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GENETIC EVIDENCE FOR RELATIONSHIPS IN THE AVIAN FAMILY VIREONIDAE[1]

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Abstract. Using starch gel electrophoresis we analyzed variation at 29 genetic loci in 32 taxa (20 species in four genera) of Vireonidae. Two species of Corvidae and two species of Emberizidae (Parulinae) served as outgroups. Twenty-three loci (83%) were either polymorphic or were fixed at alternative alleles among taxa. Cladistic analyses were weakly informative of relationships. In contrast, UPGMA phenograms and Wagner trees, generated from Rogers' genetic distances, provided hypotheses of relationship at the species level for the following taxa: (1) *Vireo huttonii* and *V. carmioli* appear to be sister taxa; (2) *V. leucophrys* is a species distinct from *V. gilvus*; (3) *V. solitarius* and *V. flavifrons* are not necessarily sister taxa; (4) *V. "chivi" solimoensis* and *V. "chivi" chivi* are allied to *V. olivaceus*, not with *V. flavoviridis*. Traditional *Vireo* consists of four clusters of species: (1) an eye-ringed group (*griseus, solitarius, flavifrons, vicinior, huttoni,* and *carmioli*); (2) the eye-lined *olivaceus* group (*olivaceus* and *flavoviridis*); (3) an eye-lined *gilvus* group (*gilvus, leucophrys,* and *philadelphicus*); and (4) *"Vireo" bellii*. Each cluster is genetically distinct at a level comparable to that which distinguishes *Cyclarhis* and *Vireolanius* from other vireonids. Traditional *Hylophilus* appears to be either polyphyletic or paraphyletic. The six species examined fell into two groups: *hypoxanthus, decurtatus, aurantiifrons,* and *ochraceiceps* cluster in the vicinity of eye-ringed *Vireo*; *poicilotis* and *thoracicus* form a remote sister clade to all other vireonids. The exceptionally large interspecific genetic distances reported for *Vireo* are an artifact resulting from taxonomic undersplitting. Subgenera for *Vireo,* and separate subfamilies for peppershrikes, shrike-vireos, and vireos and greenlets, respectively, cannot be justified by the genetic evidence. Vireonids are more closely related to corvids than to paruline warblers.

Key words: Vireonidae; Vireo; Hylophilus; Cyclarhis; Vireolanius; *allozymes; phylogenetic inference; genetic distance.*

INTRODUCTION

Avise et al. (1982) reported that five species of *Vireo* were distinguished by strikingly large Nei's genetic distances in comparison with those reported for other birds. In a study of the *Vireo olivaceus-flavoviridis-"chivi"* complex, Johnson and Zink (1985) established that substantial genetic distances in the genus could occur even when phenotypically very similar forms were compared. These discoveries suggested that electrophoretic analysis could be applied profitably to a host of other systematic problems in the Vireonidae. Accordingly, we assembled tissue of 95 specimens of 32 taxa, including four genera represented by 20 species. Twenty-four of these taxa had not been analyzed previously by either Avise et al. (1982) or Johnson and Zink (1985). The new comparisons allowed us to consider questions of relationship at all taxonomic levels up to and including that of the family.

Johnson and Zink (1985) compared *Cyclarhis gujanensis ochrocephala* (subfamily Cyclarhinae) with three taxa of *Vireo* (subfamily Vireoninae). Here we extend that preliminary "macrotaxonomic" analysis (Barrowclough 1983) to the familial level by comparing all represented taxa of Vireonidae to two species of Corvidae (Steller's Jay [*Cyanocitta stelleri*] and Black-billed Magpie [*Pica pica*]) and two species of paruline warblers in the family Emberizidae (Tropical Parula [*Par-*

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ula pitiayumi] and Golden-crowned Warbler [*Basileuterus culicivorus*]). The choice of these families for higher level comparisons was dictated by the findings of Sibley and Ahlquist (1982). They proposed on the basis of DNA-DNA hybridization studies that vireos and their close allies (peppershrikes, shrike-vireos, and greenlets) are related to a "corvine assemblage," rather than to wood warblers, next to which they traditionally have been placed.

MATERIALS AND METHODS

Taxa studied, sample sizes, and geographic sources of specimens are listed in Table 1. Nomenclature follows Blake (1968), except that we regard *Vireo flavoviridis* and *V. leucophrys* as full species.

Procedures for the collection and storage of tissue samples have been described elsewhere (Johnson et al. 1984). Electrophoretic methods followed Selander et al. (1971), with the slight modifications outlined by Johnson et al. (1984). Twenty-nine presumptive genetic loci were scored. Unscorable loci were G-6-Pdh, Ck-1, Got-1, Got-2, and Gsr. Alleles at a locus were coded by their mobility from the origin. The most anodal allele was designated a, with successively slower alleles denoted as b, c, etc. Isozyme nomenclature follows Yang and Patton (1981). From banding patterns on gels (presumptive individual genotypes), we derived a table of genotypic frequencies. Observed heterozygosity (H_{obs}) was determined by direct count for each specimen and then averaged (+SE) for each sample. The computer program BIOSYS-1 (Swofford and Selander 1981) was used to compute allelic frequencies (Table 2), expected heterozygosity (H_{exp}) per sample, percentage polymorphic loci, average number of alleles per polymorphic locus, Nei's (1978) and Rogers' (1972) genetic distances (Table 3), UPGMA and WPGMA phenograms (Sneath and Sokal 1973), and distance Wagner trees (Farris 1972, 1981; Swofford 1981). Robustness of distance Wagner trees was evaluated by (a) reordering the data input sequence and (b) producing several trees with the multiple addition criterion of Swofford (1981) and optimizing the resultant trees by minimizing the Prager and Wilson *F*-value. The various branching diagrams portray patterns of genetic similarity and provide estimates, under differing assumptions, of the evolutionary relationships among taxa (Felsenstein 1982, 1985). The distribution of observed and expected number of heterozygotes over all loci in a sample (Table 4) was examined for departure from Hardy-Weinberg expectation (Hartl 1981) with a χ^2 test (Barrowclough 1980).

Because the use of distance matrices for the inference of phylogenetic patterns is controversial (Farris 1986, Felsenstein 1986), we also conducted two cladistic analyses (Mickevich and Mitter 1981, Patton and Avise 1983) with the computer program PAUP (Swofford 1985). One used alleles as characters and the other used loci as characters and constituent alleles as character states. For the first, any allele not present in any of the four outgroup taxa was considered derived, and each taxon was coded as either possessing (1) or lacking (0) the allele; the "ancestor" was coded as all 0s. In the case of a polymorphism, the taxon was coded as 1 (even if the allele was present in only a single heterozygote). For the second method, alleles at each locus were numbered consecutively beginning with the outgroup taxa; in the case of polymorphism, a taxon was assigned the state for its most common allele. The outgroup taxa were designated as such in the analysis, and the character states (alleles at each locus) were input as "unordered" to indicate that we lacked information on the direction of character state transformation. For both analyses we used the addition sequence CLOSEST, the branch swapping option ALT, and the method of detecting all equally parsimonious trees, MULPARS. We specified the maximum number of trees at 50. All equally parsimonious trees were input into CONTREE, the subroutine that produces a strict consensus tree.

RESULTS

GENETIC VARIATION

Of the 29 loci scored, 15 showed at least a single heterozygote (Table 2). At nine other loci (Sod-2, Gda, Ck-2, Ldh-1, Ald, Gpt, Mdh-1, Eap, and Sdh) at least some species, including outgroup taxa, were fixed at alternative alleles. Therefore, 24 (83%) of the total loci were variable (Table 2). Five loci (Sod-1, Ldh-2, Mdh-2, Glud, and Adh) were monomorphic and fixed for the same allele in all taxa.

