SPECIATION IN SAPSUCKERS (*SPHYRAPICUS*): I. GENETIC DIFFERENTIATION

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ABSTRACT.—We report the results of an electrophoretic analysis at 39 presumptive genetic loci of 88 specimens in the picid genus *Sphyrapicus*, here treated as comprising the following species: Williamson's Sapsucker (*S. thyroideus*), Yellow-bellied Sapsucker (*S. varius*), Red-naped Sapsucker (*S. nuchalis*), and Red-breasted Sapsucker (*S. ruber*). Seventeen loci (43.5%) were polymorphic. Values for observed heterozygosity in *S. ruber ruber*, *S. ruber daggetti*, and in three populations of *S. nuchalis* were uniform and averaged 0.043, the mean value reported for other birds. However, *S. varius*, at 0.022, and *S. thyroideus*, at 0.016, have low values of *H*. Values for percentage of polymorphic loci ranged from 12.8 to 20.5 in *S. ruber* and *S. nuchalis*, 12.8 in *S. varius*, and 7.7 in *S. thyroideus*. In contrast, all four species exhibited a similar mean number of alleles per locus (1.12–1.21). Genetic distances between populations of the same species are either very low or zero. Genetic distances are also very low between the phenotypically dissimilar *ruber* and *nuchalis* ($\bar{D} = 0.004$), two forms we consider to be biological species based on their assortative mating in sympatry. This is the lowest avian interspecific *D*-value ever reported and is similar to that found between avian subspecies. Phenotypically, *varius* and *nuchalis* are very similar, yet the average genetic distance between samples of these forms, 0.029, is comparable to interspecific values reported for other avian congeners and is our chief basis for treating the two forms as separate species. *S. thyroideus* is strongly differentiated genetically from *varius* ($\bar{D} = 0.142$), from *nuchalis* ($\bar{D} = 0.197$), and from *ruber* ($\bar{D} = 0.186$). F_{ST} statistics point to little or no population subdivision within *ruber* and *nuchalis* but substantial subdivision within the superspecies *varius* (*S. varius* + *S. nuchalis* + *S. ruber*). The genetic information suggests that the Williamson's Sapsucker represents the oldest lineage in the genus. *S. thyroideus* split from its sister clade (superspecies *S. varius*) between 3 and 4 MYBP. *S. ruber* and *S. nuchalis* are sister species that evolved very recently, either during or since the Pleistocene and sometime after their common ancestor diverged from *S. varius*. *Received 3 January 1983, accepted 4 May 1983.*

THE recent upswing in the number of avian electrophoretic studies has included few titles by authors (e.g. Martin and Selander 1975, Barrowclough 1980) concerned specifically with taxa known to hybridize, despite the potential of this approach for clarifying the genetic aspects of hybridization and the speciation process. In the present paper, we offer the results of an electrophoretic analysis of the major taxa of North American sapsuckers (genus *Sphyrapicus*), specialized woodpeckers that illustrate prominent patterns of geographic variation and hybridization. Many past publications (Howell 1952, A.O.U. 1957) have treated *S. varius*, *nuchalis*, and *ruber*, three basically allopatric forms, as geographic representatives of a single species, the Yellow-bellied Sapsucker (*S. varius*). We regard these forms as specifically distinct, however, and consider the genus to comprise the following four species: Williamson's Sapsucker (*S. thyroideus*), Yellow-bellied Sapsucker (*S. varius*), Red-naped Sapsucker (*S. nuchalis*), and Red-breasted Sapsucker (*S. ruber*). This treatment follows Mayr and Short (1970), Short and Morony (1970) and Short (1982). Our view that *nuchalis* is specifically distinct from *ruber* is based on a detailed field study (Johnson and Johnson in prep.) in south-central Oregon and northeastern California, where these two forms nest in local sympatry and mate assortatively, with infrequent hybridization. The opinion that *nuchalis* is a distinct species is supported here by analysis of allozymic variation.

The four species of sapsuckers are similar in size but fall into three categories of coloration (Fig. 1). *S. thyroideus*, in which the male is strongly melanic and the female barred, is the most divergent in plumage. *S. ruber* is basically sexually monomorphic, with the striking red of the head and upper breast superimposed over subtle whitish lateral head stripes and a faint dusky breast band resulting from melanic

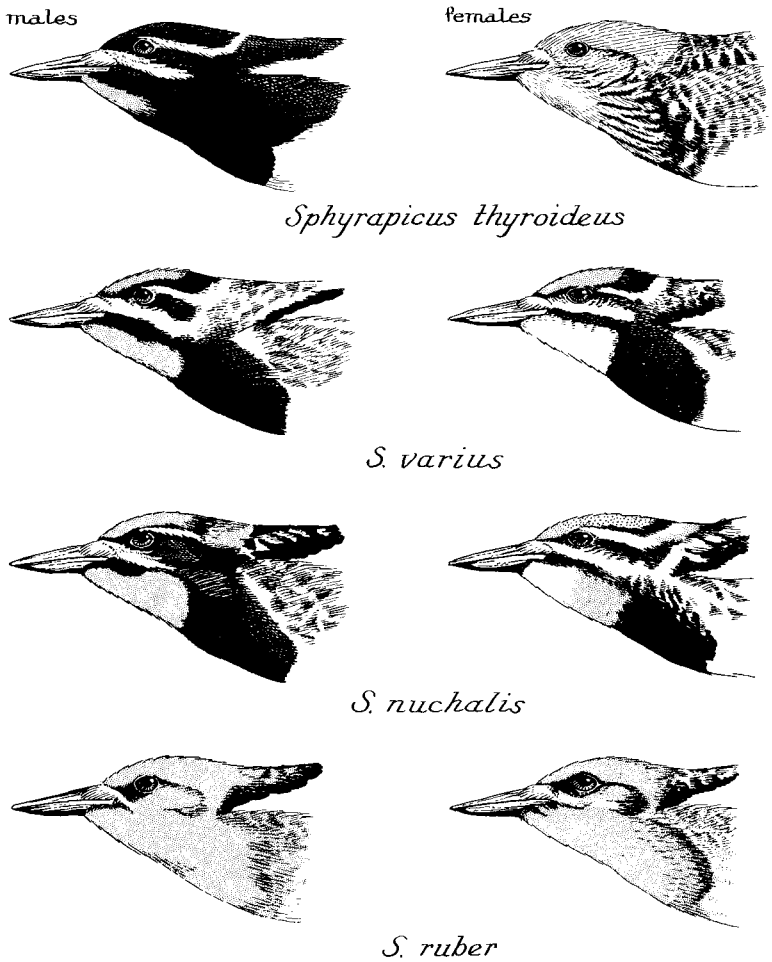

Fig. 1. Lateral aspect of head and breast plumage of males and females of four species of *Sphyrapicus*.

