SPECIATION IN VIREOS. I. MACROGEOGRAPHIC PATTERNS OF ALLOZYMIC VARIATION IN THE *VIREO SOLITARIUS* COMPLEX IN THE CONTIGUOUS UNITED STATES[1]

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Abstract. Using allozymes, I examined genetic variation in 23 populations of four currently recognized subspecies of the Solitary Vireo (*Vireo solitarius solitarius, V. s. alticola, V. s. cassinii,* and *V. s. plumbeus*), with emphasis on the latter two taxa. Eighteen of 38 genetic loci (47.4%) were polymorphic. Intrataxon Nei's genetic distances were low ($D =$ 0.0008) among populations of *V. s. cassinii* and *V. s. plumbeus.* Average intertaxon Nei's D ranged from zero (*V. s. solitarius* vs. *V. s. alticola*) to 0.0030 (*V. s. solitarius* vs. *V. s. cassinii*), 0.0033 (*V. s. cassinii* vs. *V. s. alticola*), 0.0283 (*V. s. plumbeus* vs. *V. s. solitarius*), 0.0288 (*V. s. plumbeus* vs. *V. s. alticola*), and 0.0294 (*V. s. plumbeus* vs. *V. s. cassinii*). Nei's D between the Yellow-throated Vireo (*Vireo flavifrons*) and the four taxa of Solitary Vireo ranged from 0.0430–0.0743. A mean F_{st} value of 0.289 over all populations of *V. solitarius,* and across combined populations of *V. s. cassinii* and *V. s. plumbeus,* indicated pronounced genetic discontinuity between these two forms. Gene flow estimates between populations representing the subset of *V. s. cassinii* and *V. s. plumbeus* ranged from one individual every two generations (Wright's 1951 formula) to an average of 12 immigrants per generation over all populations (Slatkin's rare allele method). Based on strong allozymic divergence from the three other allopatric forms treated here, the Plumbeous Vireo (*Vireo plumbeus*) is a species. Although similar allozymically, the Cassin's Vireo (*Vireo cassinii*) and the Blue-headed Vireo (*V. s. solitarius* plus the "Mountain Vireo," *V. s. alticola*) also may deserve species status because data published by others demonstrate trenchant differences in voice and mtDNA base sequences.

Key words: Speciation; allozymes; geographic variation; gene flow; Vireonidae; Solitary Vireo; Vireo solitarius; Blue-headed Vireo; Mountain Vireo; Cassin's Vireo; Plumbeous Vireo.

INTRODUCTION

The ongoing discovery of previously unrecognized, probable or definite cryptic species of Vireonidae (Johnson and Zink 1985, Johnson et al. 1988, Willis 1991, Cicero and Johnson 1992) suggests that the group has been taxonomically undersplit and that additional forms need modern biosystematic attention. The various taxa currently included in the Solitary Vireo (*Vireo solitarius*), a common passerine of mixed coniferous-deciduous woodlands across North America, comprise a complex deserving such detailed study. Each form is strongly characterized in features of size, coloration, habitat selection, and voice. This unusual combination of attributes allows a multidimensional examination of variation across geography that would clarify the nature of character change in the vicinity of boundaries between these taxa. In turn, this information could illuminate both the current taxonomic

status and the evolutionary history of the several allopatric units. The present paper initiates such a study by presenting the results of a broad-scale population analysis of allozymic variation based on an electrophoretic survey of protein-coding loci. Future papers, based on research in progress, will examine geographic variation in mtDNA nucleotide sequences, morphology, coloration, and male advertising song and will focus on the two most divergent forms in the complex, *V. s. cassinii* and *V. s. plumbeus.* This paper does not treat the several additional named subspecies that breed in Mexico and Belize.

I discuss four taxa. *Vireo s. alticola,* a large, dorsally blackish-gray form with bright yellowish sides, lives in forests of mixed hardwoods and conifers in Appalachia. *Vireo s. solitarius,* a smaller, gray-headed form with an olive-green back and rump and yellow flanks, inhabits similar open woods across the northeastern United States and southern Canada. *Vireo s. cassinii,* although basically similar to the previous form, is paler and has a less contrasting head and dor-

sum. It breeds in open ponderosa pines and pine-oak woodland from British Columbia south-eastward to western Montana and southward to California. A fourth taxon, *V. s. plumbeus,* a rel-atively large, clean gray and white form, nests in arid pinyon-juniper and open ponderosa pine-scrub oak woodland from central Montana south through the interior of the western United States.

Since first described, all forms have been treat-ed as subspecies in the Check-list of North Amer-ican birds (American Ornithologists' Union 1886, 1889, 1895, 1910, 1931, 1957, 1983). Nonethe-less, the vernacular names used for subspecies in four editions of that series also have been applied commonly in other literature: "Blue-headed Vir-eo" (*V. s. solitarius*), "Mountain Solitary Vireo" or "Mountain Vireo" (*V. s. alticola*), "Cassin's Vireo" (*V. s. cassinii*), and "Plumbeous Vireo" (*V. s. plumbeus*). Furthermore, with the excep-tion of *V. s. alticola,* relatively unworn adults of the North American forms can often be identi-fied safely under ideal field conditions and are occasionally illustrated separately in field guides. Such treatment by both the professional and pop-ular literature implies a degree of distinctiveness greater than that usually associated with subspe-cies. The statement in American Ornithologists' Union (1983:594) that more than one species may be presently included under *V. solitarius* and the treatment of *V. s. solitarius, V. s. cassinii,* and *V. s. plumbeus* as separate species by Sibley and Monroe (1990) and Monroe and Sibley (1993) are based upon results of the present paper and others in progress.

METHODS AND MATERIALS

COLLECTION OF SPECIMENS

This study was based upon 335 specimens taken on their breeding grounds during the spring and early summer of 1977–1987. I divided this total into 23 sample areas for analysis (Fig. 1). Eleven sample areas (Ravalli through Shaver Lake) rep-resent *V. s. cassinii,* nine (Wassuk-Paradise through Mogollon) represent *V. s. plumbeus,* two (Minnesota and Maine) are of *V. s. solitarius,* and one composite sample area (Virginia-North Carolina) represents *V. s. alticola.*

ALLOZYME ELECTROPHORESIS

I analyzed 38 protein-coding loci in tissue ex-tracts using standard techniques described by Se-lander et al. (1971) and Yang and Patton (1981), with the slight modifications of Johnson et al.

(1984). Protein assays were prepared according to Harris and Hopkinson (1976) and Selander et al. (1971). Electromorphs (alleles) at each locus were designated alphabetically in order of de-creasing mobility. Six additional loci (Acp, Ak, Ck-3, Est-2, Lap, and Mdh-1) could not be scored consistently and were excluded. Two specimens of the Yellow-throated Vireo (*Vireo flavifrons*) from Louisiana were used as an outgroup. With 335 specimens from 23 populations at 38 loci (two alleles per locus per individual), 25,460 al-leles were scored.

STATISTICAL ANALYSES

Using BIOSYS-1 (Swofford and Selander 1981), I calculated observed and expected heterozygos-ities for each sample, Chi-square tests for de-partures from Hardy-Weinberg equilibrium, al-lelic frequencies, genetic distances (with the methods of Nei 1978, Rogers 1972, and Cavalli-Sforza and Edwards 1967, as modified by Nei 1983), and Wright's F_{st} (1965), using the modi-fications of Wright (1978) for small sample size and of Nei (1975) for multiple alleles. Because observed values of heterozygosity (*H*obs.) did not differ from expected values based on Hardy-Weinberg equilibrium (Nei 1975) in any com-parison, only values for *H*obs. are presented.

