilson Bull. 96:543–560.

JOHNSTON, R. F. AND R. K. SELANDER. 1964. House sparrows: rapid evolution of races in North America. Science 144:548–550.

———. 1971. Evolution of the house sparrow. II. Adaptive differentiation in North American populations. Evolution 25:1–28.

KIMURA, M. 1979. The neutral theory of molecular evolution. Sci. Am. 241:98–126.

———. 1982. The neutral theory as a basis for understanding the mechanism of evolution and variation at the molecular level. Pp. 3–56 *in* Molecular evolution, protein polymorphism and the neutral theory (M. Kimura, ed.). Japan Scientific Societies Press, Tokyo, Japan.

MORTON, E. S. 1977. Intratropical migration in the Yellow-green Vireo and Piratic Flycatcher. Auk 94:97–106.

NATIONAL GEOGRAPHIC SOCIETY. 1983. Field guide to the birds of North America. National Geographic Society, Washington, D.C.

NEI, M. 1975. Molecular population genetics and evolution. North Holland Publ. Co., Amsterdam, Netherlands.

———. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

PRESCH, W. 1979. Phenetic analysis of a single data set: phylogenetic implications. Syst. Zool. 28:366–371.

REMSEN, J. V., JR. 1984. High incidence of "leapfrog" pattern of geographic variation in Andean Birds: implication for the speciation process. Science 224:171–173.

ROBBINS, C. S., B. BRUUN, AND H. S. ZIM. 1966. Birds of North America. Golden Press, New York, New York.

ROGERS, J. S. 1972. Measures of genetic similarity and genetic distance. Univ. Texas Publ. 7213:145–153.

SARICH, V. M. 1977. Rates, sample sizes, and the neutrality hypothesis for electrophoresis in evolutionary studies. Nature 265:24–28.

SELANDER, R. K., M. H. SMITH, S. Y. YANG, W. E. JOHNSON, AND J. B. GENTRY. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Univ. Texas Publ. 7103:49–90.

SIBLEY, C. G. AND J. E. AHLQUIST. 1982. The relationships of the vireos (Vireoninae) as indicated by DNA-DNA hybridization. Wilson Bull. 94:114–128.

SLUD, P. 1964. The birds of Costa Rica. Bull. Am. Mus. Nat. Hist. 128:1–430.

SNEATH, P. H. A. AND R. R. SOKAL. 1973. Numerical taxonomy. W. H. Freeman and Co., San Francisco, California.

SWOFFORD, D. L. 1981. Utility of the distance Wagner procedure. Pp. 25–44 *in* Advances in cladistics, Vol. 1 (V. A. Funk and D. R. Brooks, eds.). Ann. N.Y. Bot. Gardens, New York, New York.

THORPE, J. P. 1982. The molecular clock hypothesis: biochemical evolution, genetic differentiation and systematics. Ann. Rev. Ecol. Syst. 13:139–168.

WELTY, J. C. 1982. The life of birds, Third ed. Saunders, Philadelphia, Pennsylvania.

WETMORE, A. 1934. A fossil quail from Nebraska. Condor 36:30.

WILSON, A. C., S. S. CARLSON, AND T. J. WHITE. 1977. Biochemical evolution. Ann. Rev. Biochem. 46:573–639.

YANG, S. Y. AND J. L. PATTON. 1981. Genic variability and differentiation in the Galapagos finches. Auk 98:230–242.

MUSEUM OF VERTEBRATE ZOOLOGY AND DEPT. ZOOLOGY, UNIV. CALIFORNIA, BERKELEY, CALIFORNIA 94720. (PRESENT ADDRESS OF RMZ: MUSEUM OF ZOOLOGY, LOUISIANA STATE UNIV., BATON ROUGE, LOUISIANA 70803.) ACCEPTED 8 AUG. 1985.

COLOR PLATE

The Frontispiece painting of *Tangara meyerdeschauenseei* has been made possible by an endowment established by George Miksch Sutton (1896–1982). The painting is by J. P. O'Neill.