feather bases under the red tips. *S. varius* and *S. nuchalis* are extremely similar in ventral and lateral plumage, the only significant difference being in the females. In the former species the female has a white throat whereas in *nuchalis* the throat patch of the female is basally red with a varying amount of white near the base of the bill. Dorsally, the head patterning of the Yellow-bellied Sapsucker and Red-naped Sapsucker is different in both sexes. In *S. varius*, a well-defined white mark begins behind the eyes and encircles the nape. In *S. nuchalis*, a subtle white mark remains on the feather bases of the nape, but the tips of the feather barbs are red, resulting in a crimson nuchal patch. The dorsum of *S. varius* is also much whiter than that of *S. nuchalis*.

In addition to delimiting species' boundaries, the electrophoretic data also throw light on the phylogenetic relationships of sapsuckers, a topic discussed by both Howell (1952) and Short and Morony (1970), who had no access to quantitative genetic information bearing on phylogenetic estimation. Finally, values here reported for heterozygosity, percentage polymorphism, number of alleles per locus, and population structure (genetic distances and *F*-statistics) add to the growing body of genetic information on populations of birds.

Materials and Methods

This analysis is based on 88 specimens of *Sphyrapicus* (Table 1). A list of collecting localities can be

obtained from the authors. Data from two *S. thyroideus nataliae* from Montana were combined with those from *S. thyroideus thyroideus* ($n = 16$) from Oregon and California after initial analysis showed the results to be indistinguishable.

Samples of heart, liver, kidney, and pectoral muscle were removed from specimens 1–4 h after collection in the field and placed in liquid nitrogen before permanent storage at −76°C in Berkeley. Tissue extracts were prepared for electrophoresis as described by Selander et al. (1971) and Zink (1982). Thirty-nine presumptive genetic loci were scored in this study. Electrophoretic procedures (i.e. gel and buffer combinations) essentially follow Yang and Patton (1981). We have basically used the "one-pass" approach (Aquadro and Avise 1982), although many of the loci have been scored on more than one gel type. Precise conditions are available from the authors. The most common allele at a locus was designated M, and alleles with more anodal or cathodal migrations were coded as F or S, respectively; pluses and minuses were used to code further different allelic states.

The distribution of observed and expected numbers of heterozygotes, over all loci in a sample, was tested for departure from Hardy-Weinberg expectation (Hartl 1981) with a χ^2-test (see Barrowclough 1980). Individual heterozygosity was calculated by dividing the number of loci at which an individual was heterozygous by the total number of loci scored (39 for all birds). Two measures of average individual heterozygosity per population sample were calculated: first, the mean (±SD) of the observed individual heterozygosities (H_{obs}) and, second, the heterozygosity expected under conditions of Hardy-Weinberg equilibrium (i.e. $H_{exp} = 1 - \sum_{i=1}^{k} x_i^2$, where x_i is the frequency of the ith allele summed over k alleles at a locus). Individual genotypes were converted to allelic frequencies (Appendix) for each sample. Genetic distances between samples were calculated using the methods of Nei (1978) and Rogers (1972). Hierarchical F-statistics, which measure population subdivision in the absence of selection, were calculated according to Wright (1978). Branching diagrams, summarizing the pattern of genic similarity among samples, were constructed using the methods of Sneath and Sokal (1973; UPGMA and WPGMA phenograms), Farris [1972; distance Wagner networks, optimized according to Swofford (1981)], and Fitch and Margoliash (1967; "F-M" trees).

Results

Genetic Variation and Differentiation

Variation at loci and heterozygosity.—Of the 39 loci scored, 14 loci showed at least a single heterozygote. At three other loci, the species were fixed at alternative alleles. Thus, we regard 17 of the total loci as being variable within the clade (43.5%). Allelic frequencies at the polymorphic loci are listed by population or taxon in the Appendix. The 22 monomorphic loci (within and between populations) are: EAP, Hemoglobin, GPT, ICD-2, EST-4 ($4M_\mu$), ALD, SDH, ADA, ADH, LA-1, SOD-1, SOD-2, MDH-1, MDH-2, GOT-2, GLUD (= GDH), GDA, LDH-2 (muscle form), CK-1 (heart form), CK-3 (muscle), and two general proteins (stained with amido black). Acronyms for enzymes follow Harris and Hopkinson (1976).

The observed and expected heterozygosities, the percentage of polymorphic loci, and the mean number of alleles per locus are given for five taxa of *Sphyrapicus* in Table 1. Values for observed heterozygosity for *S. ruber ruber, S. ruber daggetti,* and three populations of *nuchalis* are all remarkably uniform and average 0.043, the mean value reported for birds in general (Barrowclough 1980). *S. varius,* at 0.022, and *thyroideus,* at 0.016, have low values of H. The values for the percentage of polymorphic loci range from 12.8 to 20.5 ($\bar{x} = 16.4$) in *ruber* and *nuchalis.* Again, *S. varius* is lower than this average, at 12.8%, and *thyroideus* has only one-half as many polymorphic loci, at 7.7%. In mean number of alleles per locus, however, *ruber* and *nuchalis* range from 1.13 to 1.21 ($\bar{x} = 1.17$), *varius* has a value of 1.13, and *thyroideus* is 1.18. All four species are therefore very similar in this feature.