Based on Nei (1983) modified arc distances of Cavalli-Sforza and Edwards (1967), I produced two trees depicting relationships among popu-lations: a UPGMA dendrogram (with BIOSYS-1 [Swofford and Selander 1981]) and a neighbor-joining (N-J) tree (with the method of Saitou and Nei [1987]) by application of the program MEGA (Kumar et al. 1993). Using the same distance values, interpopulation genetic variation was further examined through multidimensional scaling (MDS) with the program ALSCAL (SPSS Inc. 1990). Lessa (1990) discussed the merits of ordination techniques such as MDS, as opposed to hierarchical approaches, to analyze allelic variation across geography.

Contemporary levels of gene flow were esti-mated with Wright's (1951) formula, $Nm = \frac{1}{4}$ $(1/F_{st} - 1)$, and with Slatkin's (1981, 1985a, 1985b) rare allele method, which uses the for-mula $\ln p(1) = -0.505 \ln(Nm) + (-2.44)$, where $p(1)$ is the average frequency of private alleles and Nm is the product of the population size and immigration rate. Because sample sizes differed among populations, I applied Slatkin's (1985a) recommended correction, in which Nm is divid-
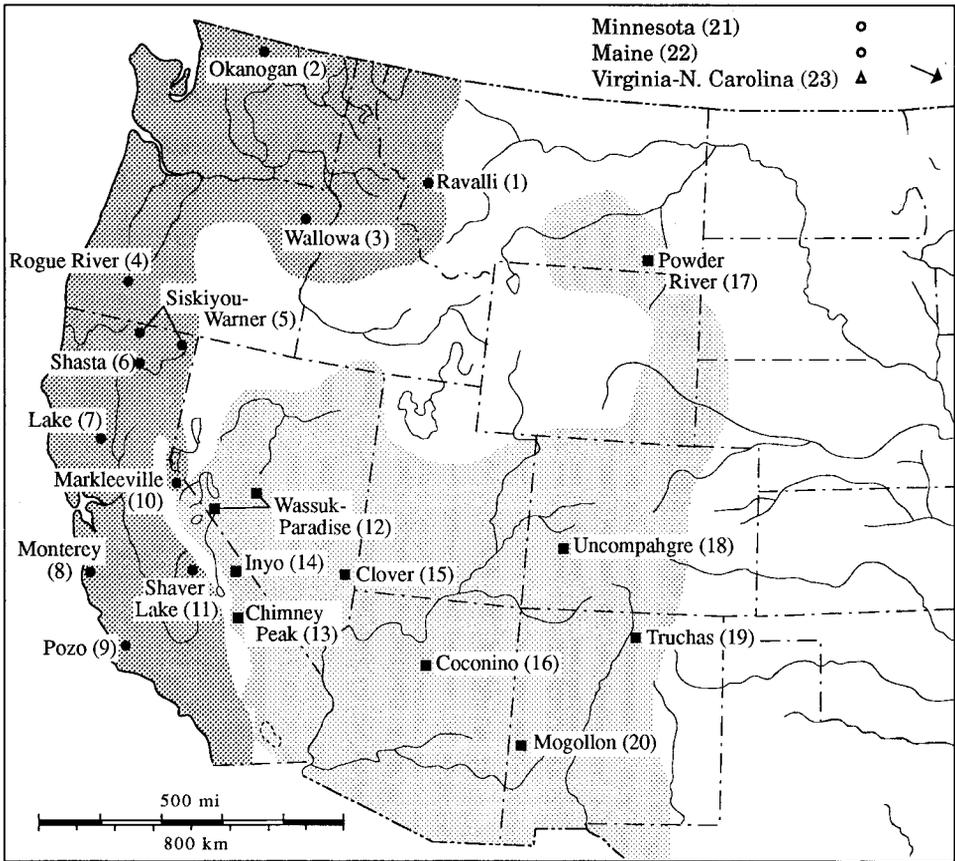
FIGURE 1.   Gross approximation of the nesting distributions of *V. s. cassinii* (dark shading) and *V. s. plumbeus* (pale shading) in the contiguous western United States. Within the shaded regions, areas which are actually occupied by either form consist of irregular islands of suitable habitat surrounded by vast regions of unsuitable terrain. Specimen sampling sites are identified by solid dots (*V. s. cassinii*), and solid squares (*V. s. plumbeus*). The two sampling sites for *V. s. solitarius* (open circles) and the single combined site for *V. s. alticola* (triangle), in the eastern United States, are not mapped.

ed by the ratio of the average sample size to 25. Thus, $Nm_c = Nm(\bar{N}/25)^{-1}$, where $\bar{N} = (\Sigma \, N_i/n)$, the average sample size. N_i is the number of specimens in a given sample and n is the number of samples studied. For my average sample size of $\bar{N} = 14.6$, the correction factor was 0.584. Slatkin and Barton (1989) verified the suitability of both F_{st} and rare alleles, as opposed to maximum likelihood methods, for estimating the average level of gene flow in natural populations.

RESULTS

VARIATION AMONG LOCI AND HETEROZYGOSITY

Eighteen of 38 scorable loci (47.4%) were polymorphic. Twenty loci were monomorphic: AB-

1, 2, 3, 4; ALD; CK-1, 2; EST-1; GAPDH; GDA; GLO; GLUD; GOT-2; GPT; G-6-PDH; ICD-2; LDH-1, 2; MDH-2; SDH.

Levels of genetic variability within populations of the *Vireo solitarius* complex are listed in Table 1. Observed heterozygosity ranged from 0.023 (Virginia-North Carolina) to 0.056 (Wallowa). Average Hobs. was 0.039. Percentage of polymorphic loci ranged from 7.9% (four populations) to 21.1% (Pozo), with a mean of 12.94%. Mean number of alleles per locus ranged from 1.1 (three populations) to 1.4 (Clover), with an average of 1.23. Mean Hobs. for *V. s. cassinii* (0.0438) was significantly different from that for *V. s. plumbeus* (0.0350) based on a Mann-Whitney-U statistic of 82 ($0.05 > P > 0.02$). Sample

TABLE 1. Genetic variability measures for 23 samples representing four taxa of the *Vireo solitarius* complex.

Sample number and name	n	Mean no. alleles per locus	Percentage of loci polymorphic[a]	H_{obs}
1. Ravalli	21	1.3	13.2	0.036 ± 0.014
2. Okanogan	11	1.2	7.9	0.038 ± 0.018
3. Wallowa	16	1.2	15.8	0.056 ± 0.022
4. Rogue River	14	1.2	10.5	0.041 ± 0.020
5. Siskiyou-Warner	11	1.2	10.5	0.043 ± 0.020
6. Shasta	19	1.3	18.4	0.047 ± 0.018
7. Lake	18	1.3	15.8	0.050 ± 0.019
8. Monterey	13	1.1	7.9	0.030 ± 0.017
9. Pozo	10	1.2	21.1	0.045 ± 0.018
10. Markleeville	3	1.1	13.2	0.053 ± 0.024
11. Shaver Lake	20	1.3	10.5	0.043 ± 0.021
12. Wassuk-Paradise	7	1.1	13.2	0.030 ± 0.016
13. Chimney Peak	10	1.2	13.2	0.037 ± 0.017
14. Inyo	16	1.3	15.8	0.030 ± 0.013
15. Clover	27	1.4	18.4	0.036 ± 0.013
16. Coconino	20	1.3	13.2	0.030 ± 0.011
17. Powder River	18	1.2	13.2	0.041 ± 0.019
18. Uncompahgre	16	1.3	13.2	0.030 ± 0.012
19. Truchas	15	1.3	13.2	0.037 ± 0.015
20. Mogollon	15	1.3	10.5	0.044 ± 0.020
21. Minnesota	6	1.2	13.2	0.039 ± 0.024
22. Maine	11	1.2	7.9	0.041 ± 0.018
23. Virginia-North Carolina	18	1.2	7.9	0.023 ± 0.012
Mean	14.6	1.23	12.94	0.0391

[a] Frequency of most common allele ≤ 0.95.

size was uncorrelated ($P > 0.05$) with either mean observed heterozygosity ($r = -0.1783$) or percentage of polymorphic loci ($r = 0.2129$), but was significantly correlated with mean number of alleles per locus ($r = 0.7952$; $P < 0.01$). The lack of dependence of either mean levels of H or percentage of polymorphic loci on sample size, when the number of loci surveyed is relatively large, agrees with both the theoretical predictions of Nei (1978) and the empirical results of Gorman and Renzi (1979).