Correlations between sample size and number of alleles at polymorphic loci ($r = 0.9504$), H_{obs} ($r = 0.4517$), percentage of polymorphic loci ($r = 0.8276$), and average number of alleles ($r = 0.9352$), respectively, are all statistically signifi-

TABLE 1. Taxa studied, sample sizes, and sources of specimens.

	n	Source of specimens
Family Vireonidae[1]		
Subfamily Cyclarhinae		
Genus *Cyclarhis*		
Rufous-browed Peppershrike		
1. *Cyclarhis gujanensis ochrocephala*	1	Paraguay
2. *C. g. contrerasi*	1	Peru
3. *C. g. dorsalis*	1	Bolivia
4. *C. g. gujanensis*	1	Bolivia
Subfamily Vireolaninae		
Genus *Vireolanius*		
Slaty-capped Shrike-Vireo		
5. *Vireolanius leucotis leucotis*	1	Ecuador
6. *V. l. bolivianus*	1	Bolivia
7. *V. l. simplex*	1	Peru
Subfamily Vireoninae		
Genus *Vireo*		
Subgenus *Vireo*		
Hutton's Vireo		
8. *Vireo huttoni huttoni*	5	California
White-eyed Vireo		
9. *Vireo griseus noveboracensis*	1	Oklahoma
10. *V. g. griseus*	5	Louisiana
Bell's Vireo		
11. *Vireo bellii bellii*	1	Oklahoma
	1	Louisiana
Gray Vireo		
12. *Vireo vicinior*	5	Nevada
Yellow-winged Vireo		
13. *Vireo carmioli*	1	Costa Rica
Solitary Vireo		
14. *Vireo solitarius solitarius*	6	Minnesota
Yellow-throated Vireo		
15. *Vireo flavifrons*	2	Louisiana
Subgenus *Vireosylva*		
Philadelphia Vireo		
16. *Vireo philadelphicus*	3	Louisiana
Red-eyed Vireo		
17. *Vireo olivaceus olivaceus*	15	Minnesota
18. *V. o. solimoensis*	7	Peru
19. *V. o. diversus*	14	Paraguay
20. *V. o. chivi*	1	Bolivia
Yellow-green Vireo		
21. *Vireo flavoviridis flavoviridis*	1	Costa Rica
Warbling Vireo		
22. *Vireo gilvus swainsonii*	1	California
23. *V. g. leucopolius*	4	California
24. *V. g. gilvus*	1	Louisiana
Brown-capped Vireo		
25. *Vireo leucophrys leucophrys*	2	Peru
Genus *Hylophilus*		
Rufous-crowned Greenlet		
26. *Hylophilus poicilotis poicilotis*	1	Paraguay
Lemon-chested Greenlet		
27. *Hylophilus thoracicus aemulus*	2	Peru
Golden-fronted Greenlet		
28. *Hylophilus aurantiifrons aurantiifrons*	1	Panama
Dusky-capped Greenlet		
29. *Hylophilus hypoxanthus flaviventris*	1	Peru
Tawny-crowned Greenlet		
30. *Hylophilus ochraceiceps ferrugineifrons*	5	Peru
31. *H. o. viridior*	1	Bolivia
Lesser Greenlet		
32. *Hylophilus decurtatus darienensis*	1	Panama
Family Corvidae		
Steller's Jay		
33. *Cyanocitta stelleri*	1	California
Black-billed Magpie		
34. *Pica pica hudsonia*	1	Idaho
Family Emberizidae		
Subfamily Parulinae		
Tropical Parula		
35. *Parula pitiayumi pitiayumi*	1	Paraguay
Golden-crowned Warbler		
36. *Basileuterus culicivorus azarae*	1	Paraguay
Total	99	

[1] Classification and sequence of species follows Blake (1968), who covers all taxa in the family Vireonidae. Hamilton (1962), Barlow (1981), and the American Ornithologists' Union (1983) also present classifications of many of the species listed here; in addition to sequence, these treatments differ from that of Blake mainly with regard to the recognition and composition of subgenera within *Vireo*.

cant ($P < 0.01$, df = 34). Therefore, to reduce impact of sampling bias we present calculations for these parameters for samples of at least five individuals. Table 4 provides data on variation at loci and heterozygosity for eight taxa represented by our largest samples. H_{obs} ranged from 0.014 in *Vireo h. huttoni* to 0.078 in *V. o. olivaceus*. Average H_{obs} over all taxa, 0.046, is very similar to the average value reported for birds in general, 0.053 (Barrowclough 1983). H_{obs} for *V. g. griseus* and *V. s. solitarius* were virtually identical to values given for those species by Avise et al. (1982), whereas our figure for *V. o. olivaceus* is higher (H_{obs} = 0.078 vs. 0.048). In fact, all

subspecies of *V. olivaceus* averaged unexpectedly high ($H_{obs} = 0.072$). In contrast, H_{obs} for *V. huttoni* (0.014) and *H. o. ferrugineifrons* (0.021) were 26 to 40% of Barrowclough's (1983:228–229) average for large single breeding populations of 30 species. Percentage of polymorphic loci ranged from 6.90 in *V. h. huttoni* to 44.83 in *V. o. olivaceus*, with a mean of 16.85. Two species, *V. h. huttoni* and *V. vicinior*, had low average numbers of alleles per locus, 1.07; *V. o. diversus*, at 1.62, was highest in this measure. For all comparisons, H_{obs} and H_{exp} are similar within each taxon and chi-square tests revealed no significant departure from Hardy-Weinberg expectations ($P > 0.05$).

GENETIC DISTANCES

Good estimates of genetic distances between taxa can be obtained even from single individuals (Nei 1978, Gorman and Renzi 1979), when large numbers of loci are examined. Based on traditional classification, Nei's (1978) genetic distances (Table 3) progressively increase when taxa representing successively more inclusive taxonomic levels are compared (Table 5). Subspecies differ at mean Nei's D of 0.018; congeneric species, $\bar{D} = 0.293$; noncongeneric species in same family, $\bar{D} = 0.354$; and species in different families, $\bar{D} = 0.984$. Values for comparison of species within either *Vireo* or *Hylophilus* are very similar ($\bar{D} = 0.291$ vs. 0.302, respectively). Vireonids are closer to corvids ($\bar{D} = 0.798$) than to parulines ($\bar{D} = 1.180$).

BRANCHING DIAGRAMS

Because preliminary analyses revealed a complex relationship of taxa in the four genera of vireonids examined, we constructed (admittedly a posteriori) three separate UPGMA phenograms to search for congruence between traditional classificaton and our genetic data. Analysis A (Fig. 1) covered all taxa (including outgroups) except *Hylophilus*; Analysis B (Fig. 2) included all taxa except *Vireo*; and Analysis C (Fig. 3) involved all taxa.

Analysis A gave eight clearly defined clusters: (1) taxa of *Cyclarhis*; (2) taxa of "spectacled" or "eye-ringed" forms of *Vireo* (*V. solitarius, griseus, flavifrons, vicinior, huttoni,* and *carmioli*); (3) the three subspecies of *Vireolanius leucotis*; (4) a first cluster of "eye-lined" forms of *Vireo*, including the subspecies of *V. olivaceus* and *V. flavoviridis*; (5) a second cluster of eye-lined forms of *Vireo*, including the subspecies of *V. gilvus, V. leucophrys,* and *V. philadelphicus*; (6) *V. bellii*; (7) the two corvids; and (8) the two paruline warblers. This analysis revealed several major surprises that conflict with traditional classification. *Vireo* is split into several sections. The eye-ringed vireos combine first with *Vireolanius* and then with *Cyclarhis* to form a major cluster, the eye-lined vireos are divided into two major groups and *V. bellii* is included with the vireos only as a distant outlier. All vireonids comprise a sister group with the two corvids, which in turn form a sister group with the two wood warblers.

Six clusters emerged from analysis B (Fig. 2): (1) the subspecies of *Cyclarhis gujanensis*; (2) a first cluster of *Hylophilus*, including *H. hypoxanthus, decurtatus, aurantiifrons,* and *ochraceiceps*; (3) the subspecies of *Vireolanius leucotis*; (4) a second cluster of *Hylophilus*, including *H. poicilotis* and *H. thoracicus*; (5) the jay and the magpie; and (6) the two species of New World warblers. The division of *Hylophilus* into two major components, with the first component shown as being more closely related to both *Vireolanius* and *Cyclarhis* than to the second component conflicts strikingly with traditional classification. As in analysis A, in this analysis the vireonids are shown as being closer to the corvids than to the emberizids.

Analysis C (Fig. 3) maintains every major cluster revealed by analyses A and B and, in addition, shows the complex interrelationship of one cluster of species of *Hylophilus* with the eye-ringed members of *Vireo*. Nine major clusters can be identified: (1) the subspecies of *Cyclarhis gujanensis*; (2) a large cluster with two subcomponents, the first combining *H. hypoxanthus* and *H. decurtatus* with the eye-ringed members of *Vireo*, and the second comprised of *H. aurantiifrons* and *H. ochraceiceps*; (3) the subspecies of *Vireolanius leucotis*; (4) the "*gilvus*" cluster of eye-lined members of *Vireo*; (5) the "*olivaceus*" cluster of eye-lined members of *Vireo*; (6) *V. bellii*; (7) a second cluster of *Hylophilus*, including *H. poicilotis* and *H. thoracicus*; (8) the two species of Corvidae; and (9) the two species of wood warblers. To aid interpretation, the first seven major clusters, including all vireonids represented, are numbered in Figure 3. This analysis portrays the complex alliance of certain species traditionally placed in *Hylophilus* with the eye-ringed species of *Vireo*.

Of 10 distance Wagner trees produced, five had similar F-values (7.45 to 7.50) and lengths (3.43); these five trees were combined into a strict consensus tree. The consensus distance Wagner

TABLE 2. Allelic frequencies for polymorphic loci. Numbers in parentheses are frequencies for alleles (coded as letters) when a particular allele was not fixed.