The observed number of heterozygotes in each population did not differ ($P > 0.05$) from Hardy-Weinberg expectation: *S. r. ruber,* $\chi^2_7 = 1.91$); *S. r. daggetti,* $\chi^2_6 = 0.92$; *S. nuchalis,* Warner Mountains, $\chi^2_8 = 1.95$; *S. nuchalis,* Montana, $\chi^2_5 = 0.807$; *S. nuchalis,* Black Hills, $\chi^2_7 = 1.100$; *S. varius,* $\chi^2_3 = 1.05$, and *S. thyroideus,* $\chi^2_4 = 0.002$. Because of small sample sizes, these tests should be interpreted with caution. Suffice it to say that there is no evidence for significant departures from Hardy-Weinberg equilibrium.

A multiple regression analysis (BMDP6R; Frane 1977) showed that the number of animals per sample was not a significant predictor (two-tailed t-tests, $P > 0.05$) of estimates of genetic variation, such as average heterozygosity, number of alleles per locus, and percentage of polymorphic loci. For example, Gorman and Renzi (1979) and Nei (1978) showed both on empirical and theoretical bases, respectively, that mean levels of H are not as dependent on

TABLE 1. Genetic variability measures for eight samples representing five taxa of sapsuckers (*Sphyrapicus*).

Taxon	*n*	Sample area[a]	$H_{obs} \pm$ SE	$H_{exp} \pm$ SE	Percentage polymorphic loci[b]	Average number of alleles[c]
S. ruber ruber	13	Oregon	0.045 ± 0.021	0.046 ± 0.021	15.38	1.18
S. ruber daggetti	15	Oregon (3), California (12)	0.041 ± 0.008	0.044 ± 0.019	15.38	1.15
S. nuchalis × *S. daggetti* (hybrid)	1	California	0.077	0.077 ± 0.043	7.69	1.08
S. nuchalis	12	California (Warner)	0.045 ± 0.008	0.050 ± 0.020	20.51	1.21
S. nuchalis	7	Montana	0.047 ± 0.009	0.048 ± 0.022	12.82	1.13
S. nuchalis	15	South Dakota (Black Hills)	0.038 ± 0.006	0.045 ± 0.019	17.95	1.18
S. v. varius	7	Minnesota	0.022 ± 0.007	0.032 ± 0.015	12.82	1.13
S. thyroideus	18	Oregon (1), California (15), Montana (2)	0.016 ± 0.004	0.015 ± 0.013	7.69	1.18
Total	88					
Mean[d]			0.041	0.045	13.78	1.16

[a] Breeding specimens only (see Figs. 2 and 3). Exact localities available from authors.
[b] Frequency of most common allele ≤0.99.
[c] Per locus.
[d] Unweighted by sample size.

number of animals surveyed as on the number of loci. Our insignificant multiple R^2 (0.59, $P > 0.05$) of H on sample size corroborates the findings of Gorman and Renzi and of Nei. Thus, we compare estimates of genetic variation directly and without correction for differences in sample sizes.

Three patterns are evident from summarized information on polymorphism at specific loci (see Appendix): (a) loci that are polymorphic in one species but monomorphic in the other three species (*thyroideus*, ICD-1 and GPI; *ruber*, PGM-1; *nuchalis*, MPI and GOT-1; and *varius*, G-6-PDH and αGPD); (b) loci that are polymorphic in two species (*ruber* and *nuchalis*) and monomorphic in the other two species (LDH-1, NP, 6-PGD, and LAP); and (c) loci that are polymorphic in three species and monomorphic in the remaining species (*ruber*, *nuchalis*, and *varius* at LGG and LA-2; *nuchalis*, *varius*, and *thyroideus* at GR). No locus was found to be polymorphic in all four species, although this is probably dependent on sample size. The Williamson's Sapsucker is monomorphic at a series of loci showing substantial variation in two and sometimes three of its congeners. In contrast, *thyroideus* has six alleles at GPI, which is monomorphic in all three other species of sapsuckers.

Geographic trends in allelic frequency.—Several loci illustrate geographic trends in the frequency of alleles. Four of these patterns are mapped (Figs. 2 and 3). All represent two-allele systems.

At GR (= GSR; Fig. 2, upper), the M allele is widespread in all taxa. In *nuchalis*, *varius*, and *thyroideus*, but not in *ruber* (where M is apparently fixed), a fast allele is present at low frequencies, reaching 17% in the Black Hills of South Dakota. At 6-PGD (Fig. 2, lower), both *ruber* and *nuchalis* have an F allele at a frequency of 20–31%, but in *varius* and *thyroideus* only the M allele is found. At LGG (Fig. 3, upper), the S allele occurs at a level of 3–13% in both populations of *ruber* and in two of the three samples of *nuchalis*. The Montana sample of *nuchalis* has a fairly high frequency of the S allele (36%). In *varius*, however, the S allele increases to 71%, and *thyroideus* is fixed for the S allele. The most striking pattern is revealed at NP (Fig. 3, lower). Here, unique allelic proportions of M and F alleles are shown by *ruber* and by *nuchalis*. In contrast, *varius* and *thyroideus* are both fixed for the M allele.

Genetic distances.—Genetic distances between populations of the same species are either very low or zero (Table 2). For example, a D of 0.0 results when *S. r. ruber* and *S. r. daggetti* are compared. *S. nuchalis*, with no named subspecies, is represented in this study by three samples from the far western (Warner), central