GEOGRAPHIC TRENDS IN ALLELIC FREQUENCY

A pronounced pattern in the frequency of alleles occurred at two loci, Acon and 6-Pgd (Table 2, Fig. 2). Acon *b* was fixed in all populations of *V. s. cassinii*, *V. s. solitarius*, *V. s. alticola* and in the outgroup, *V. flavifrons*. In sharp contrast, seven samples of *V. s. plumbeus*, were fixed at Acon *a* and in two others (Inyo, Clover) was nearing fixation. At 6-Pgd, allele *a* occurred in *V. s. cassinii* at a frequency of 16.7–54.5%, with a mean of 37.71%. In *V. s. plumbeus*, the same allele occurred from 10.0–44.4% (mean 21.4%). The frequency of 6-Pgd *a* was much reduced in

V. s. solitarius, at 6.4%, and this allele was almost lost (2.8%) in the single population of *V. s. alticola*. A geographic difference also occurred at Lgg, where allele *d* was found in only one of 11 populations of *V. s. cassinii*, but turned up in seven of nine samples of *V. s. plumbeus* and was missing in *V. s. solitarius* and *V. s. alticola*. Eleven populations had unique (private) alleles. Although their distribution was probably random, an unusual concentration occurred at Ravalli (4) and Lake (3). Chimney Peak had two unique alleles. Unexpectedly, the number of private alleles was uncorrelated with either sample size ($r = 0.215$; $P > 0.05$) or with H_{obs}. ($r = -0.054$; $P > 0.05$). The outgroup, *V. flavifrons*, showed major allelic frequency differences from *V. solitarius* at Ada, Gpi and Np.

GENETIC DISTANCES

Matrices of 276 pairwise comparisons of Nei's (1978) genetic distances and Nei's (1983) modification of Cavalli-Sforza and Edward's (1967) arc distances are presented in Table 3. Mean intrataxon population differences in Nei's D were 0.0008 + 0.00013 (*V. s. cassinii*), 0.0008 + 0.00019 (*V. s. plumbeus*), and zero between the
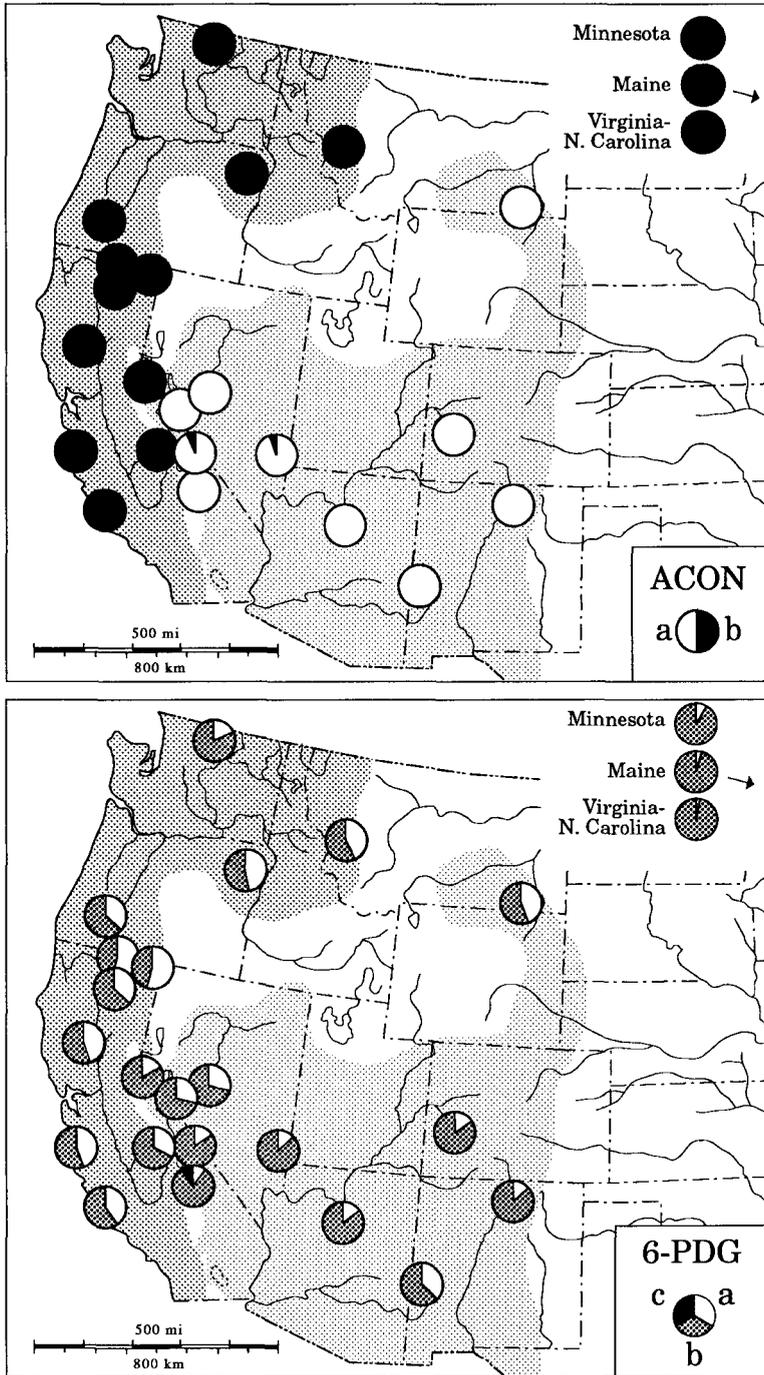
FIGURE 2.   Geographic and taxonomic occurrence and percentage frequency of alleles at Acon (upper) and 6-Pgd (lower) in the *Vireo solitarius* complex. The key to alleles is located in the lower right of each map. Note that at 6-Pgd a single private allele (*c*) occurred in the sample from Chimney Peak and that allele *b* is nearly fixed in the three samples from the eastern United States. Sample sizes are given in Table 1.

TABLE 2. Allelic frequencies at 18 variable loci in 23 populations of *Vireo solitarius* and one population of *V. flavifrons*.