Locus	*Cyclarhis gujanensis ochrocephala*	*Cyclarhis gujanensis contrerasi*	*Cyclarhis gujanensis dorsalis*	*Cyclarhis gujanensis gujanensis*	*Vireolanius leucotis leucotis*	*Vireolanius leucotis bolivianus*	*Vireolanius leucotis simplex*	*Vireo huttoni huttoni*
1. Sod 2	a	a	a	a	b	c	b	a
2. 6-Pgd	a	a	a	a	a	a	a	a
3. Gda	a	a	a	a	a	a	a	a
4. Est-D	b	b	b	b	a (0.50) b (0.50)	a	a	a
5. La-1	a (0.50) b (0.50)	c	a (0.50) d (0.50)	c	b	b	b	a
6. CK-2	b	b	b	b	a	a	a	a
7. Ldh-1	b	b	b	b	b	b	b	b
8. Gpi	a	a	a	a	a (0.50) c (0.50)	a	a	b
9. Ald	b	b	b	b	b	b	b	b
10. αGpd	b	b (0.50) c (0.50)	b	b	a	a	a	a
11. Mpi	a	a	a	a	a	a	a	a
12. Gpt	b	b	b	b	b	b	b	b
13. Mdh-1	a	a	a	a	a	a	a	a
14. Pgm-1	b	b	b	b (0.50) c (0.50)	b	b (0.50) c (0.50)	b	b
15. Icd-2	a	a	a	a	a	a	a	a
16. Eap	a	a	a	a	a	a	a	a
17. Sdh	b	b	b	b	a	a	a	a
18. Ada	b (0.50) c (0.50)	b (0.50) c (0.50)	b	b	f	f	f	f (0.90) h (0.10)
19. La-2	a	a	a	a	a	a	a	a
20. Np	b	b	b	b	a	a	a	n
21. Icd-1	a	a	a	a	e	e	e (0.50) g (0.50)	a
22. Lgg	a	a	a	a	a	a	a	a (0.90) d (0.10)
23. Pgm-2	b	b	b	b	d	d	d	b
24. Acon	b	b	b	b	b	b	b	a

tree does not differ appreciably from the tree shown (Fig. 4). This approach in essence supports relationships obtained in the UPGMA analyses except that certain taxa of *Hylophilus,* the eye-ringed members of *Vireo, Vireolanius,* and *Cyclarhis* are intertwined. Otherwise, most major clusters already identified reappear: (a) the *olivaceus* group of eye-lined vireos; (b) the *gilvus* group of eye-lined vireos; (c) *V. bellii*; (d) the two warblers; and (e) the jay and the magpie.

TABLE 2. Extended.

Vireo griseus noveboracensis	*Vireo griseus griseus*	*Vireo bellii bellii*	*Vireo vicinior*	*Vireo carmioli*	*Vireo solitarius solitarius*	*Vireo flavifrons*	*Vireo philadelphicus*	*Vireo olivaceus olivaceus*	*Vireo olivaceus solimoensis*
a	a	a	a	a	a	a	a	a	a
a	a (0.90) e (0.10)	f	a	a	a (0.92) d (0.08)	a	a (0.83) e (0.17)	a (0.90) d (0.07) g (0.03)	a
a	a	a	a	a	a	a	a	a	a
a	a	a	a (0.90) c (0.10)	a	a	a	a (0.83) b (0.17)	a (0.97) b (0.03)	a
a	a	f	a	a	a (0.92) e (0.08)	a	d	a (0.97) e (0.03)	a (0.93) d (0.07)
a	a	a	a	a	a	a	a	a	a
b	b	b	b	e	b	b	e	a	a
a	a	a	a	a	a	a (0.75) c (0.25)	a	a (0.97) b (0.03)	a (0.86) b (0.07) c (0.07)
b	b	b	b	b	b	b	b	a	a
a	a	a	a (0.30) b (0.70)	a	a	a	a	a	a
a	a	a	a	a	a	a	c (0.67) h (0.33)	a (0.97) e (0.03)	a
b	b	c	b	b	b	b	a	a	a
a	a	a	a	a	a	a	a	a	a
b	a (0.10) b (0.90)	e	b	b	b	b	b	a (0.77) b (0.23)	a (0.86) f (0.14)
a	a	a	b	a	a	a	a	a (0.97) c (0.03)	a
a	a	a	a	a	a	a	a	a	a
a	a	a	a	a	a	a	a	a	a
f	f	a (0.25) e (0.75)	f	f	e	f (0.75) g (0.25)	a (0.83) e (0.17)	a (0.97) i (0.03)	a
a	a	a	a	a	a	a	f	a (0.93) b (0.07)	a
l	a (0.30) h (0.10) k (0.10) l (0.50)	m	n	b	a (0.75) b (0.08) g (0.17)	e	e	a (0.77) g (0.23)	a (0.57) h (0.36) l (0.07)
a	a	d	a	a	a	a	a	a (0.80) e (0.03) f (0.17)	a
a	a	d (0.50) g (0.50)	a	a	a (0.59) b (0.08) c (0.25) d (0.08)	a (0.75) d (0.25)	a	a (0.80) e (0.20)	a (0.64) c (0.14) d (0.14) h (0.08)
b	b	c	b	b	b	b	a	a (0.93) b (0.07)	a (0.93) g (0.07)
b	b	b	b	a	b	b	b	a	a

CLADISTIC ANALYSES

Considering presumably derived alleles as characters, the PAUP program found over 50 equally parsimonious trees of length 70. A strict consensus tree (not shown) was similar to the distance Wagner analysis in revealing the following clusters: (1) a large heterogeneous cluster comprised of *Cyclarhis, Vireolanius,* the eye-ringed vireos, the *olivaceus* group of *Vireo* and four species of

TABLE 2. Continued.

Locus	*Vireo olivaceus diversus*	*Vireo olivaceus chivi*	*Vireo flavoviridis flavoviridis*	*Vireo gilvus swainsonii*	*Vireo gilvus leucopolius*	*Vireo gilvus gilvus*	*Vireo leucophrys leucophrys*	*Hylophilus poicilotis poicilotis*
1. Sod-2	a	a	a	a	a	a	a	a
2. 6-Pgd	a	a	a	a	a	a	a	b
3. Gda	a	a	b	a	a	a	a	a
4. Est-D	a (0.96) c (0.04)	a	a	c	a	a	a (0.75) b (0.25)	a
5. La-1	a (0.96) d (0.04)	a	a (0.50) d (0.50)	a (0.50) d (0.50)	a (0.13) d (0.87)	d	d	a
6. CK-2	a	a	a	a	a	a	a	b
7. Ldh-1	a	a	c	e	e	e	e	c
8. Gpi	a (0.96) b (0.04)	a	a	a	a	a	a	a
9. Ald	a	a	a	b	b	b	b	b
10. αGpd	a	a	d	a	a	b	a	a
11. Mpi	a (0.94) e (0.03) f (0.03)	a (0.50) f (0.50)	a	a	a (0.75) b (0.25)	a	a	a
12. Gpt	a	a	a	a	a	a	a	c
13. Mdh-1	a	a	a	a	a	a	a	a
14. Pgm-1	a (0.96) b (0.04)	a	a	b	b	b	b	d
15. Icd-2	a	a	a	a	a	a	a	a
16. Eap	a	a	a	a	a	a	a	a
17. Sdh	a	a	a	a	a	a	a	c
18. Ada	a	a	a	e	e	e	i	d
19. La-2	a (0.86) b (0.04) c (0.10)	a	a	e	e	e	e	a
20. Np	a (0.46) g (0.28) h (0.18) i (0.04) j (0.04)	a	a	b	b	b	o	b
21. Icd-1	a	a	a	a (0.50) f (0.50)	a (0.75) f (0.25)	a	a	b
22. Lgg	a (0.68) c (0.04) e (0.04) f (0.24)	a	a	a	a (0.50) d (0.37) g (0.13)	d	d	a
23. Pgm-2	a (0.32) b (0.60) d (0.04) e (0.04)	a	b	a	a	a	a	c
24. Acon	a	a	a	b	b	b	b	b

Hylophilus; (2) the *gilvus* cluster as a sister group to cluster 1; (3) a cluster comprised of *H. poicilotis* and *H. thoracicus*; and (4) *V. bellii*. The consensus tree differs from the Wagner tree in that the *olivaceus* cluster, although remaining cohesive, is part of cluster 1 in the cladistic analysis. In the cladistic analysis by alleles, subspecific taxa in *Cyclarhis, Vireolanius,* and *V. olivaceus* were grouped together. In the *gilvus* cluster, in contrast, although *V. g. leucopolius* and *V. g.*

TABLE 2. Extended.