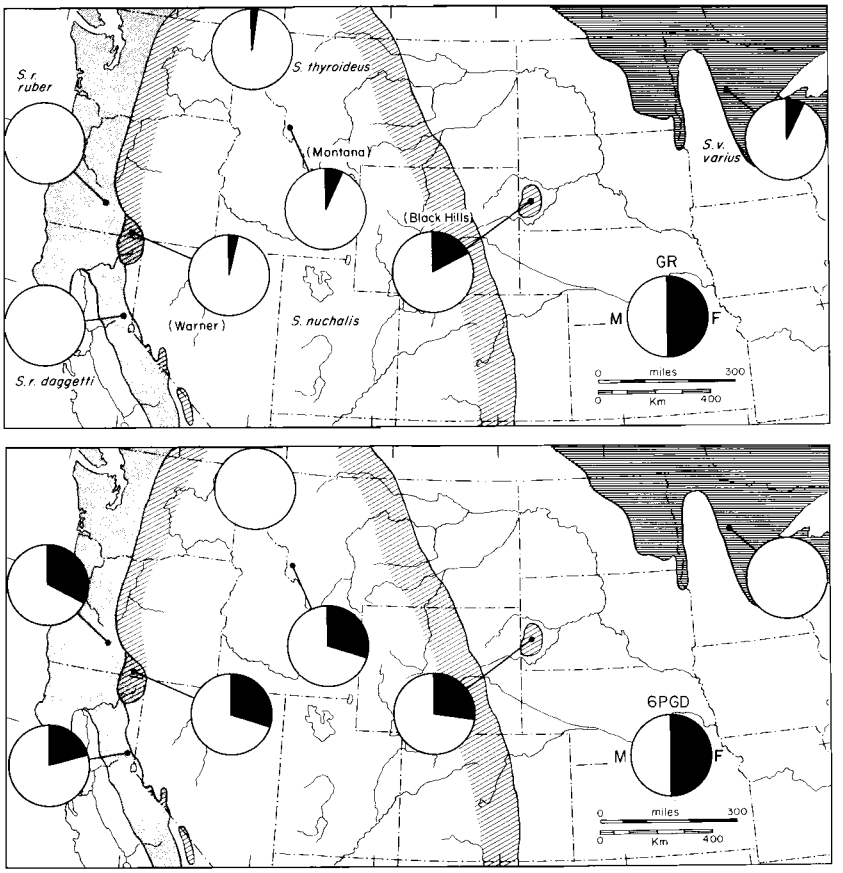

Fig. 2. Upper: proportions of M and F alleles at GR (=GSR) in samples of two subspecies of *Sphyrapicus ruber*, three populations of *S. nuchalis*, one population of *S. varius*, and one sample of S. *thyroideus*. The diagram for the latter sample is not pinpointed geographically. Although positioned in northern Idaho, specimens comprising this sample were taken mostly in California (Table 1). Lower: proportions of M and F alleles at 6-PGD in samples of sapsuckers.

(Montana), and far eastern (Black Hills) portions of the breeding range. Despite (1) the great distances separating these populations (from approximately 660 to 860 km), (2) the fact that the Warner population is sympatric and hybridizes at a low level with a large population of *S. ruber* (Johnson and Johnson in prep.), and (3) the fact that the Black Hills population of *S. nuchalis* is a disjunct isolate (Figs. 2 and 3), values of *D* for the three possible comparisons within the Red-naped Sapsucker are 0, 0.0, and 0.002 (Table 2).

Genetic distances are also low between *ruber* and *nuchalis*. Values of *D* for the six possible comparisons between samples of these two species range from 0.002 to 0.006 ($\bar{D} = 0.004$). This value of *D* is surprisingly low for biological species (e.g. Johnson and Johnson in prep.) and resembles the mean value of 0.0048 reported for avian subspecies (Barrowclough 1980: 661).

Unexpectedly, *S. nuchalis* is genetically more distant from *S. varius* (*D* range 0.026–0.032; $\bar{D} = 0.029$), the species it most closely resembles phenotypically, than is *ruber* (*D* range 0.018–0.020; $\bar{D} = 0.019$), a form of very different appearance (Fig. 1). These *D*-values are consistent with other species-level comparisons (Barrowclough 1980, Avise et al. 1980a) and form our principal basis for concluding that *nuchalis* is best treated as a distinct species. Although we do not consider levels of *D* to be a perfect taxo-

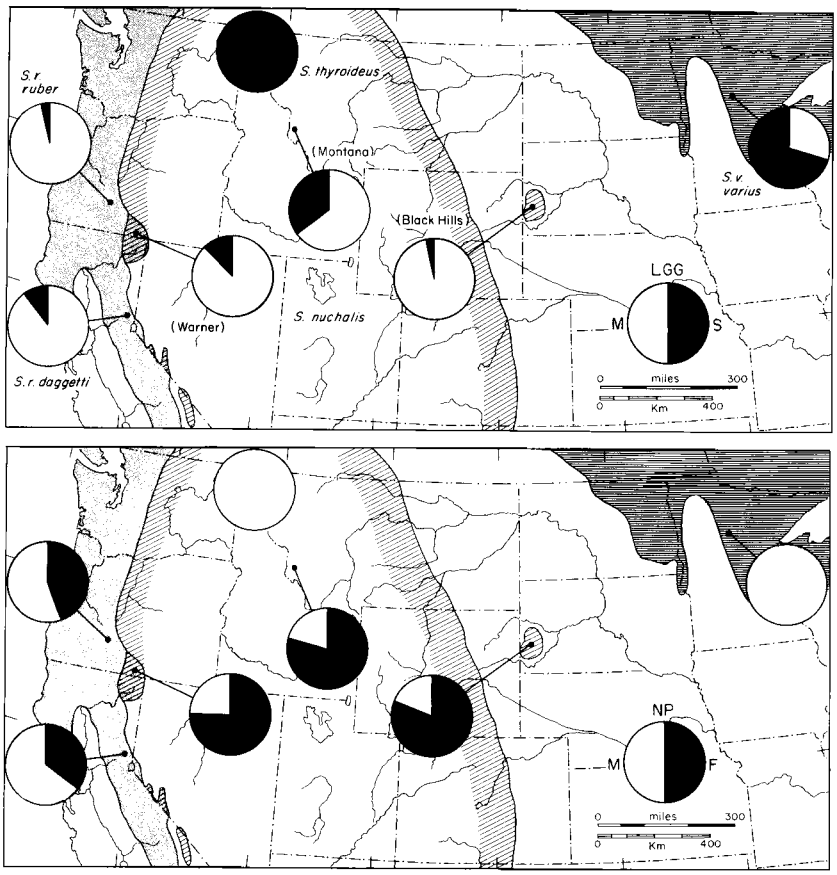

Fig. 3. Upper: proportions of M and S alleles at LGG in samples of *Sphyrapicus*. Lower: proportions of M and F alleles at NP. See legend to Fig. 2 for comments on sample of *S. thyroideus*.

nomic yardstick, clearly an inappropriate practice (see Barrowclough et al. 1981), we feel that the genic differences (summarized by the *D* statistic) between *varius* and *nuchalis*, compared to those between *nuchalis* and *ruber* (two clear species), warrant an hypothesis of species status for *nuchalis*.