Locus (EC no.)	Al-lele	1 Ravalli	2 Okanagan	3 Wallowa	4 Rogue River	5 Siskiyou-Warner	6 Shasta	7 Lake	8 Monterey	9 Pozo	10 Marklee-ville	11 Shaver Lake	12 Wassuk-Paradise
ACON	a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
(4.2.1.3)	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000
ADA	a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
(3.4.4.4)	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	c	0.929	0.909	1.000	0964	1.000	0.921	0.944	1.000	0.950	1.000	1.000	1.000
	d	0.048	0.091	0.000	0.000	0.000	0.079	0.056	0.000	0.050	0.000	0.000	0.000
	e	0.024	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	f	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
ADH	a	0.000	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
(1.1.1.1)	b	1.000	1.000	1.000	0.893	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	c	0.000	0.000	0.000	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
A-GPD	a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
(1.1.1.8)	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	c	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
EAP	a	1.000	1.000	1.000	1.000	1.000	1.000	0.972	1.000	1.000	1.000	1.000	0.929
(3.1.3.2)	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.071
	c	0.000	0.000	0.000	0.000	0.000	0.000	0.028	0.000	0.000	0.000	0.000	0.000
EST-4	a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
(3.1.1.1)	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GOT-1	a	0.000	0.000	0.000	0.000	0.000	0.000	0.028	0.000	0.000	0.000	0.000	0.000
(2.6.1.1)	b	1.000	1.000	1.000	1.000	1.000	1.000	0.972	1.000	1.000	1.000	1.000	1.000
GPI	a	0.000	0.000	0.094	0.000	0.045	0.000	0.000	0.000	0.000	0.167	0.000	0.000
(5.3.1.9)	b	1.000	1.000	0.906	0.964	0.955	0.974	1.000	1.000	1.000	0.833	1.000	1.000
	c	0.000	0.000	0.000	0.036	0.000	0.026	0.000	0.000	0.000	0.000	0.000	0.000
ICD-1	a	0.024	0.000	0.031	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000
(1.1.1.42)	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000
	c	0.976	0.955	0.969	1.000	1.000	1.000	1.000	1.000	0.950	1.000	0.975	1.000
	d	0.000	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LA-1	a	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
(3.4.11)	b	0.000	0.000	0.000	0.000	0.091	0.000	0.000	0.077	0.050	0.333	0.125	0.071
	c	0.095	0.045	0.188	0.107	0.227	0.079	0.028	0.000	0.000	0.000	0.075	0.000
	d	0.881	0.955	0.781	0.857	0.682	0.868	0.889	0.846	0.950	0.667	0.725	0.929
	e	0.000	0.000	0.000	0.000	0.000	0.000	0.028	0.000	0.000	0.000	0.000	0.000
	f	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	g	0.000	0.000	0.000	0.036	0.000	0.053	0.056	0.077	0.000	0.000	0.075	0.000
	h	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LA-2	a	0.000	0.000	0.156	0.000	0.136	0.211	0.167	0.000	0.050	0.000	0.000	0.000
(3.4.11)	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	c	1.000	1.000	0.844	1.000	0.864	0.789	0.833	1.000	0.950	1.000	0.975	1.000
	d	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000
LGG	a	0.000	0.000	0.000	0.000	0.000	0.000	0.028	0.000	0.000	0.000	0.000	0.000
(3.4.11)	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	c	0.786	0.727	0.563	0.607	0.727	0.632	0.583	0.654	0.600	0.833	0.750	0.929
	d	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.071
	e	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	f	0.214	0.273	0.438	0.393	0.273	0.368	0.389	0.346	0.400	0.167	0.225	0.000
MDH-1	a	0.976	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
(1.1.1.37)	b	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MPI	a	0.000	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
(5.3.1.8)	b	0.976	0.955	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	c	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NP	a	0.024	0.000	0.000	0.000	0.000	0.026	0.056	0.000	0.050	0.167	0.000	0.000
(2.4.2.1)	b	0.881	0.955	0.938	1.000	0.955	0.921	0.833	1.000	0.900	0.833	0.925	0.929
	c	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	d	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000

TABLE 2. Extended.

Locus (EC no.)	Al-lele	13 Chimney Peak	14 Inyo	15 Clover	16 Coconino	17 Powder River	18 Uncom-pahgre	19 Truchas	20 Mogollon	21 Min-nesota	22 Maine	23 Virginia-N. Carol.	*Vireo flavi-frons*
ACON	a	1.000	0.938	0.944	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000
	b	0.000	0.063	0.056	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000
ADA	a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.250
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.750
	c	0.900	1.000	1.000	0.975	0.861	0.906	0.967	1.000	0.833	1.000	1.000	0.000
	d	01.00	0.000	0.000	0.025	0.139	0.031	0.033	0.000	0.167	0.000	0.000	0.000
	e	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.000	0.000
	f	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.000	0.000
ADH	a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
A-GPD	a	0.000	0.000	0.019	0.025	0.000	0.031	0.033	0.033	0.000	0.045	0.000	0.000
	b	0.000	0.000	0.019	0.000	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000
	c	1.000	1.000	0.963	0.975	1.000	0.969	0.933	0.967	1.000	0.955	1.000	1.000
EAP	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
EST-4	a	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.033	0.000	0.045	0.000	0.000
	b	1.000	1.000	1.000	0.975	1.000	1.000	1.000	0.967	1.000	0.955	1.000	1.000
GOT-1	a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GPI	a	0.000	0.000	0.019	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	b	1.000	1.000	0.981	0.950	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.750
	c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.250
ICD-1	a	0.000	0.000	0.056	0.025	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	c	1.000	1.000	0.944	0.975	1.000	1.000	0.967	1.000	1.000	1.000	1.000	1.000
	d	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LA-1	a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	b	0.000	0.000	0.056	0.000	0.000	0.000	0.067	0.000	0.000	0.000	0.000	0.000
	c	0.000	0.031	0.019	0.000	0.056	0.000	0.000	0.000	0.000	0.182	0.083	0.000
	d	1.000	0.906	0.926	1.000	0.806	0.938	0.867	0.833	0.917	0.727	0.861	1.000
	e	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	f	0.000	0.000	0.000	0.000	0.000	0.063	0.000	0.000	0.000	0.000	0.000	0.000
	g	0.000	0.000	0.000	0.000	0.139	0.000	0.067	0.167	0.083	0.091	0.056	0.000
	h	0.000	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LA-2	a	0.000	0.000	0.056	0.100	0.000	0.000	0.000	0.033	0.000	0.000	0.028	0.000
	b	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	c	0.950	1.000	0.926	0.900	0.944	1.000	1.000	0.967	1.000	1.000	0.972	1.000
	d	0.050	0.000	0.000	0.000	0.056	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LGG	a	0.000	0.031	0.056	0.025	0.000	0.000	0.033	0.033	0.083	0.000	0.000	0.250
	b	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	c	0.750	0.844	0.759	0.825	0.833	0.813	0.833	0.833	0.583	0.773	0.694	0.750
	d	0.000	0.031	0.056	0.025	0.000	0.031	0.033	0.067	0.000	0.000	0.000	0.000
	e	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.083	0.000	0.000	0.000
	f	0.200	0.094	0.130	0.125	0.167	0.156	0.100	0.067	0.250	0.227	0.306	0.000
MDH-1	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MPI	a	0.000	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	b	1.000	0.938	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NP	a	0.200	0.031	0.037	0.025	0.000	0.063	0.067	0.033	0.083	0.045	0.056	0.000
	b	0.750	0.875	0.944	0.925	1.000	0.906	0.867	0.833	0.833	0.864	0.917	0.000
	c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	d	0.000	0.000	0.000	0.000	0.000	0.000	0.067	0.133	0.000	0.000	0.000	0.000

TABLE 2.   Continued.