Hylophilus thoracicus aemulus	*Hylophilus auranti-ifrons auranti-ifrons*	*Hylophilus hypoxanthus flaviventris*	*Hylophilus ochrace-iceps ferrugine-ifrons*	*Hylophilus ochrace-iceps viridior*	*Hylophilus decurtatus darienensis*	*Cyanocitta stelleri*	*Pica pica hudsonia*	*Parula pitiayumi pitiayumi*	*Basile-uterus culicivorus azarae*
a	a	a	a	a	a	e	f	d	d
c	a	a	a	a	a	i	h	h	h
a	a	a	a	a	a	a	c	c	c
b	a	a	a	a	a	a (0.50) b (0.50)	a (0.50) b (0.50)	a	a
a	a	a	a	a	a	b	b	b	b
b	a	b	a	a	a	b	a	b	b
d	b	b	b	b	b	b	b	d	f
a (0.75) b (0.25)	a	a	a	a	a	a	a	a	b
b	c	b	b	b	b	b	d	b	b
a	a	a	a (0.90) d (0.10)	a	a	f	b	e	b
a	a (0.50) b (0.50)	a	c (0.90) d (0.10)	c	a	a	i	c	c
d	e	b	e	e	b	e	b	c	c
a	a	a	a	a	a	b	b	b	b
d	b	b	b (0.90) c (0.10)	b	b	b	g	c	c
a	a	a	a	a	a	a	a	b	b
a	a	a	a	a	a	a	a	b	b
d	a	a	a	a	a	f	f	c	e
e	e	f	e	e	f	k	k	a	a
a	a	a	a	a	a	a	a	a	g
b	c	b	e	e	d	e	e (0.50) p (0.50)	e	e
c	a	a	a	a	a	f	b	b	h
a	a	a	a	a	a	k	l	i	j
c	b	b	b	b	b	i	g	h	h
b	b	b	b	b	b	c	c	b	c

swainsonii grouped together, *V. g. gilvus* allied instead with *V. l. leucophrys*. Because this method may yield intermediate taxa with no alleles, Buth (1984) recommended the following analysis.

In contrast to previous branching diagrams, the second cladistic analysis, which considered loci as characters (Fig. 5), only partially recovered an interpretable taxonomic structure. Although the subspecies of *C. gujanensis* are

TABLE 3. Nei's (1978) genetic distances *below* diagonal. Rogers' (1972) genetic distances *above* diagonal.

	1	2	3	4	5	6	7	8	9
1. *C. g. ochrocephala*	—	0.047	0.034	0.064	0.323	0.340	0.318	0.290	0.220
2. *C. g. contrerasi*	0.009	—	0.064	0.052	0.336	0.353	0.331	0.303	0.232
3. *C. g. dorsalis*	0.0	0.027	—	0.047	0.340	0.357	0.336	0.295	0.224
4. *C. g. gujanensis*	0.018	0.009	0.018	—	0.362	0.345	0.357	0.329	0.259
5. *V. l. leucotis*	0.325	0.360	0.370	0.396	—	0.086	0.052	0.261	0.207
6. *V. l. bolivianus*	0.370	0.405	0.414	0.414	0.037	—	0.069	0.266	0.190
7. *V. l. simplex*	0.344	0.378	0.388	0.414	0.0	0.036	—	0.244	0.168
8. *V. h. huttoni*	0.309	0.343	0.327	0.378	0.266	0.284	0.261	—	0.110
9. *V. g. noveboracensis*	0.218	0.249	0.236	0.282	0.196	0.193	0.172	0.110	—
10. *V. g. griseus*	0.207	0.239	0.226	0.269	0.172	0.167	0.148	0.098	0.005
11. *V. b. bellii*	0.538	0.547	0.557	0.557	0.421	0.385	0.385	0.447	0.351
12. *V. vicinior*	0.220	0.268	0.238	0.284	0.264	0.261	0.238	0.130	0.090
13. *V. carmioli*	0.264	0.296	0.282	0.329	0.287	0.282	0.259	0.110	0.109
14. *V. s. solitarius*	0.214	0.243	0.232	0.276	0.204	0.200	0.179	0.142	0.068
15. *V. flavifrons*	0.214	0.246	0.232	0.279	0.191	0.199	0.177	0.100	0.036
16. *V. philadelphicus*	0.471	0.480	0.460	0.519	0.423	0.423	0.396	0.412	0.312
17. *V. o. olivaceus*	0.510	0.549	0.528	0.566	0.425	0.397	0.391	0.297	0.303
18. *V. o. solimoensis*	0.526	0.564	0.542	0.573	0.452	0.420	0.420	0.300	0.311
19. *V. o. diversus*	0.481	0.520	0.499	0.530	0.457	0.421	0.420	0.272	0.279
20. *V. o. chivi*	0.541	0.582	0.560	0.591	0.452	0.414	0.414	0.327	0.329
21. *V. f. flavoviridis*	0.526	0.550	0.529	0.560	0.541	0.499	0.499	0.378	0.379
22. *V. g. swainsonii*	0.365	0.387	0.370	0.424	0.351	0.344	0.318	0.334	0.241
23. *V. g. leucopolius*	0.406	0.418	0.400	0.456	0.396	0.387	0.360	0.378	0.285
24. *V. g. gilvus*	0.387	0.422	0.379	0.432	0.498	0.488	0.459	0.464	0.372
25. *V. l. leucophrys*	0.475	0.484	0.465	0.523	0.432	0.437	0.410	0.415	0.326
26. *H. p. poicilotis*	0.414	0.450	0.432	0.459	0.559	0.517	0.517	0.533	0.423
27. *H. t. aemulus*	0.365	0.400	0.384	0.410	0.558	0.584	0.584	0.570	0.482
28. *H. a. aurantiifrons*	0.318	0.352	0.336	0.388	0.344	0.336	0.312	0.234	0.151
29. *H. h. flaviventris*	0.133	0.162	0.151	0.193	0.241	0.236	0.214	0.150	0.071
30. *H. o. ferrugineifrons*	0.306	0.339	0.324	0.369	0.335	0.324	0.304	0.228	0.146
31. *H. o. viridior*	0.311	0.345	0.329	0.379	0.336	0.329	0.305	0.229	0.148
32. *H. d. darienensis*	0.218	0.249	0.236	0.282	0.196	0.193	0.172	0.110	0.035
33. *C. stelleri*	0.541	0.582	0.591	0.624	0.606	0.624	0.591	0.740	0.676
34. *P. p. hudsonia*	0.731	0.820	0.788	0.788	0.731	0.711	0.711	0.882	0.806
35. *P. p. pitiayumi*	0.980	1.038	1.047	0.998	1.029	0.907	0.952	1.281	1.065
36. *B. c. azarae*	1.191	1.325	1.270	1.210	1.317	1.210	1.270	1.281	1.421

grouped together, those of *V. leucotis* and *V. griseus* are divided illogically. Species of *Hylophilus* and of the eye-ringed forms of *Vireo* are mixed. *Vireo bellii,* instead of forming a distinct category as in all other analyses, joins in an unresolved trichotomy with two species of *Hylophilus.* The only groups congruent with those identified by other branching algorithms are the *olivaceus* and *gilvus* clusters of eye-lined vireos, which form sister categories, and the four outgroup taxa. Oddly, even the latter are confounded in that the magpie is shown as being more closely related to the two wood warblers than to the other corvid. Thus, the method of inferring evolutionary history by locus recovers only some of the groups of vireonids recognized by current taxonomic practice. That is, by ignoring frequency information, phylogenetic patterns may be obscured. Additionally, our cladistic analysis implies the existence of parallel or convergent allelic states.

DISCUSSION

LARGE GENETIC DISTANCES IN THE VIREONIDAE

Our results corroborate the finding of Avise et al. (1982), obtained from five species of *Vireo* (*griseus, solitarius, flavifrons, philadelphicus,* and *olivaceus*), that several of these forms are separated by genetic distances that are unusually large according to traditional classification. Furthermore, the present findings show that unusually great *D*s occur not only in this group of species but in seven additional species of *Vireo* and six species of *Hylophilus.* Therefore the evidence is strong (Tables 3 and 5) that large interspecific

TABLE 3. Extended.