The phenotypically distinct *thyroideus* is strongly differentiated genetically from *ruber* ($\bar{D} = 0.186$), *nuchalis* ($\bar{D} = 0.197$), and *varius* ($\bar{D} = 0.142$).

F-statistics and the analysis of genetic population structure.—As described by Barrowclough (1980: 657), "F_{ST} is a measure of the departure from panmixia among populations, reflecting local differentiation into subpopulations or demes." An F_{ST} "value of one indicates fixation of alternate alleles among populations, while a value of zero indicates an absence of subdivision" (Barrowclough 1980: 658). A detailed discussion of *F*-statistics can be found in Wright (1978). For the seven samples (including the hybrid individual) of the superspecies *S. varius*, the average F_{ST} across polymorphic loci was 0.053 ± 0.029 (SE). Variance in allelic frequency at LGG ($F_{ST} = 0.325$) contributed most to the overall F_{ST}, followed by NP ($F_{ST} = 0.200$), 6-PGD ($F_{ST} = 0.054$), GR ($F_{ST} = 0.019$), PGM-1 ($F_{ST} = 0.015$), LDH-1 ($F_{ST} = 0.013$), and LAP ($F_{ST} = 0.007$).

We also computed F_{ST} within *nuchalis* (three populations) and within *ruber* (two populations). As seen from the allelic frequency data in the Appendix, some of the loci, such as LDH-1, PGM, GSR, GOT-1, and LAP, are most variable within species. The remaining loci are variable between species. The average F_{ST} within *ruber* and within *nuchalis*, across the poly-

TABLE 2. Matrix of genetic distances between eight samples representing four species of sapsuckers (*Sphyrapicus*) and one interspecific hybrid (*S. nuchalis* × *ruber*). Nei's (1978) *D*-values are above the diagonal and Rogers' (1972) *D*-values are below the diagonal.

Taxon	1	2	3	4	5	6	7	8
1. *S. ruber ruber*	—	0.0	0.005	0.002	0.005	0.003	0.020	0.187
2. *S. ruber daggetti*	0.014	—	0.006	0.003	0.005	0.006	0.018	0.184
3. *S. nucahlis* × *S. r. daggetti* Hybrid	0.017	0.025	—	0.007	0.010	0.008	0.029	0.197
4. *S. nuchalis* (Warner)	0.020	0.020	0.028	—	0.0	0.0	0.029	0.197
5. *S. nuchalis* (Montana)	0.028	0.028	0.035	0.015	—	0.002	0.026	0.192
6. *S. nuchalis* (Black Hills)	0.020	0.029	0.026	0.015	0.020	—	0.032	0.201
7. *S. v. varius*	0.052	0.049	0.057	0.060	0.053	0.059	—	0.142
8. *S. thyroideus*	0.194	0.192	0.199	0.203	0.198	0.204	0.149	—

morphic loci, is 0.019, considerably less than the value reported above for the superspecies *varius*. Therefore, genetic differentiation within species is slight compared to variation among species.

Cladistic analysis.—It is useful to assess genetic relationships among taxa from a cladistic standpoint (e.g. Nelson and Platnick 1981) in which unique alleles (autapomorphies) and shared-derived alleles (synapomorphies) are tallied (Avise et al. 1980b). All taxa possess unique alleles. *S. thyroideus* is the most distinctive species, with autapomorphies at ICD-1 (F and F+), EST-1 (S), PHE-PRO peptidase (S), ACON (F), GPI (S, M+, F−, F, and F+), and G-6-PDH (S). *S. ruber* has unique alleles at LDH-1 (heart form) (F) and PGM-1 (S and F); *nuchalis* is autapomorphic at MPI (F), GOT-1 (S), and LDH-1 (S); and *varius* has a unique F allele at (αGPD) and a unique F allele at G-6-PDH. The unique F+ allele at 6-PGD occurs in the hybrid of *ruber* × *nuchalis*.

Although *thyroideus*, *varius*, and *nuchalis* share a distinctive F allele at GSR, the most impressive series of synapomorphies occurs in the superspecies *varius* (*varius* + *nuchalis* + *ruber*): ICD-1 (M allele), EST-1 (M), PHE-PRO (M), ACON (M), LGG (M), LA-2 (M), and G-6-PDH (M). In other words, these alleles serve to unite the superspecies *varius* as a monophyletic group, relative to *thyroideus*, which we presume to represent the ancestral condition relative to the other three species. (Undoubtedly, there are alleles that unite all four species as a monophyletic unit. We have not extended our survey to other closely related species of woodpeckers, however, in a search for such alleles.) The sister species *ruber* and *nuchalis* share distinctive alleles at NP (F), 6-PGD (F), and LAP (S). The distribution of autapomorphies and synapomorphies is concordant with a pattern of relationships depicted in both the dendrogram and phylogenetic tree (Fig. 4; discussed below).

Inheritance of electromorphs.—Specimens representing two complete family groups (1 mated pair of *nuchalis* and their 3 young; 1 interspecifically mated pair of *nuchalis* × *ruber* and their 2 hybrid young) and two partial family groups (adult female *ruber* and 1 young; adult male *ruber* and 1 hybrid young) were collected and analyzed electrophoretically. In each example, the parents and their offspring showed electromorph patterns at all polymorphic loci that were expected according to a system of Mendelian inheritance.