Locus (EC no.)	Allele	1 Ravalli	2 Okanagan	3 Wallowa	4 Rogue River	5 Siskiyou-Warner	6 Shasta	7 Lake	8 Monterey	9 Pozo	10 Marklee-ville	11 Shaver Lake	12 Wassuk-Paradise
	e	0.048	0.045	0.063	0.000	0.045	0.053	0.111	0.000	0.050	0.000	0.050	0.071
	f	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6-PGD	a	0.429	0.182	0.469	0.357	0.545	0.368	0.444	0.462	0.400	0.167	0.325	0.286
(1.1.1.44)	b	0.571	0.818	0.531	0.643	0.455	0.632	0.556	0.538	0.600	0.833	0.675	0.714
	c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PGM-2	a	0.000	0.000	0.000	0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.025	0.000
(2.7.5.1)	b	1.000	1.000	0.969	1.000	1.000	0.947	1.000	1.000	0.950	1.000	0.975	1.000
	c	0.000	0.000	0.031	0.000	0.000	0.026	0.000	0.000	0.050	0.000	0.000	0.000
SOD-1	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
(1.15.1.1)	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

two populations of *V. s. solitarius*. Intertaxon differences were absent between *V. s. solitarius* and *V. s. alticola*, modest between *V. s. cassinii* and *V. s. solitarius* ($D = 0.0030 \pm 0.00046$) and between *V. s. cassinii* and *V. s. alticola* ($D = 0.0033 \pm 0.00071$), and pronounced in all three comparisons involving *V. s. plumbeus* ($D = 0.0294 \pm 0.00023$ vs. *V. s. cassinii*, 0.0283 ± 0.0056 vs. *V. s. solitarius*, and 0.0288 ± 0.00097 vs. *V. s. alticola*). Values for Nei's D in all four comparisons of *Vireo flavifrons* with different subspecies of *Vireo solitarius* were also high: $D = 0.0515 \pm 0.00137$ vs. *V. s. cassinii*, 0.0743 ± 0.00127 vs. *V. s. plumbeus*, 0.0430 ± 0.00424 vs. *V. s. solitarius*, and 0.0470 vs. *V. s. alticola*.

GENETIC POPULATION STRUCTURE AND GENE FLOW

Wright's F_{st} statistic for all 23 populations of *V. solitarius* was 0.289. Therefore, 29% of the genetic variation present was distributed among populations, indicating significant fragmentation. Calculation of F_{st} in population subsets yielded relatively low values within *V. s. cassinii* (0.055), *V. s. plumbeus* (0.050), and *V. s. solitarius* (0.033). When pairs of subspecies were combined, relatively low F_{st} values still occurred in *V. s. cassinii* + *V. s. solitarius* (0.070) and in *V. s. solitarius* + *V. s. alticola* (0.032). In sharp contrast, overall mean $F_{st} = 0.289$ when only *V. s. cassinii* and *V. s. plumbeus* were analyzed, pointing to the major source of the genetic fragmentation. As anticipated, Acon, which is nearly fixed at alternative alleles between *V. s. cassinii* and *V. s. plumbeus*, had the highest F_{st} (0.980) of any locus and thus contributed most significantly to the overall F_{st} value. The second highest F_{st} (0.119) was found at 6-Pgd.

With Wright's (1951) formula, Nm equalled 0.62 over all populations of *V. solitarius*. An identical estimate of gene flow, equivalent to approximately one migrant per two generations, was obtained for the subset *V. s. cassinii* + *V. s. plumbeus*, the two most divergent populations. All other estimates of Nm were substantially higher: 4.30 immigrants per generation within *V. s. cassinii*, 4.75 within *V. s. plumbeus*, and 7.33 within *V. s. solitarius*. Combining either *V. s. cassinii* with *V. s. solitarius*, or *V. s. solitarius* with *V. s. alticola*, yielded similar numbers of immigrants per generation, 3.32 and 7.56, respectively. Thus, according to Wright's method, gene flow is considerably restricted between the two phenotypically contrasting forms in the western United States. Under the assumption of both genetic and demographic equilibrium, Slatkin's (1985a, 1985b) rare allele method pointed to variable and much higher rates of gene flow in the Solitary Vireo, with Nm estimated at from two (Minnesota) to 35 (Clover), with a mean over all populations of 12 immigrants per generation (Table 4).

DENDROGRAMS AND MULTIDIMENSIONAL SCALING PLOTS

Both the UPGMA (Fig. 3) and Neighbor-joining (Fig. 4) trees show: (a) the outgroup, *V. flavifrons*, segregated at a large distance from all populations of *V. solitarius*; (b) all populations of *V. s. plumbeus* in a distinct major cluster; (c) all populations of *V. s. cassinii*, both populations of *V. s. solitarius* (except for Minnesota in the N-J tree), and the single population of *V. s. alticola* in a second major cluster; and (d) miniscule branch lengths separating individual populations within each major cluster. Although the very high co-

TABLE 2. Extended. Continued.

Locus (EC no.)	Al-lele	13 Chimney Peak	14 Inyo	15 Clover	16 Coconino	17 Powder River	18 Uncom-pahgre	19 Truchas	20 Mogollon	21 Min-nesota	22 Maine	23 Virginia-N. Carol.	Vireo flavi-frons
	e	0.050	0.094	0.019	0.050	0.000	0.031	0.000	0.000	0.083	0.091	0.028	0.000
	f	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
6-PGD	a	0.100	0.156	0.130	0.150	0.444	0.156	0.133	0.367	0.083	0.045	0.028	0.000
	b	0.800	0.844	0.870	0.850	0.556	0.844	0.867	0.633	0.917	0.955	0.972	1.000
	c	0.100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PGM-2	a	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	b	1.000	1.000	0.981	1.000	1.000	1.000	1.000	1.000	1.000	0.955	1.000	1.000
	c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.045	0.000	0.000
SOD-1	a	1.000	1.000	0.981	0.975	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	b	0.000	0.000	0.019	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

phenetic correlation coefficient of 0.98 indicated that the UPGMA tree faithfully represented the genetic distance matrix, such trees are most accurate at the branch tip level. In contrast, the multidimensional scaling plot (Fig. 5), best portrayed major clusters. In that plot note the great separation between, and the tight association of populations within, the two clusters representing *V. s. plumbeus* and *V. s. cassinii-solitarius-alticola,* respectively.
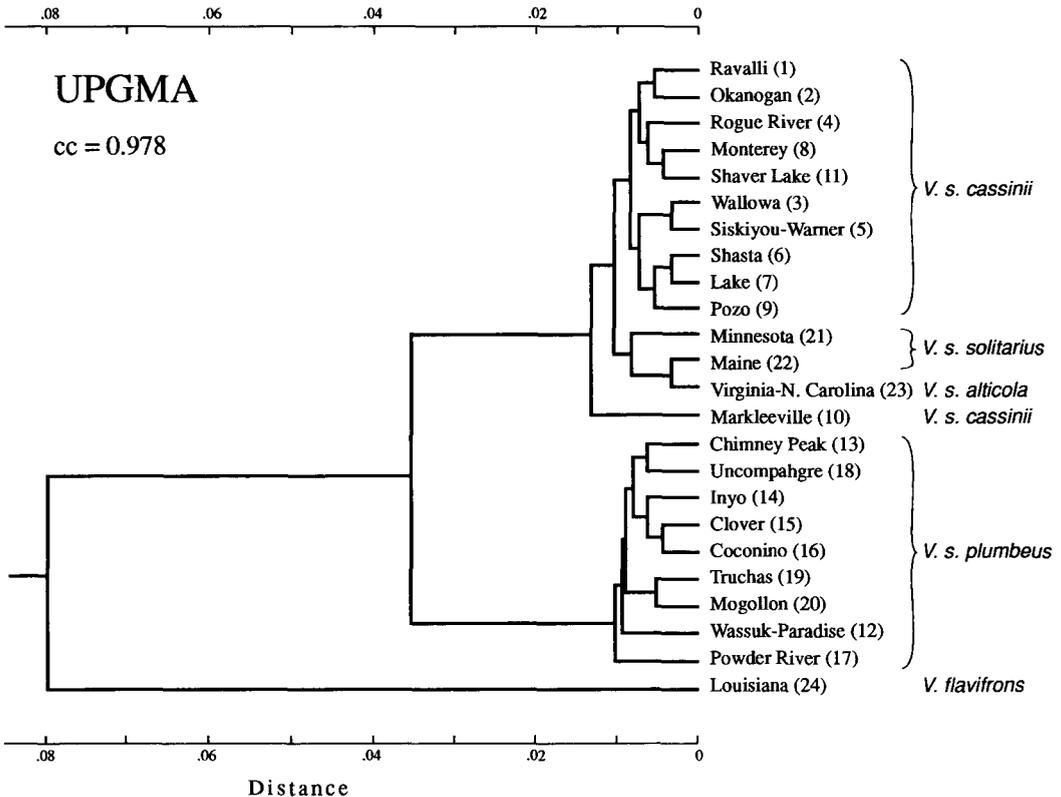
FIGURE 3. UPGMA dendrogram based on Nei (1983) modified arc distances of Cavalli-Sforza and Edward's (1967) among 23 population samples of the *Vireo solitarius* complex and one of *Vireo flavifrons.* The high cophenetic correlation coefficient (0.978) indicates excellent agreement between the original data matrix and the topology of the dendrogram.