	10	11	12	13	14	15	16	17	18
1. *C. g. ochrocephala*	0.220	0.430	0.228	0.254	0.228	0.233	0.398	0.433	0.431
2. *C. g. contrerasi*	0.233	0.430	0.250	0.267	0.241	0.245	0.398	0.446	0.444
3. *C. g. dorsalis*	0.225	0.436	0.233	0.259	0.233	0.238	0.390	0.438	0.435
4. *C. g. gujanensis*	0.255	0.436	0.267	0.293	0.267	0.272	0.424	0.452	0.448
5. *V. l. leucotis*	0.201	0.371	0.264	0.276	0.228	0.216	0.373	0.387	0.395
6. *V. l. bolivianus*	0.178	0.332	0.252	0.259	0.211	0.216	0.367	0.353	0.363
7. *V. l. simplex*	0.161	0.332	0.230	0.237	0.189	0.194	0.345	0.352	0.364
8. *V. h. huttoni*	0.111	0.367	0.138	0.110	0.148	0.113	0.351	0.282	0.272
9. *V. g. noveboracensis*	0.022	0.302	0.097	0.103	0.083	0.060	0.281	0.287	0.283
10. *V. g. griseus*	—	0.293	0.097	0.104	0.074	0.061	0.275	0.270	0.269
11. *V. b. bellii*	0.331	—	0.364	0.371	0.266	0.300	0.394	0.392	0.387
12. *V. vicinior*	0.078	0.434	—	0.166	0.145	0.122	0.339	0.346	0.346
13. *V. carmioli*	0.097	0.456	0.169	—	0.151	0.129	0.281	0.252	0.250
14. *V. s. solitarius*	0.048	0.287	0.127	0.144	—	0.085	0.284	0.260	0.263
15. *V. flavifrons*	0.024	0.331	0.092	0.112	0.056	—	0.260	0.291	0.277
16. *V. philadelphicus*	0.300	0.477	0.390	0.312	0.302	0.261	—	0.290	0.296
17. *V. o. olivaceus*	0.275	0.471	0.382	0.256	0.272	0.299	0.299	—	0.052
18. *V. o. solimoensis*	0.284	0.468	0.395	0.267	0.288	0.304	0.309	0.006	—
19. *V. o. diversus*	0.253	0.472	0.357	0.233	0.256	0.272	0.326	0.018	0.016
20. *V. o. chivi*	0.295	0.511	0.410	0.282	0.296	0.327	0.302	0.005	0.008
21. *V. f. flavoviridis*	0.345	0.605	0.426	0.282	0.344	0.379	0.378	0.159	0.160
22. *V. g. swainsonii*	0.230	0.380	0.314	0.196	0.191	0.237	0.133	0.260	0.279
23. *V. g. leucopolius*	0.275	0.384	0.362	0.239	0.222	0.272	0.132	0.305	0.312
24. *V. g. gilvus*	0.362	0.443	0.380	0.323	0.294	0.345	0.212	0.395	0.387
25. *V. l. leucophrys*	0.316	0.434	0.404	0.326	0.301	0.299	0.174	0.346	0.340
26. *H. p. poicilotis*	0.403	0.402	0.511	0.423	0.420	0.423	0.578	0.520	0.533
27. *H. t. aemulus*	0.463	0.476	0.571	0.482	0.425	0.481	0.618	0.585	0.600
28. *H. a. aurantiifrons*	0.140	0.372	0.216	0.236	0.109	0.145	0.335	0.310	0.322
29. *H. h. flaviventris*	0.059	0.402	0.129	0.109	0.104	0.073	0.361	0.353	0.365
30. *H. o. ferrugineifrons*	0.134	0.358	0.207	0.230	0.104	0.099	0.228	0.348	0.360
31. *H. o. viridior*	0.137	0.364	0.212	0.232	0.107	0.102	0.227	0.352	0.365
32. *H. d. darienensis*	0.023	0.351	0.090	0.109	0.068	0.036	0.312	0.303	0.315
33. *C. stelleri*	0.663	0.727	0.739	0.747	0.651	0.599	0.797	0.848	0.884
34. *P. p. hudsonia*	0.786	0.869	0.823	0.889	0.781	0.759	1.002	0.954	0.951
35. *P. p. pitiayumi*	1.045	0.926	0.967	1.170	1.040	0.966	0.912	1.151	1.151
36. *B. c. azarae*	1.402	1.237	1.204	1.421	1.396	1.261	1.107	1.384	1.376

genetic distances characterize the entire family Vireonidae.

Avise et al. (1982) suggested that the observed genetic distances within *Vireo* could result if the species were older on the average than congeneric bird species in general. They further argued that "conventional thought about the origin and relative age of the Vireonidae appears compatible with this explanation." However, the relative age of a family is not necessarily reflected clearly in the ages of all existing species. Of the five major clusters of species we identify in the Vireonidae, two existing species, *Cyclarhis gujanensis* and "*Vireo*" *bellii,* probably represent old lineages. In contrast, the great similarity in general behavior, voice, and ecology of a number of other species (e.g., *Vireo huttoni* and *Vireo carmioli*) would argue for their recency of origin. Thus, the relative age of existing species probably varies widely and provides little evidence for the belief that vireo species are "older on the average" than congeneric species in other families of birds. We can offer another explanation for these large genetic distances that does not require greater average age of species. Stated simply, vireonids are probably taxonomically undersplit at both the generic and specific levels.

At the generic level, for example, both the UPGMA and Wagner procedures expose clusters within *Vireo* that are demarcated by genetic distances of a magnitude equivalent to those distinguishing *Cyclarhis* and *Vireolanius* from other vireonids. In the past these forms have often been placed by systematists into either separate families or subfamilies. The simplest explanation for this is that traditional *Vireo* is currently

TABLE 3. Continued.

	19	20	21	22	23	24	25	26	27
1. *C. g. ochrocephala*	0.413	0.444	0.426	0.340	0.365	0.336	0.396	0.357	0.332
2. *C. g. contrerasi*	0.426	0.456	0.434	0.348	0.366	0.357	0.396	0.370	0.344
3. *C. g. dorsalis*	0.417	0.448	0.414	0.328	0.356	0.328	0.388	0.362	0.336
4. *C. g. gujanensis*	0.431	0.461	0.439	0.375	0.393	0.362	0.422	0.375	0.349
5. *V. l. leucotis*	0.403	0.397	0.444	0.336	0.364	0.414	0.371	0.448	0.446
6. *V. l. bolivianus*	0.367	0.357	0.405	0.318	0.346	0.397	0.371	0.409	0.452
7. *V. l. simplex*	0.367	0.357	0.405	0.296	0.324	0.375	0.349	0.409	0.452
8. *V. h. huttoni*	0.261	0.295	0.329	0.312	0.334	0.374	0.348	0.416	0.442
9. *V. g. noveboracensis*	0.267	0.293	0.328	0.241	0.270	0.310	0.284	0.345	0.388
10. *V. g. griseus*	0.253	0.280	0.314	0.242	0.271	0.311	0.285	0.336	0.379
11. *V. b. bellii*	0.392	0.418	0.465	0.340	0.338	0.371	0.367	0.337	0.392
12. *V. vicinior*	0.326	0.355	0.362	0.303	0.332	0.324	0.342	0.407	0.445
13. *V. carmioli*	0.232	0.259	0.259	0.207	0.235	0.276	0.284	0.345	0.388
14. *V. s. solitarius*	0.250	0.284	0.315	0.215	0.226	0.265	0.275	0.353	0.362
15. *V. flavifrons*	0.269	0.307	0.341	0.255	0.267	0.307	0.281	0.359	0.393
16. *V. philadelphicus*	0.310	0.284	0.341	0.174	0.164	0.209	0.174	0.445	0.473
17. *V. o. olivaceus*	0.062	0.060	0.194	0.272	0.290	0.344	0.316	0.417	0.455
18. *V. o. solimoensis*	0.054	0.055	0.185	0.283	0.294	0.334	0.308	0.422	0.458
19. *V. o. diversus*	—	0.073	0.168	0.302	0.314	0.356	0.329	0.422	0.461
20. *V. o. chivi*	0.024	—	0.172	0.293	0.311	0.362	0.336	0.431	0.474
21. *V. f. flavoviridis*	0.131	0.154	—	0.328	0.356	0.362	0.371	0.500	0.543
22. *V. g. swainsonii*	0.298	0.294	0.370	—	0.046	0.103	0.147	0.357	0.366
23. *V. g. leucopolius*	0.336	0.335	0.400	0.0	—	0.076	0.119	0.396	0.404
24. *V. g. gilvus*	0.420	0.432	0.432	0.073	0.049	—	0.112	0.448	0.457
25. *V. l. leucophrys*	0.370	0.384	0.437	0.114	0.088	0.110	—	0.457	0.483
26. *H. p. poicilotis*	0.529	0.546	0.676	0.414	0.480	0.595	0.602	—	0.250
27. *H. t. aemulus*	0.596	0.616	0.757	0.419	0.486	0.602	0.642	0.279	—
28. *H. a. aurantiifrons*	0.284	0.324	0.388	0.246	0.279	0.379	0.384	0.488	0.493
29. *H. h. flaviventris*	0.328	0.379	0.432	0.241	0.285	0.372	0.375	0.323	0.375
30. *H. o. ferrugineifrons*	0.319	0.349	0.416	0.239	0.272	0.366	0.375	0.472	0.477
31. *H. o. viridior*	0.324	0.354	0.432	0.241	0.273	0.372	0.375	0.477	0.482
32. *H. d. darienensis*	0.279	0.329	0.379	0.241	0.285	0.372	0.326	0.423	0.482
33. *C. stelleri*	0.884	0.934	0.981	0.749	0.792	0.824	0.815	0.747	0.757
34. *P. p. hudsonia*	0.956	0.963	0.963	1.045	1.048	0.980	1.072	0.980	1.098
35. *P. p. pitiayumi*	1.151	1.153	1.153	1.134	1.138	1.170	1.190	0.728	0.984
36. *B. c. azarae*	1.378	1.404	1.404	1.386	1.390	1.288	1.449	1.170	1.378