ANALYSIS OF RELATIONSHIPS

Phenograms and phylogenetic trees.—Despite the objections of Farris (1981), we feel that branching diagrams derived from distance matrices are useful in showing overall structure in the data. Based on the matrix of Rogers' *D*-values, the WPGMA and UPGMA clustering algorithms (Sneath and Sokal 1973) yielded virtually identical results. Therefore, only the UPGMA phenogram is shown (Fig. 4, upper). In both phenograms, *varius* splits from the main cluster containing *ruber* and *nuchalis* at approximately 0.055, and *ruber* divides from *nuchalis* at approximately 0.028. Within *ruber*, the forms *S. r. ruber* and *S. r. daggetti* separate at a distance of 0.015. The hybrid specimen joins the *ruber* cluster at 0.020. Within *nuchalis*, the three geographic samples divide at low *D*-values, roughly equivalent to the values where the subspecies of *ruber* separate, at 0.013–0.018. In the WPGMA phenogram, the branch leading to

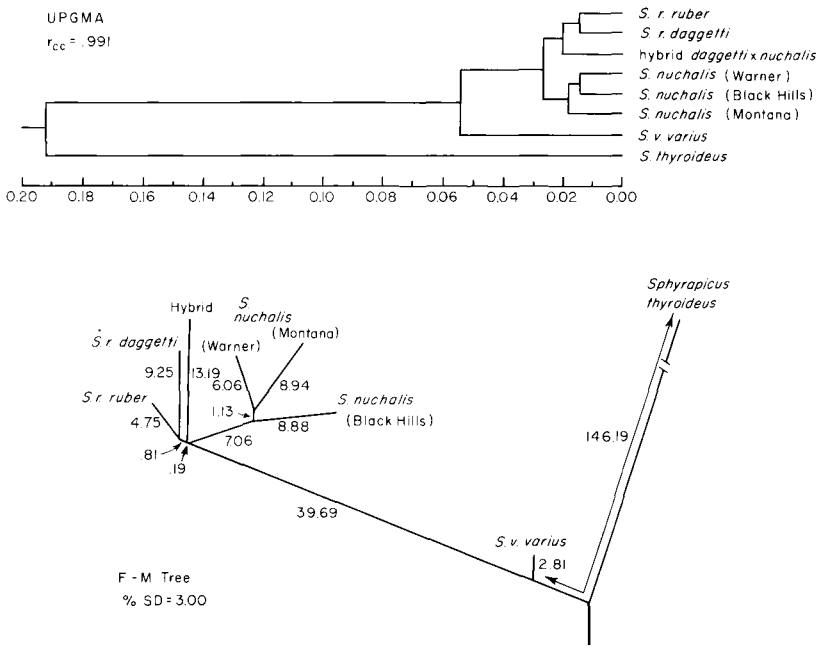

Fig. 4. Upper: phenogram based on Rogers' *D*-values (Table 2) derived by the UPGMA method. The cophenetic correlation coefficient (r_{cc}) evaluates the accuracy with which the phenogram summarizes the distance matrix (Sneath and Sokal 1973); a value of 1.00 indicates perfect agreement between the distances implied by the phenogram and the original distance matrix. Lower: branching diagram based on Rogers' *D*-values and constructed according to the method of Fitch and Margoliash (1967). The branch lengths are in units of Rogers' *D* (×1,000). The tree is "rooted" (see Farris 1972) at *S. thyroideus*.

thyroideus arises at a distance of 0.175 (Rogers' *D*), whereas in the UPGMA method this branch arises at a *D* of 0.192; this trivial difference is of a kind commonly noted when the same data set is analyzed by these two clustering algorithms (Sneath and Sokal 1973).

An assumption in phenetic clustering algorithms is constancy of rates of (allozymic) change. Thus, we used both the distance Wagner and the F-M methods to construct phylogenetic trees (rooted at *S. thyroideus*) that are not constrained by the homogeneity of rates assumption. In addition, we wished to determine whether or not a consistent branching pattern emerged when different procedures for constructing trees were employed. The F-M tree (Fig. 4, lower) suggests that *ruber, nuchalis,* and the hybrid form an unresolved trichotomy; the distances separating these three branches are too small to allow resolution of their correct relationships. As in the phenogram, the genic distinctness of *S. thyroideus* and *S. varius* is apparent. A distance Wagner tree (not shown) was also constructed. The branching pattern was similar to the phenogram and the F-M tree, although the branch lengths differed from those implied by the phenograms. The strong separation of *varius* from *ruber* and *nuchalis* is apparent, as is the close similarity of both forms of *ruber*, the three populations of *nuchalis*, and the hybrid. Within *ruber* and within *nuchalis*, population samples cluster together.

Thus, with regard to the relationships of *ruber-nuchalis, varius,* and *thyroideus*, the four methods used to construct branching diagrams produced very similar results. Therefore, the branching structure at this level is not simply a function of the method used to construct the tree (see Presch 1979). We hypothesize that *ruber* and *nuchalis* are sister taxa relative to *varius*.

Yang and Patton (1981) and Zink (1982) used calibrations of genetic distances to estimate the ages of various avian clades. For *ruber* and *nuchalis*, the genetic distances are so low that we do not make such an attempt. At any rate, the divergence of *ruber* and *nuchalis* occurred very recently, sometime after their common ancestor split from *varius* (or "pre-*varius*"), probably

during or since the Pleistocene. The values of D between *thyroideus* and the other three species (0.186–0.142), however, suggest that the separation of the *thyroideus* lineage occurred from about 3.0 to 3.7 MYBP, or in the late Pliocene. For this calculation, we use the calibration $t = 26.3 \times 10^6 D$, where t is time since divergence and D is Nei's (1978) genetic distance (Gutiérrez et al. 1983).

Discussion and Conclusions

Genetic variation and differentiation.—Species differences in H are evident in *Sphyrapicus*, with the value for *thyroideus* being one of the lowest observed for birds; only *Ardea herodias* ($H = 0.007$; Guttman et al. 1980) and *Olor buccinator* ($H = 0.01$; Barrett and Vyse 1982) exhibited lower H-values. Low values of H are expected if the total population of a species has passed through a bottleneck or exists at present at low densities (Nei et al. 1975). Although we cannot rule out such a past bottleneck, we note that at the present time the Williamson's Sapsucker is easily the least numerous species in its genus (NKJ pers. obs.).

Low observed heterozygosities are also reported for insular species (Selander 1976, Yang and Patton 1981). Two of the populations of *nuchalis* here surveyed are "insular" in that they are from continental mountaintops (Warner Mountains, Black Hills). Sapsuckers in the Black Hills are especially well-isolated during the breeding period from those in the main Rocky Mountains of Colorado, Wyoming, and Montana. In spite of isolation, the three populations of *nuchalis* exhibited very similar values of H, perhaps suggesting that neither are these isolated populations of low density nor have they passed through bottlenecks. *S. nuchalis* is strongly migratory (Howell 1952), however, and significant annual mixing of populations could occur during the nonbreeding period, possibly resulting in weak Ortstreue.