TABLE 3. Matrix of pairwise genetic distances between 23 populations of *Vireo solitarius* and one population of *V. flavifrons*. Nei's (1978) unbiased *D* is given above the diagonal. Cavalli-Sforza and Edwards' (1967) arc distance (modified by Nei 1983) is given below the diagonal.

Sample location and number	1	2	3	4	5	6	7	8	9	10	11	12
1 Ravalli	—	0.001	0.001	0.000	0.001	0.001	0.001	0.000	0.000	0.001	0.000	0.028
2 Okanogan	0.005	—	0.003	0.000	0.004	0.001	0.002	0.002	0.000	0.000	0.001	0.028
3 Wallowa	0.008	0.010	—	0.009	0.000	0.000	0.000	0.000	0.000	0.003	0.002	0.033
4 Rogue River	0.006	0.007	0.009	—	0.001	0.000	0.001	0.000	0.000	0.001	0.000	0.031
5 Siskiyou-Warner	0.008	0.009	0.003	0.008	—	0.001	0.001	0.001	0.001	0.002	0.001	0.031
6 Shasta	0.007	0.007	0.005	0.008	0.006	—	0.000	0.000	0.000	0.002	0.000	0.031
7 Lake	0.008	0.008	0.007	0.010	0.008	0.003	—	0.000	0.000	0.003	0.001	0.032
8 Monterey	0.007	0.008	0.010	0.005	0.007	0.008	0.008	—	0.005	0.001	0.001	0.030
9 Pozo	0.006	0.007	0.007	0.009	0.007	0.008	0.005	0.005	—	0.001	0.000	0.030
10 Markleeville	0.014	0.006	0.016	0.015	0.012	0.005	0.016	0.009	0.010	—	0.000	0.027
11 Shaver Lake	0.007	0.013	0.010	0.007	0.006	0.017	0.010	0.004	0.008	0.010	—	0.028
12 Wassuk-Paradise	0.037	0.036	0.043	0.040	0.039	0.042	0.042	0.036	0.038	0.038	0.034	—
13 Chimney Peak	0.036	0.034	0.043	0.040	0.043	0.038	0.037	0.038	0.035	0.038	0.039	0.013
14 Inyo	0.027	0.024	0.032	0.029	0.031	0.030	0.030	0.029	0.029	0.032	0.027	0.007
15 Clover	0.029	0.029	0.030	0.031	0.029	0.030	0.030	0.029	0.027	0.028	0.028	0.009
16 Coconino	0.035	0.033	0.036	0.038	0.036	0.034	0.034	0.036	0.033	0.037	0.037	0.008
17 Powder River	0.033	0.032	0.038	0.033	0.036	0.033	0.035	0.031	0.035	0.042	0.032	0.012
18 Uncompahgre	0.033	0.032	0.039	0.036	0.040	0.036	0.036	0.035	0.033	0.037	0.035	0.008
19 Truchas	0.035	0.035	0.043	0.038	0.041	0.039	0.038	0.034	0.035	0.035	0.033	0.009
20 Mogollon	0.037	0.039	0.042	0.038	0.040	0.037	0.037	0.034	0.038	0.041	0.034	0.010
21 Minnesota	0.010	0.007	0.017	0.012	0.018	0.010	0.008	0.011	0.009	0.014	0.012	0.041
22 Maine	0.009	0.008	0.012	0.009	0.012	0.009	0.011	0.011	0.011	0.014	0.008	0.040
23 Virginia-N. Carolina	0.008	0.005	0.010	0.007	0.010	0.007	0.008	0.008	0.008	0.011	0.007	0.039
24 Louisiana	0.071	0.067	0.080	0.071	0.079	0.073	0.074	0.073	0.073	0.071	0.072	0.093

TABLE 3. Extended.

Sample location and number	13	14	15	16	17	18	19	20	21	22	23	24
1 Ravalli	0.030	0.026	0.027	0.030	0.028	0.029	0.030	0.028	0.002	0.004	0.004	0.049
2 Okanogan	0.028	0.024	0.025	0.028	0.029	0.027	0.028	0.030	0.000	0.001	0.000	0.046
3 Wallowa	0.034	0.030	0.030	0.033	0.031	0.033	0.034	0.032	0.005	0.006	0.006	0.057
4 Rogue River	0.031	0.027	0.027	0.031	0.029	0.030	0.031	0.030	0.002	0.003	0.002	0.053
5 Siskiyou-Warner	0.035	0.030	0.030	0.033	0.029	0.033	0.034	0.030	0.007	0.006	0.007	0.058
6 Shasta	0.031	0.028	0.028	0.030	0.030	0.031	0.032	0.030	0.002	0.004	0.003	0.051
7 Lake	0.031	0.029	0.029	0.032	0.028	0.032	0.033	0.030	0.003	0.005	0.005	0.051
8 Monterey	0.032	0.028	0.028	0.031	0.029	0.031	0.031	0.029	0.003	0.005	0.004	0.056
9 Pozo	0.029	0.027	0.027	0.030	0.029	0.030	0.031	0.030	0.001	0.004	0.003	0.051
10 Markleeville	0.027	0.023	0.024	0.027	0.028	0.027	0.026	0.028	0.000	0.000	0.000	0.044
11 Shaver Lake	0.030	0.025	0.026	0.030	0.029	0.029	0.029	0.028	0.002	0.001	0.002	0.051
12 Wassuk-Paradise	0.001	0.000	0.001	0.000	0.001	0.000	0.000	0.000	0.030	0.030	0.031	0.077
13 Chimney Peak	—	0.000	0.001	0.000	0.003	0.000	0.000	0.002	0.027	0.029	0.028	0.069
14 Inyo	0.009	—	0.000	0.000	0.003	0.000	0.000	0.001	0.025	0.025	0.025	0.071
15 Clover	0.011	0.006	—	0.000	0.003	0.000	0.000	0.002	0.025	0.025	0.025	0.073
16 Coconino	0.008	0.007	0.004	—	0.003	0.000	0.000	0.002	0.028	0.029	0.029	0.075
17 Powder River	0.010	0.011	0.013	0.011	—	0.002	0.003	0.000	0.031	0.032	0.033	0.081
18 Uncompahgre	0.006	0.006	0.007	0.005	0.009	—	0.000	0.001	0.027	0.028	0.028	0.073
19 Truchas	0.009	0.009	0.005	0.007	0.009	0.006	—	0.001	0.028	0.028	0.028	0.073
20 Mogollon	0.014	0.010	0.010	0.008	0.009	0.009	0.005	—	0.031	0.031	0.032	0.077
21 Minnesota	0.033	0.028	0.030	0.034	0.035	0.034	0.034	0.038	—	0.009	0.000	0.040
22 Maine	0.037	0.027	0.030	0.035	0.037	0.035	0.036	0.037	0.009	—	0.000	0.046
23 Virginia-N. Carolina	0.034	0.025	0.027	0.033	0.035	0.033	0.034	0.036	0.007	0.003	—	0.047
24 Louisiana	0.092	0.083	0.085	0.091	0.098	0.092	0.091	0.095	0.064	0.069	0.066	—