comprised of several distinct radiations worthy of recognition at the generic level. If these groups are considered to be genera, then the calculation of new interspecific genetic distances yields values comparable to those known for other birds (Table 5): eye-ringed *Vireo,* 14 comparisons, $\bar{D} \pm SE = 0.086 \pm 0.0096$; *olivaceus* group of eye-lined *"Vireo,"* four comparisons, $\bar{D} \pm SE = 0.151 \pm 0.0068$; and *gilvus* group of eye-lined *Vireo,* $\bar{D} \pm SE = 0.138 \pm 0.0160$. The new intergeneric confamilial value (254 comparisons, $\bar{D} \pm SE = 0.358 \pm 0.0065$, range = 0.132–0.605) is nearly identical to that found under the old classification (Table 5). These new values show conclusively that members of the genus *Vireo* need not have values at either level that fundamentally disagree with those determined for other taxa of birds.

At the species level as well several clues support an hypothesis of taxonomic undersplitting. First, genetic distances between subspecies of vireonids average substantially higher than among birds in general (Table 5). Second, the family (especially the genera *Vireo* and *Hylophilus*) contains many morphologically very similar forms that have demonstrated their certain biologic species status through sympatry. Therefore, phenotypic evolution has been only slight or modest in these two genera. Finally, even very similar allopatric forms can differ by substantial genetic distances. Consider *V. flavoviridis,* a species which is fixed at an allele different from the predominant one shared by its allopatric and very similar close relative, *V. o. olivaceus,* at six loci (Johnson and Zink 1985). Therefore, additional allopatric cryptic species could be present

TABLE 3. Extended.

	28	29	30	31	32	33	34	35	36
1. *C. g. ochrocephala*	0.306	0.151	0.289	0.288	0.220	0.444	0.542	0.633	0.702
2. *C. g. contrerasi*	0.318	0.163	0.301	0.301	0.232	0.456	0.577	0.646	0.737
3. *C. g. dorsalis*	0.310	0.155	0.293	0.293	0.224	0.461	0.560	0.651	0.720
4. *C. g. gujanensis*	0.345	0.190	0.321	0.328	0.259	0.483	0.560	0.638	0.707
5. *V. l. leucotis*	0.328	0.241	0.316	0.310	0.207	0.466	0.530	0.655	0.737
6. *V. l. bolivianus*	0.310	0.224	0.292	0.293	0.190	0.483	0.525	0.603	0.707
7. *V. l. simplex*	0.288	0.202	0.277	0.271	0.168	0.461	0.525	0.616	0.720
8. *V. h. huttoni*	0.226	0.145	0.214	0.209	0.110	0.531	0.596	0.721	0.721
9. *V. g. noveboracensis*	0.155	0.069	0.143	0.138	0.034	0.500	0.564	0.655	0.759
10. *V. g. griseus*	0.156	0.070	0.141	0.139	0.035	0.496	0.554	0.646	0.749
11. *V. b. bellii*	0.332	0.337	0.315	0.314	0.302	0.526	0.591	0.607	0.711
12. *V. vicinior*	0.217	0.131	0.200	0.200	0.097	0.529	0.573	0.620	0.703
13. *V. carmioli*	0.224	0.103	0.212	0.207	0.103	0.534	0.599	0.690	0.759
14. *V. s. solitarius*	0.135	0.116	0.123	0.118	0.083	0.488	0.552	0.643	0.747
15. *V. flavifrons*	0.169	0.095	0.123	0.117	0.060	0.467	0.554	0.623	0.714
16. *V. philadelphicus*	0.307	0.315	0.230	0.224	0.281	0.554	0.640	0.607	0.676
17. *V. o. olivaceus*	0.302	0.321	0.320	0.320	0.287	0.584	0.620	0.679	0.741
18. *V. o. solimoensis*	0.302	0.319	0.319	0.319	0.284	0.593	0.621	0.679	0.740
19. *V. o. diversus*	0.280	0.301	0.298	0.298	0.267	0.593	0.619	0.679	0.738
20. *V. o. chivi*	0.293	0.328	0.306	0.306	0.293	0.621	0.629	0.685	0.754
21. *V. f. flavoviridis*	0.345	0.362	0.355	0.362	0.328	0.633	0.629	0.685	0.754
22. *V. g. swainsonii*	0.259	0.241	0.247	0.241	0.241	0.547	0.659	0.680	0.749
23. *V. g. leucopolius*	0.270	0.270	0.263	0.258	0.270	0.561	0.653	0.675	0.744
24. *V. g. gilvus*	0.328	0.310	0.311	0.310	0.310	0.569	0.633	0.690	0.724
25. *V. l. leucophrys*	0.336	0.319	0.324	0.319	0.284	0.560	0.659	0.698	0.767
26. *H. p. poicilotis*	0.397	0.276	0.380	0.379	0.345	0.534	0.633	0.517	0.690
27. *H. t. aemulus*	0.405	0.319	0.388	0.388	0.388	0.543	0.676	0.629	0.750
28. *H. a. aurantiifrons*	—	0.190	0.104	0.099	0.155	0.517	0.594	0.685	0.788
29. *H. h. flaviventris*	0.193	—	0.178	0.172	0.069	0.466	0.599	0.621	0.724
30. *H. o. ferrugineifrons*	0.089	0.187	—	0.010	0.143	0.466	0.581	0.585	0.688
31. *H. o. viridior*	0.092	0.189	0.0	—	0.138	0.466	0.586	0.586	0.690
32. *H. d. darienensis*	0.151	0.071	0.146	0.148	—	0.500	0.564	0.655	0.759
33. *C. stelleri*	0.693	0.609	0.605	0.609	0.676	—	0.431	0.569	0.603
34. *P. p. hudsonia*	0.872	0.889	0.836	0.847	0.806	0.541	—	0.552	0.586
35. *P. p. pitiayumi*	1.153	0.969	0.872	0.882	1.065	0.824	0.767	—	0.276
36. *B. c. azarae*	1.558	1.288	1.160	1.170	1.421	0.907	0.847	0.323	—

TABLE 4. Genetic variability measures for selected[a] taxa of Vireonidae.

Taxon	No. alleles at polymorphic loci	$H_{obs} \pm$ SE	$H_{exp} \pm$ SE	Percent polymorphic loci[b]	Average number of alleles[c]
Vireo h. huttoni	31	0.014 ± 0.010	0.014 ± 0.010	6.90	1.07
Vireo g. griseus	34	0.034 ± 0.022	0.038 ± 0.026	10.34	1.17
Vireo vicinior	31	0.028 ± 0.022	0.023 ± 0.017	6.90	1.07
Vireo s. solitarius	36	0.052 ± 0.031	0.049 ± 0.027	13.79	1.24
Vireo o. olivaceus[d]	45	0.078 ± 0.025	0.080 ± 0.023	44.83	1.55
Vireo o. solimoensis	39	0.064 ± 0.034	0.069 ± 0.030	20.69	1.34
Vireo o. diversus[d]	47	0.074 ± 0.030	0.083 ± 0.034	31.03	1.62
Hylophilus o. ferrugineifrons	32	0.021 ± 0.012	0.021 ± 0.012	10.34	1.10
$\bar{x}$[e]	36.9	0.046	0.047	16.85	1.27

[a] Taxa represented by samples ≥ 5.
[b] Frequency of most common allele ≤ 0.99.
[c] Per locus.
[d] Values for this taxon differ from those given in Johnson and Zink (1985:423) because that study was based on the analysis of 38 rather than 29 loci.
[e] Unweighted by sample size.

TABLE 5. Mean genetic distances (Nei 1978) among samples from populations differentiated at several taxonomic levels.