Corbin (1981) postulated that interactions between genetically differentiated subspecies could result in increased levels of H. Corbin's hypothesis would predict higher H-values in *S. ruber ruber*, because our sample is from a locality near the zone of intergradation with *S. r. daggetti*. Nonetheless, *S. r. ruber* has a typical level of heterozygosity. But, because our two samples of *ruber* are not genically differentiated, Corbin's hypothesis may not apply. At least our values of H do not indicate a zone of interaction between these populations.

We clearly lack a convincing explanation for the range of variation in H, both in sapsuckers and for birds in general. For the present, we favor the opinion of Barrowclough et al. (MS) that the maintenance of genic polymorphism (i.e. heterozygosity) *within* avian populations is not influenced by natural selection. We feel, therefore, that alternative alleles segregating at a locus are selectively neutral and that gene flow, genetic drift, and fluctuations in effective population size are the primary factors contributing to intrapopulational genetic variation. One of the population samples examined by Barrowclough et al. (MS, their Fig. 1) is from this study (Black Hills), and their analysis shows that the distribution of allelic frequencies within the Black Hills sample does not differ from expectations of the neutral model.

Values of genetic distance are notable from several standpoints: (1) the lack of genetic differentiation between the two subspecies of *ruber*, (2) the essential lack of genetic differentiation among the three widely spaced samples of *nuchalis*, (3) the close genetic identity of two biological species of very dissimilar appearance (*ruber* and *nuchalis*), and (4) the genetic divergence of two taxa of very similar phenotype (*nuchalis* and *varius*). The first two points, considered together with the F_{ST} values, indicate that both *ruber* and *nuchalis* are genetically uniform *inter se*. Regarding (3), we note that the plumage differences between *ruber* and *nuchalis* have a very superficial basis (Howell 1952, Johnson and Johnson in prep.). A simple reduction in the amount of red carotenoid on the barb tips of the head feathers of *ruber*, permitting the exposure and elaboration of the underlying white throat and neck stripes and the underlying black breast band typical of *nuchalis*, is sufficient to "transform" phenotypically one species into the other. Johnson and Brush (1972) have commented on several other species and morphs of birds in which striking plumage differences are achieved through very simple pigmentary control. The genetic information strongly suggests that *ruber* and *nuchalis* are recently diverged, sister species.

We emphasize another aspect of (3), the low genetic distance between *ruber* and *nuchalis*. Because a detailed field analysis revealed a situation of assortative mating in sympatry (Johnson and Johnson in prep.), these forms have

"proved" their biological species status. We therefore report the lowest avian interspecific genetic distance. Thus, low interspecific genetic distances *per se* do not suggest or prove conspecificity in the absence of information on mating preference. Furthermore, the trivial *D*-values suggest that speciation in the *ruber-nuchalis* example has not been accompanied by a "genetic revolution," as envisioned by Mayr (1970), at least among the structural genes we surveyed.

Concerning point (4), we note that there is no *a priori* reason to expect genic and external phenotypic patterns of variation to be congruent (see Barrowclough and Corbin 1978, Barrowclough et al. 1981, Zink 1982, Gutiérrez et al. 1983). Evolution can clearly proceed independently at these two levels. Therefore, the finding of genic divergence and phenotypic similarity (mosaic evolution) suggests that *varius* and *nuchalis* have simply retained the ancestral plumage condition, while evolution has proceeded at the genic level, probably in a more-or-less uniform, time-dependent manner.

Few studies have assessed protein variation in nonpasserines (Guttman et al. 1980, Barrowclough et al. 1981, Barrett and Vyse 1982, Gutiérrez et al. 1983), and none has been published previously for any member of the Piciformes. Our findings show that sapsuckers have levels of heterozygosity typical of other birds (Barrowclough and Corbin 1978) and that the genetic distances between *ruber* and *nuchalis* are low. The *D*-values for comparisons of *varius* vs. *ruber-nuchalis* are typical for congeneric avian species. The $\bar{D}$-value of 0.175 found between *thyroideus* and the other three species of sapsuckers, however, is higher than those generally found among avian congeners ($\bar{D}$ = 0.044; Barrowclough 1980). Genera can include genetically divergent species, however. For example, Zink (1982) found that the Rufous-collared Sparrow (*Zonotrichia capensis*) differs from its congeners at a $\bar{D}$ of 0.199.

Relationships among the taxa.—Based on the appearance of the adult and juvenile plumages, geographic distribution, and ecology, Short and Morony (1970) presented a hypothetical evolutionary history of sapsuckers in North America. They envisioned an ancestral *Sphyrapicus* stock as stemming from a melanerpine woodpecker line. Because *thyroideus*, in both adult and juvenile plumages, most closely resembles certain forms of *Melanerpes*, it is thought to be the oldest existing lineage of *Sphyrapicus*. Short and Morony postulated that a continuous ancestral sapsucker population gave rise to two isolates, one in western North America, which evolved into *thyroideus*, and one in eastern North America, which gave rise to the *varius* complex. A westward dispersal of *varius* occurred, effecting current distributions. Short and Morony state that, "the distinctive plumages of *thyroideus* and the occurrence of two hybrids between *thyroideus* and *nuchalis* suggest that *thyroideus* is very closely related to the *varius* complex and that interactions between them have affected the evolution of their distinctive plumages." Finally, they question whether or not as many as three species of sapsuckers can coexist in view of their possible interspecific competition and vulnerability to hybridization. (Although members of the superspecies *varius* are essentially allopatric, both *nuchalis*, and to a lesser extent *ruber*, occur sympatrically with *thyroideus* in the western United States and southwestern Canada).

The data on protein variation presented here form the basis for hypotheses of relationships and evolutionary history that may be usefully compared with the views of Short and Morony. First, although *thyroideus* has always been considered to be an unquestioned member of the genus *Sphyrapicus*, the genetic distance data indicate that it is not "very closely related to the *varius* complex," as Short and Morony suggest, although we presume a sister-group relationship between *thyroideus* and the superspecies *varius*. Indeed, *thyroideus* is as different from its congeners as are species of different genera in the Parulidae (Barrowclough 1980). Whether the distinctness of *thyroideus* is due to a relatively old divergence time or to differential rates of genic evolution is unknown. If our dating is reasonable, the split between *thyroideus* and "pre" *varius-ruber-nuchalis*, postulated by Short and Morony and our data (Fig. 4), occurred at least 3 MYBP. The ecological and/or geological factors responsible for this split are unknown.