TABLE 4. Number and average frequency of private alleles and estimates of gene flow in populations of *Vireo solitarius*. See Table 1 for sample size of each population. Average sample size was 14.6. Only populations with private alleles were included in the analysis.

Sample location	No. private alleles	$p(1)$	Nm_c[a]
Ravalli	4	0.024	22.02
Okanogan	1	0.045	6.34
Rogue River	1	0.036	9.86
Lake	3	0.028	16.23
Shaver Lake	1	0.025	20.31
Wassuk-Paradise	1	0.071	2.57
Chimney Peak	2	0.075	2.31
Inyo	1	0.063	3.26
Clover	1	0.019	34.96
Uncompahgre	1	0.031	13.26
Minnesota	1	0.083	1.89
Mean	1.5	0.0455	12.092

[a] $Nm_c = Nm$ corrected for sample size.

Because of its proximity to the Wassuk-Paradise sample of *V. s. plumbeus* in western Nevada, placement of the small sample (3 birds) of *V. s. cassinii* from Markleeville was of unusual interest. In the UPGMA tree this sample fell somewhat outside the principal cluster of *V. s. cassinii*. In the MDS plot, however, Markleeville is clearly included within that taxon.

DISCUSSION AND CONCLUSIONS

GENETIC VARIATION, POPULATION STRUCTURE, AND GENE FLOW

The overall level of genetic variability in *V. solitarius* (*H*obs. = 3.9%), is lower than the value of 5.3% given in the most recent compilation by Barrowclough (1983). Moreover, the values for *V. s. alticola* (2.3%) and *V. s. plumbeus* (3.5%) are both significantly lower than that for *V. s. cassinii* (4.4%), possibly indicating past population bottlenecks (Nei et al. 1975). Excluding *V. s. alticola*, which was represented by only a single sample area, each of the three other taxa was remarkably uniform, reflecting an overall cohesiveness and essential lack of genetic population structure. Such intra-taxon uniformity stands in sharp contrast to the abrupt genetic discontinuity between *V. s. plumbeus* and the other forms.

Gene flow estimates for many populations of the *V. solitarius* complex are comparable to other recent figures (Zink and Remsen 1986; Rockwell and Barrowclough 1987; Johnson and Marten 1988, 1991), and are in keeping with Barrowclough and Johnson's (1988) conclusion that North American temperate zone birds are characterized by moderate to large effective population sizes and significant levels of gene flow. Nonetheless, several aspects of the gene flow estimates provided here are perplexing. First, the contrast between values provided by Wright's formula versus Slatkin's rare allele method remains to be explained. Second, the geographic occurrence of private alleles, whose frequency forms the basis for the rare allele method, appears to be stochastic. That such unique alleles are sensitive to weak selection (Slatkin 1985b) implies a vulnerability to random extinction with an unknown consequence to gene flow estimates. Third, values of *Nm* calculated by the rare allele approach vary widely, from 2–35. Although such variance in estimates could reflect real differences in gene flow among populations, several (e.g., Ravalli and Clover at 22 and 35, respectively) are probably unrealistically high. A likely explanation is that some populations of vireos are neither in demographic nor genetic equilibrium, an important assumption of the rare allele approach. Evidence for demographic non-equilibrium is seen clearly in *V. s. plumbeus*. In recent decades this form has vigorously expanded its nesting distribution westwardly (Johnson 1994), an incursion that continues. Another source of unusually high estimates of gene flow could be the use of data from allozymes. Mitton (1994), for example, summarized evidence from studies of organisms as diverse as horseshoe crabs, oysters, and coniferous trees in which DNA markers pointed to much greater population differentiation than did allozymes, suggesting significantly less gene flow than indicated by the latter method. Indeed, in some examples the allozyme results cited by Mitton (1994) pointed to substantial gene flow among certain populations where genetic contact would have been impossible. One pair of taxa analyzed here, *V. s. solitarius* and *V. s. cassinii*, may illustrate the phenomenon described by Mitton (1994). Although both forms are very similar allozymically, Murray et al. (1994, Table 3) reported tallies of mtDNA base substitutions equivalent to 4.7%, suggesting significant genetic subdivision between these taxa. This value is intermediate between levels of sequence difference derived from comparisons of two other species pairs, the (Black-whiskered Vireo [*V. altiloquus*] vs. Red-eyed Vireo [*V. olivaceus*], 4.0%) and (White-eyed Vireo [*V. gri-*
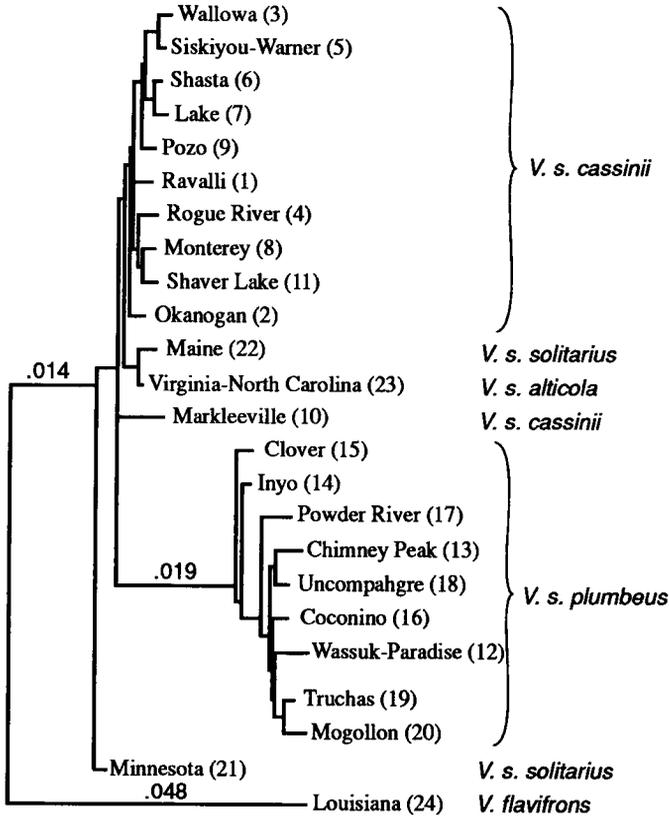
FIGURE 4. Neighbor-joining tree showing the relationship of 23 population samples of the *Vireo solitarius* complex and one of *Vireo flavifrons,* based on Nei (1983) modified arc distances of Cavalli-Sforza and Edwards (1967). Branch lengths are proportional to the distances between populations. Computed lengths of the three longest branches are provided.

seus] vs. Bell's Vireo [*V. belli*], 5.5%). In view of these uncertainties, I regard all such indirect estimates of gene flow as tentative and exploratory.