Traditional taxonomic level	Number of comparisons	D ± SE	Range	*D* for other passerine birds[1]
Intraspecific (subspecies)	20	0.0179 ± 0.0042	0.000–0.073	0.0048
Interspecific congeners	162	0.2926 ± 0.0095	0.071–0.605	0.0809
Within *Vireo*	142	0.2914 ± 0.0096	0.088–0.605	
Within subgenus *Vireo*[2]	2	0.3410 ± 0.0100	0.331–0.351	
Within subgenus *Lanivireo*	10	0.1182 ± 0.0100	0.056–0.169	
Within subgenus *Vireosylva*	34	0.2793 ± 0.0182	0.088–0.437	
Within *Hylophilus*	20	0.3018 ± 0.0362	0.071–0.493	
Intergeneric confamilial	322	0.3538 ± 0.0076	0.023–0.757	0.3264
Interfamilial	132	0.9839 ± 0.0215	0.541–1.558	0.7580
Vireonidae vs. Corvidae	64	0.7975 ± 0.0173	0.541–1.098	
Vireonidae vs. Emberizidae	64	1.1796 ± 0.0222	0.728–1.558	
Corvidae vs. Emberizidae	4	0.8363 ± 0.0290	0.767–0.907	

[1] Weighted means calculated from data in Barrowclough (1980:661), Avise et al. (1982:95), Zink (1982:638–639), Zink and Johnson (1984:209), and Marten and Johnson (1986:415).
[2] Subgeneric classification follows Barlow (1981) not Hamilton (1962). Only *V. griseus* and *V. bellii* are represented.

in the Vireonidae and an unknown number of currently recognized "subspecies" may be distinct species. If such is the case, average interspecific genetic distances based on current taxonomy would be inflated relative to typical passerine genera. Several supposed subspecies shown in this study to have suspiciously high genetic distances actually could be cryptic species (i.e., *C. g. contrerasi* and/or *C. g. gujanensis, V. l. bolivianus, V. o. diversus, V. g. gilvus*). Other candidates for possible species status, although not investigated in this study, are some of the currently recognized forms of *V. huttoni, V. griseus, V. bellii, V. solitarius* (currently under study by the first author), and *V. altiloquus*.

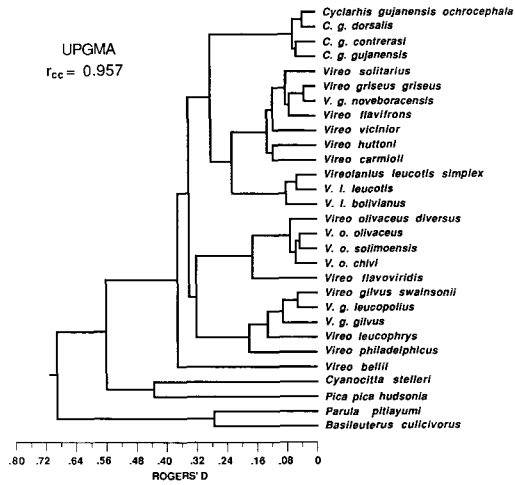

FIGURE 1. Phenogram based on Rogers' *D*-values and derived by the UPGMA method. All taxa of Vireonidae studied, exclusive of *Hylophilus*, plus the four outgroup species, are included in this diagram (analysis A of text). The high cophenetic correlation coefficient (r_{cc} = 0.957) indicates excellent agreement between the distances shown in the phenogram and the original data matrix.

EVOLUTIONARY RELATIONSHIPS OF TAXA

For several reasons we do not rely heavily on the results of the cladistic approaches in the discussion to follow. In several instances these analyses led to certain paraphyly (subspecies clustered with the wrong species). Furthermore, unresolved trichotomies and/or polychotomies are very common. We also note that cladistic approaches have not been particularly illuminating in other avian

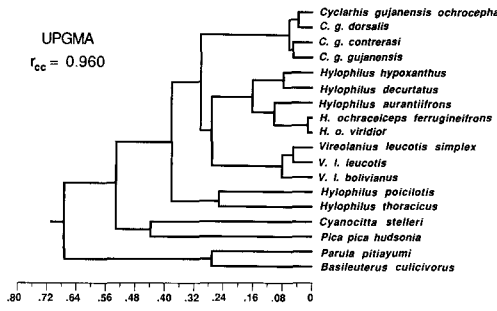

FIGURE 2. Phenogram based on Rogers' *D*-values and derived by the UPGMA method. This diagram includes all taxa of *Cyclarhis, Vireolanius, Hylophilus* and the four outgroup taxa, but excludes *Vireo* (analysis B of text).

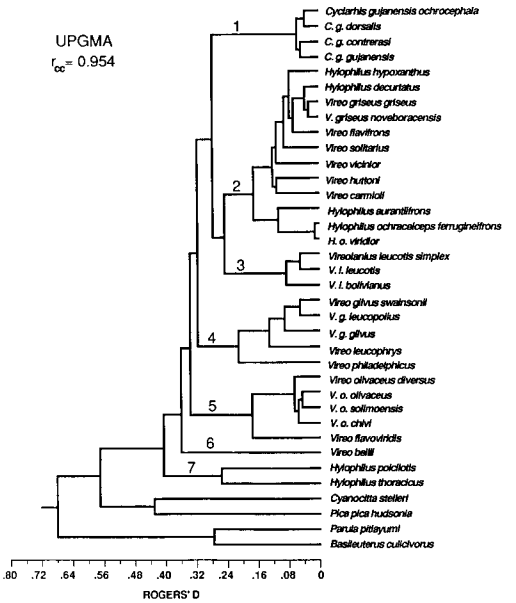

FIGURE 3. Phenogram based on Rogers' *D*-values and derived by the UPGMA method. All analyzed taxa of vireonids and the four outgroup species are treated (analysis C of text). A second cluster analysis by this method, in which Nei's genetic distances were used, gave very similar results.

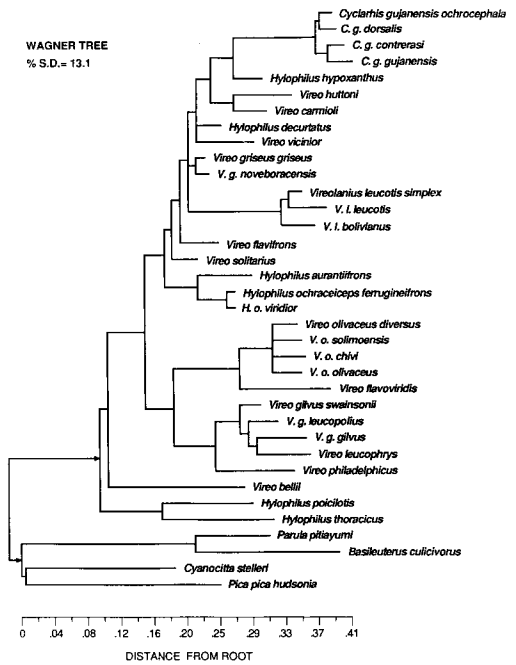

FIGURE 4. Distance Wagner tree rooted at the outgroups, *Parula pitiayumi, Basileuterus culicivorus, Cyanocitta stelleri,* and *Pica pica hudsonia.* Rogers' *D* was used.

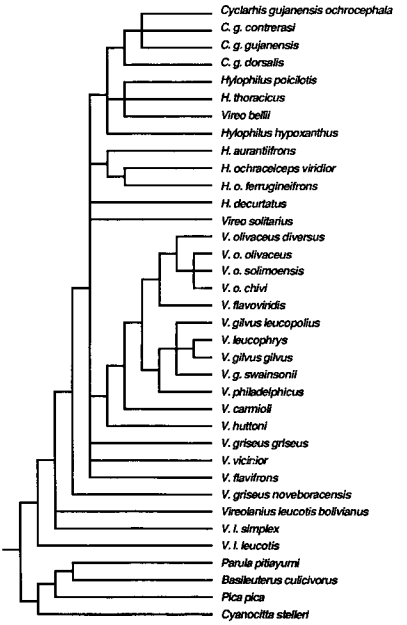

FIGURE 5. Strict consensus tree resulting from a cladistic analysis by locus. Only nodes, not branch lengths, are specified. The following statistics were derived from this tree: Consensus fork index (component count) = 22, CF (normalized) = 0.647, Term information = 186 and Total information = 208.

studies (Avise et al. 1980, Zink and Johnson 1984). Our interpretations, therefore, emphasize the genetic distance analyses (UPGMA and Wagner trees). In general, we equate congruence of branching patterns with relative robustness of results. Moreover, in interpreting the topology of the various trees and the definition of clusters, long branches are assumed to be more reliable than short branches because substantial standard errors are associated with the genetic distances upon which the branch nodes are based.

Species level. The genetic results throw light on several persistent species problems. One concerns the relationship of *V. carmioli,* an isolate in the highlands of Costa Rica and Panama. Hamilton (1962) placed *carmioli* in the *griseus* group. Mayr and Short (1970:72), however, questioned this treatment. Barlow (1981) listed *carmioli* between *V. solitarius repetens* and *V. vicinior.* The American Ornithologists' Union (1983) placed *carmioli* between *V. flavifrons* and *V. huttoni.* The UPGMA and Wagner approaches consistently portray *carmioli* as a sister taxon of *huttoni.* However, in view of the short branch length separating the *carmioli-huttoni*

clade from other taxa of eye-ringed *Vireo,* this result is somewhat tentative.