Our genetic analyses confirm that *varius* is more similar to *nuchalis* and *ruber* than it is to *thyroideus*. Short and Morony (1970) concluded that "The Red-naped, Red-breasted and Yellow-bellied Sapsuckers diverged recently, and are barely specifically distinct." Our data, however, clearly show that *nuchalis* and *ruber* are more similar to each other than either is to *var-*

ius; these three taxa do not form an unresolved trichotomy, as Short and Morony's statement implies (see also Howell 1952). Biogeographically, the split of *varius* and *ruber-nuchalis* requires the westward dispersal of the *varius* complex, subsequent to the *thryoideus-*"pre-*varius*" divergence. The mechanism responsible for the divergence of *varius* and *ruber-nuchalis* is unknown. We note, however, that the genetic distance between *varius* and *ruber-nuchalis* exceeds that reported by Corbin et al. (1979) between the eastern and western components of the Northern Oriole (*Icterus galbula galbula* and *I. g. bullockii*). If genetic distance relates to age of divergence, then the sapsuckers must have speciated before the divergence of the oriole taxa. Clearly, more genic studies of avian taxa that are differentiated into such eastern and western forms are needed to ascertain if a single biogeographic event (e.g. isolation by the Great Plains, Mengel 1970) could explain common phylogenetic patterns.

Finally, despite Short and Morony's doubts of the possibility of sympatry of as many as three species of *Sphyrapicus*, coexistence does occur in extreme south-central Oregon and northeastern California. Here, presumably, the basically dissimilar environmental tolerances of these species narrowly overlap and permit coexistence. These populations of *ruber, nuchalis,* and *thyroideus* commonly nest in local sympatry, but always on interspecifically defended territories. Although infrequent hybridization between *ruber* and *nuchalis* occurs, there is no evidence of either a hybrid swarm or of the species losing their phenotypic "identity" (Howell 1952, Johnson and Johnson in prep.). Whether extensive regions of allopatry (Fig. 2) among sapsuckers result from either competitive interaction or from differences in preferred environments is unknown. If allopatric or parapatric speciation has been recent, perhaps the taxa have had insufficient time to establish broader areas of sympatry. But, because at least three species *can* coexist in substantial numbers, the allopatric distributions probably are a consequence of adaptation to differing environments rather than to interspecific competition.

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APPENDIX. Allelic frequencies for polymorphic loci in sapsuckers (*Sphyrapicus*). Relative mobilities of alleles denoted as fast (F), medium (M), and slow (S). Number of individuals shown in parentheses.

Locus (allele)	*S. thyroideus* ($n = 18$)	*s. v. varius* ($n = 7$)	*S. nuchalis* Warner Mts. ($n = 12$)	*S. nuchalis* Montana ($n = 7$)	*S. nuchalis* Black Hills ($n = 15$)	*S. varius* × *S. nuchalis* ($n = 1$)	*S. r. ruber* ($n = 13$)	*S. r. daggetti* ($n = 15$)
LDH-1								
F							0.038	0.067
M	1.00	1.00	0.917	1.00	1.00	1.00	0.962	0.933
S			0.083					
ICD-1								
F+	.028							
F	.972							
M		1.00	1.00	1.00	1.00	1.00	1.00	1.00
EST-1								
M		1.00	1.00	1.00	1.00	1.00	1.00	1.00
S	1.00							
PHE-PRO								
M		1.00	1.00	1.00	1.00	1.00	1.00	1.00
S	1.00							
ACON								
F	1.00							
M		1.00	1.00	1.00	1.00	1.00	1.00	1.00
GPI								
F+	0.028							
F	0.028							
F−	0.028							
M+	0.028							
M	0.693	1.00	1.00	1.00	1.00	1.00	1.00	1.00
S	0.195							
PGM-1								
F							0.038	
M	1.00	1.00	1.00	1.00	1.00	1.00	0.924	1.00
S							0.038	
GR								
F	0.028	0.071	0.042	0.071	0.167			
M	0.972	0.929	0.958	0.929	0.833	1.00	1.00	1.00
NP								
F			0.750	0.786	0.800	0.500	0.423	0.333
M	1.00	1.00	0.250	0.214	0.200	0.500	0.577	0.667
G-6-PDH								
F		0.071						
M		0.929	1.00	1.00	1.00	1.00	1.00	1.00
S	1.00							
αGPD								
F		0.071						
M	1.00	0.929	1.00	1.00	1.00	1.00	1.00	1.00
MPI								
F			0.042		0.033			
M	1.00	1.00	0.958	1.00	0.967	1.00	1.00	1.00
LGG								
M		0.286	0.875	0.643	0.967	1.00	0.962	0.900
S	1.00	0.714	0.125	0.357	0.033		0.038	0.100

APPENDIX. Continued.

Locus (allele)	*S. thyroideus* ($n = 18$)	*s. v. varius* ($n = 7$)	*S. nuchalis* Warner Mts. ($n = 12$)	*S. nuchalis* Montana ($n = 7$)	*S. nuchalis* Black Hills ($n = 15$)	*S. varius* × *S. nuchalis* ($n = 1$)	*S. r. ruber* ($n = 13$)	*S. r. daggetti* ($n = 15$)
LA-2								
M		0.214	0.583	0.714	0.467	0.500	0.500	0.633
S	1.00	0.786	0.417	0.286	0.533	0.500	0.500	0.367
6-PGD								
F+						0.500		
F			0.292	0.286	0.267		0.308	0.200
M	1.00	1.00	0.708	0.714	0.733	0.500	0.692	0.800
GOT-1								
M	1.00	1.00	1.00	1.00	0.967	1.00	1.00	1.00
S					0.033			
LAP								
M	1.00	1.00	0.958	1.00	1.00	1.00	1.00	0.933
S			0.042					0.067