Acon *b,* an allele that is fixed in all samples of *V. s. solitarius, V. s. alticola,* and *V. s. cassinii,* turned up in only three of 144 (=2.1%) individuals of *V. s. plumbeus.* Importantly, these three specimens of *V. s. plumbeus,* two of which were homozygous for Acon *b,* were phenotypically indistinguishable from other examples of that taxon. The potential source of this allele in the latter taxon is of interest. The simplest and most likely explanation is that Acon *b* is ancestral in all members of the complex and has been virtually eliminated from *V. s. plumbeus.* Alternatively, this allele could be the result of intermittent gene flow from its essentially allopatric relative, *V. s. cassinii.* Not only is *V. s. cassinii* the form most geographically proximal to *V. s. plumbeus* during

the nesting season, but *V. s. cassinii* also occurs widely as a spring migrant in Arizona (Phillips et al. 1964), Utah (Behle 1943, Behle and Perry 1975), Nevada (Linsdale 1936; N. K. Johnson, ms), and southeastern California (Johnson et al. 1948), moving through woodland already occupied by *nesting V. s. plumbeus.* Because male *V. s. cassinii* typically sing during morning foraging bouts at stopover sites, the potential exists for copulation with available female *V. s. plumbeus.* The reverse is also possible—the copulation of migrant female *V. s. cassinii* with locally established singing male *V. s. plumbeus.* Regardless of the potential for interbreeding, gene flow, if it occurs from this source, must be rare.

POSSIBLE RARE SYMPATRY

Two records from the region where the nesting distributions of *V. s. cassinii* and *V. s. plumbeus*
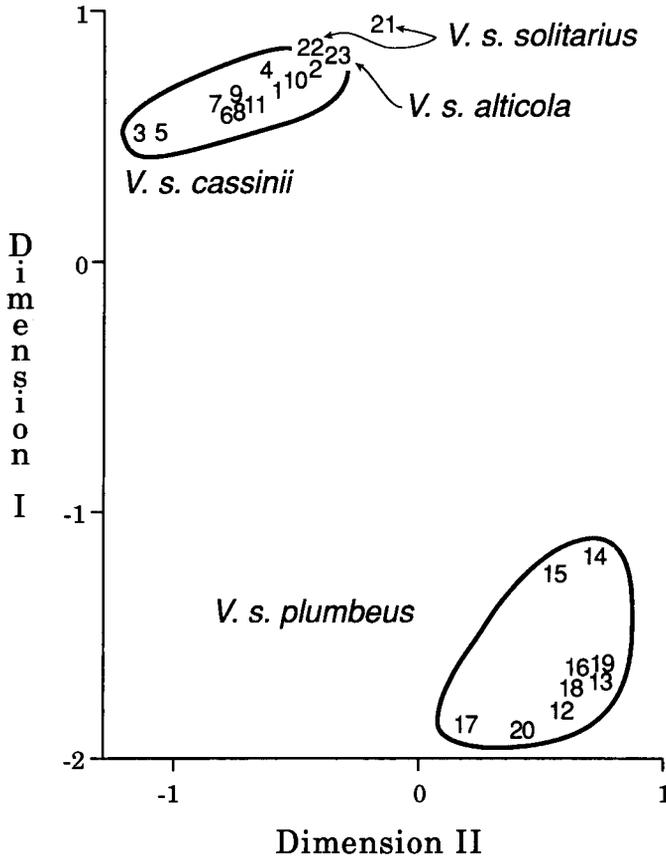
FIGURE 5. Multidimensional scaling plot based on Nei (1983) modified arc distances of Cavalli-Sforza and Edwards (1967). The coordinates for the position of *V. flavifrons* (Dimension I, 4.2667; Dimension II, 3.2127) place this sample to the upper right, far off the plot. Stress value = 0.042; R^2 = 0.996.

approach are pertinent to the issue of possible sympatry. Garrett and Dunn (1981) reported these taxa as "found together in mid-July at Big Rock Creek Canyon on the north slope of the San Gabriel Mts." [southern California], suggesting rare sympatry during the nesting season in that region. (I failed to find either form at that locality on June 19, 1985, indicating that possible sympatry there is intermittent.) The second record, one of near-sympatry, was obtained in Alpine County, California, where songs of a mated male *V. s. plumbeus* at a nest were tape-recorded at Indian Creek Reservoir Campground, 5,600 feet elevation, on June 7, 1988. The male could not be collected because campers were present. The female, although apparently also *V. s. plumbeus,* was seen too poorly for certain identification. Later that day, in a ravine approximately one-half mile above the East Fork of the Carson River, at two miles north and one-half

mile east of Markleeville, 5,600 feet elevation (and approximately two miles from Indian Creek Reservoir Campground), a singing male of *V. s. cassinii* was established on territory. The following day, songs of this male were tape-recorded and the bird was taken. No mate was evident. Later on June 8, 1988, a mated pair of *V. s. cassinii* was collected along the East Fork of the Carson River, at the mouth of the same ravine, at one and three fourth miles north and one mile east of Markleeville, 5,400 feet elevation. The male was tape-recorded prior to collection. On June 13, 1989, I returned to Indian Creek Reservoir Campground. Although no *V. s. plumbeus* were present, at 200 yards south of the site where they had nested in 1988 a singing male (songs tape-recorded) of *V. s. cassinii* was established on a territory. These records confirm the establishment of the two taxa at sites only two miles apart during the same nesting season.

TAXONOMIC AND EVOLUTIONARY STATUS OF POPULATIONS

This study focussed on the genetic relationships of *V. s. cassinii* and *V. s. plumbeus,* with limited comparative data from the eastern North American forms, *V. s. solitarius* and *V. s. alticola.* A mean genetic distance of 0.0294 between *V. s. cassinii* and *V. s. plumbeus* is comparable to the value 0.0440 distinguishing species of other birds and is much higher than the mean value of 0.0048 for subspecies (Barrowclough 1980). Furthermore, the cohesive population structure within each taxon, the lack of known intergrades where nesting populations closely approach, and the probability of at least limited sympatry further support the view that *V. s. cassinii* and *V. s. plumbeus* are independent evolutionary units. I conclude that *V. s. plumbeus* is a fully-differentiated species.

In contrast, *V. s. cassinii* and *V. s. solitarius* are similar allozymically and would not deserve species status based solely on their degree of protein divergence. Nonetheless, these taxa differ substantially in number of base pair substitutions in mtDNA (Murray et al. 1994) and show a degree of differentiation equivalent to that of at least two other definite species pairs of *Vireo* (see above). Furthermore, vocal differences are profound between *V. s. cassinii* and *V. s. solitarius,* leading Borror (1972) to state that "The songs of the eastern and western members of this species . . . are different enough to be from two different species." Although Barlow and Hardy (1981) and James (1981) corroborated this difference, the latter author concluded that such "does not indicate that eastern and western birds may be separate species." Moreover, James (1981) reported intermediate birds, apparently defined wholly on the basis of male song, in northern British Columbia. It is evident that populations in this supposed region of contact between *V. s. cassinii* and *V. s. solitarius* need intensive study before firm conclusions can be reached on the nature of their interaction and possible species status. Detailed data on voice and displays, supported by analysis of specimens of mated pairs, would be especially relevant.

ACKNOWLEDGMENTS

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