The systematic status of *V. leucophrys* has been a persistent problem. Several recent workers regarded it as a subspecies of *V. gilvus* (Mayr and Short 1970, Barlow 1981). Hamilton (1962) and the American Ornithologists' Union (1983), however, treated *leucophrys* as a full species. The genetic data indicate that *leucophrys* has differentiated to a level consistent with other species, and that it is more closely allied to *V. gilvus,* rather than to *V. philadelphicus,* within the *gilvus* group.

Another issue at the species level concerns the supposedly close relationship of *V. solitarius* and *V. flavifrons.* All recent authors have listed them in close proximity. Mayr and Short (1970) state that they are closely related and "may comprise a superspecies." The genetic data do not provide a definitive answer. Although they differ by the lowest genetic distance within *Lanivireo* (Nei's $D = 0.056$; Table 5), in no treatment are they aligned as sister taxa. We regard the question of their close relationship as unresolved.

A final species-level issue to which the electrophoretic information contributes concerns the status of members of the "*Chivi*" complex of vireos in South America. Although Johnson and Zink (1985) showed that *diversus* was more clearly allied to North American *olivaceus* than to Middle American *flavoviridis,* the relationships of members of the *chivi* complex other than *V. diversus* remained in doubt. All analyses of this study (UPGMA, Wagner and PAUP) show that the alliance of the two new forms represented, *chivi* and *solimoensis,* is clearly with *V. olivaceus,* rather than with *V. flavoviridis.*

Subgeneric and generic level. Hamilton (1962), Barlow (1981), and the American Ornithologists' Union (1983) advocated the use of subgenera in the genus *Vireo.* However, the electrophoretic results clearly indicate that the recognition of subgenera, at least for the species analyzed, is unwarranted. One traditional subgenus, *Lanivireo,* cannot be maintained because its members (*solitarius, flavifrons,* etc.) cluster closely in both the UPGMA and Wagner analyses with *griseus,* the type species of the subgenus *Vireo.* In addition, *bellii,* the other member of the subgenus *Vireo* that we examined in addition to *griseus,* is genetically so distinctive that it strains the limits of the genus *Vireo* to accommodate it there. Nor can the current composition of the subgenus *Vireosylva* be defended. The two well-defined clusters of eye-lined species, the *olivaceus* group and the *gilvus* group, differ from the eye-ringed species at distances equal to or greater than those which distinguish *Cyclarhis* and *Vireolanius* from other clusters in the family. Avise et al. (1982) also reported a large genetic distance between *V. olivaceus* and *V. philadelphicus,* representatives of the two groups of eye-lined species.

As the foregoing remarks on the status of subgenera indicate, the electrophoretic information points to the need for a complete reassessment of traditional *generic* limits in the Vireonidae. *Vireo* appears to be either polyphyletic or paraphyletic. To establish generic limits within the Vireonidae that are comparable with those in other passerines, the Bell's Vireo and the two major components of eye-lined *Vireo* (the *olivaceus* group and the *gilvus* group), each deserve full generic status. Within *Hylophilus,* species are poorly known and relationships ill-defined. Barlow (1981) states that vocal similarities of certain *Hylophilus* and species of *Vireo* "doubtless reflect the close relationship of the two genera." However, both the UPGMA and Wagner trees suggest that the genus is polyphyletic, with some species close to *Vireo* and others not. In particular, the distinctness of *poicilotis* and of *thoracicus* argues for their elevation, either as one new genus or two. Although all analyses group *poicilotis* and *thoracicus* as sister taxa, it must be borne in mind that we examined genetically only one-half of the existing species; an analysis with complete coverage of taxa might well show relationships differing from those indicated here.

In contrast, *Cyclarhis* and *Vireolanius* are clearly defined genera, according to the genetic data. Although not all of the existing species were studied, we feel that the examination of additional forms of these genera would not modify this conclusion.

Subfamilial level. It is obvious from the branching diagrams that *Cyclarhis* and *Vireolanius* do not differ from other taxa of vireonids to the degree that would justify subfamilial ranking for each. Indeed, if subfamilies are maintained for peppershrikes and shrike-vireos, consistency would require that we elevate the eye-ringed vireos, each of the two groups of eye-lined vireos, the Bell's Vireo, and the *Hylophilus poicilotis-thoracicus* cluster to an equivalent taxonomic level! Few would accept such changes. Our proposal to eliminate subfamilies in the Vireo-

nidae is not novel. Sibley and Ahlquist (1982) recently concluded from their DNA-DNA hybridization studies that *Vireo, Hylophilus,* and *Cyclarhis* were "closely enough related to one another to be placed in the same subfamily." They lacked access to the DNA of *Vireolanius.*

Familial level. The familial relationships of the Vireonidae have long been of interest, as Sibley and Ahlquist (1982) summarized. In agreement with these authors, our genetic information definitely supports a closer alliance of the vireos with the Corvidae than with the Emberizidae (specifically, the Parulinae). In only three comparisons (*V. philadelphicus, H. p. poicilotis,* and *H. t. aemulus* [each vs. *P. p. pitiayumi*]) were Nei's genetic distances smaller between a vireonid and a warbler than between any vireonid and either corvid. Again, genetic distances are accompanied by large standard errors and agreement of all comparisons is not to be expected.

Congruence of genetic and morphologic results. In several major respects the groupings of species indicated by the genetic findings agree with those reported by Orenstein and Barlow (1981) in their survey of variation in jaw musculature of vireonids. For example, they found that "*Cyclarhis* and *Vireolanius* had relatively stronger jaw musculature but were otherwise similar to *Vireo.*" The genetic information also points to an alliance of the peppershrikes and shrike-vireos to the eye-ringed species of *Vireo* rather than to *Vireosylva.* Orenstein and Barlow reported that, "Differences in relative fibre lengths in the large adductor muscles were found between the subgenera *Vireo* and *Vireosylva.*" Again, the electrophoretic results unambiguously show that there is a sharp division between these two components of traditional *Vireo.* Moreover, they report that, ". . . the jaw musculature provides no evidence for placing the arboreal short-fibred species of *V. solitarius, flavifrons, huttoni, carmioli,* and *osburni* in a subgenus *Lanivireo.*" Again, the genetic findings give no reason to recognize a separate subgenus for these species.

In two examples, however, the information from jaw musculature did not indicate a division among species that was suggested by the genetic data. For example, the species of *Vireosylva* appear to have the same basic jaw muscle patterns; the *olivaceus* group and the *gilvus* group do not segregate on this basis. Furthermore, although "*Hylophilus* spp. had significantly larger depressor musculature than the other genera studied" (Orenstein and Barlow 1981:1), they report no differences between *H. poicilotis* and its congeners. Despite these few inconsistencies, overall the morphologic results strongly support the electrophoretic findings.

EVOLUTIONARY IMPLICATIONS AND DATING OF MAJOR CLADOGENETIC EVENTS

The identification of distinct clades of vireonids suggests that the major lineages in the family each had a substantial history of independent evolution. The sequence of origin of these clades, however, was unresolved. It is thus possible that these clades arose over the same general span of time, much as envisioned for *Empidonax* and *Contopus* by Zink and Johnson (1984) and for genera of emberizid sparrows by Zink (1982).

Using Marten and Johnson's (1986:416) compromise figure of 19.7 as a substitute for the calibration of Gutiérrez et al. (1983), in the equation $t = 19.7 \times 10^6 D$, where t is the time since divergence and D is Nei's (1978) genetic distance, we can propose estimated dates for the major cladogenetic events. Based on an average Nei's D of 0.7975, the Vireonidae split from their corvid relatives at approximately 16 MYBP (million years before present). Corvids were available as ancestors at least that far back because fossils from this family are known from middle to late Miocene (Olson 1985:140). Within the Vireonidae, dates for the emergence of the major clades range from approximately 8 MYBP, when *bellii* diverged, to 4 MYBP, when *Vireolanius* split from the complex clade which includes the eye-ringed species of *Vireo.* In view of significant uncertainties in every component of the equation (Marten and Johnson 1986), these estimates serve only as gross approximations. Unfortunately, no certain Miocene or Pliocene fossil vireonids are known that could corroborate the schedule of appearance of any of these lineages.

TAXONOMIC RECOMMENDATIONS

Although the genetic results suggest that a drastic overhaul of traditional vireonid classification is in order, wholesale changes would be premature at this time. Because the present genetic survey was incomplete, final pronouncements regarding either species-level relationships or generic composition cannot yet be justified. A more complete appraisal of relationships should emerge from electrophoretic analysis of taxa not available to

us. Additional behavioral and morphologic study (e.g., Orenstein and Barlow 1981) should also be fruitful, especially if conducted with an awareness of the striking genetic discontinuities and probable polyphyly/paraphyly illuminated here.

However, not all potential changes need to be postponed. In particular the continued recognition of current subgenera within *Vireo* and of subfamilies within the Vireonidae cannot be justified. Therefore we formally recommend disuse of these categories.

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