EVOLUTIONARY GENETICS OF BIRDS I. RELATIONSHIPS AMONG NORTH AMERICAN THRUSHES AND ALLIES

JOHN C. AVISE, JOHN C. PATTON, AND CHARLES F. AQUADRO
Department of Zoology, University of Georgia, Athens, Georgia 30602 USA

ABSTRACT.—We have employed phenetic and cladistic approaches to analyze frequencies of electromorphs encoded by 25–27 loci in several species of North American thrushes (family Muscicapidae) and their relatives. In broad perspective both methods of data analysis yield similar summaries of probable relationships among these species. All four examined species of *Catharus* are nearly identical in electromorph composition. *Hylocichla mustelina* is phenetically and cladistically allied to members of *Catharus*. Both *Turdus migratorius* and *Sialia sialis* lie outside the *Hylocichla-Catharus* clade and are phenetically quite distinct from it and from each other. Despite its current placement in a distinct family, Mimidae, *Dumetella carolinensis* is cladistically and phenetically more closely allied to the Turdinae than is *Regulus calendula*, in some classifications a current member of Muscicapidae, subfamily Sylviinae. A major point of ambiguity in our data concerns the relative affinities of *Turdus migratorius* and *Sialia sialis* to the *Hylocichla-Catharus* clade. Phenetically, both are roughly equidistant to that clade, and cladistically our data can yield alternative interpretations.

The levels of genetic similarity between muscicapid species at various stages of taxonomic divergence are comparable to previous estimates for other Passeriformes but are far higher than typical estimates for other vertebrate and invertebrate taxa of equal rank. *Received 9 April 1979, accepted 20 August 1979.*

NOTWITHSTANDING the considerable work of Sibley and his colleagues on the egg-white proteins of more than 1,000 species of birds (Sibley 1970, Sibley and Ahlquist 1972), very little information exists on the genetic relationships of avian taxa. In a recent exhaustive review of the multi-locus protein electrophoretic literature, Nevo (1978, see also Powell 1975) listed studies conducted upon a total of 243 plant and animal species, yet only 10 species of birds had been examined by these techniques. This is surprising, as the utility of multilocus approaches in estimating genetic variability within and among avian species has recently been convincingly demonstrated by Smith and Zimmerman (1976), Barrowclough and Corbin (1978), and others.

In this study, we examine protein products of 25–27 loci in all seven species of thrushes that regularly inhabit eastern North America. These species are placed in Muscicapidae, subfamily Turdinae, in the recent classification of Aves by Morony et al. (1975). Also reported here are data on two additional related species that provide "outgroup" comparisons for a cladistic analysis: *Regulus calendula* (Muscicapidae, Sylviinae) and *Dumetella carolinensis* (Mimidae). With respect to the examined members of Turdinae, determination of the generic relationship of the Wood Thrush (*Hylocichla mustelina*) to members of *Catharus, Turdus,* and *Sialia* has proven to be a particularly refractory problem (Dilger 1956, Bourns 1967, Hendrickson and Yow 1973, Gibson et al. 1976). We will examine this and other issues of systematic import.

Perhaps of greater general significance is the fact that the data also yield estimates of mean levels of genetic differentiation between avian taxa at various stages of taxonomic and evolutionary divergence. These data can be compared to a large number of similar estimates previously obtained for other vertebrate and invertebrate classes (Avise 1974, 1976; Ayala 1975; Barrowclough and Corbin 1978).

TABLE 1. Electromorphs (and their frequencies) observed in North American thrushes and allies. All samples appeared monomorphic for the same electromorph at *LDH-2*, *MDH-1*, *MDH-2*, and *GOT-2*. See text for explanation of numerical designations.

Protein character	Species									Buffers[a]	Tissues
	Catharus ustulatus	*Catharus fuscescens*	*Catharus guttatus*	*Catharus minimus*	*Hylocichla mustelina*	*Turdus migratorius*	*Sialia sialis*	*Dumetella carolinensis*	*Regulus calendula*		
CK-1	100	100	100	100	100	100	95	97	80	Tris-citrate I	Heart
CK-2	100	100	100	100	100	100	100	105	80	Tris-citrate I	Heart
CK-3	100	100	100	100	100	100	100	100	200	Tris-citrate I	Muscle
EST-1	100	100	100 (0.96) 90 (0.04)	100	100	98	100	100	85 (0.85) 80 (0.15)	Tris-HCl	Liver
EST-2	A	A	A	A (0.50) B (0.50)	F	G	C (0.50) C′ (0.50)	E	D	Tris-HCl	Heart
EST-3	−100	−100	−100	−100	−100	−50	−75	−100 (0.94) −125 (0.06)	−100	Tris-HCl	Heart
GOT-1	100	100	100	100	100	100	100 (0.79) 50 (0.21)	100 (0.94) 110 (0.06)	100	Tris-citrate I	Muscle
GPD-1	100	100	100	100	100	—	125	110	—	Tris-citrate I	Muscle
GPD-2	−100	−100	−100 (0.96) 100 (0.04)	−100	−100	−100 (0.30) −200 (0.70)	−100	−20	−200	Tris-citrate I	Muscle
Hb	−100	−100	−100	−100	−100	−100	−100	−100	−250	Tris-HCl	Heart
IDH-1	100	100 (0.83) 200 (0.17)	100 (0.89) 150 (0.11)	100	100	100	100	120	50 (0.95) 100 (0.05)	Tris-citrate I	Liver
IDH-2	−100 (0.94) −130 (0.03) −120 (0.03)	−100 (0.90) −120 (0.10)	−100	−100	−100	−100	−100	−100	−120	Tris-citrate I	Heart
LDH-1	100	100	100	100	100	65	40	50	−75	Tris-citrate I	Heart
ME-1	100 (0.59) 150 (0.41)	100	100 (0.96) 175 (0.04)	100	100 (0.40) 80 (0.60)	95	100 (0.93) 90 (0.07)	89	85	Tris-citrate I	Heart
ME-2	−100 (0.97) −105 (0.03)	−100	−100	−100	−100	−100	−100	−100	−100	Tris-citrate I	Heart
NP	100 (0.78) 80 (0.18) 120 (0.04)	100 (0.80) 125 (0.20)	100 (0.08) 80 (0.84) 50 (0.08)	—	105 (0.50) 90 (0.50)	50 (0.75) 78 (0.25)	150 (0.50) 130 (0.50)	—	—	Tris-citrate I	Heart

TABLE 1. Continued.

Protein character	Species									Buffers[a]	Tissues
	Catharus ustulatus	*Catharus fuscescens*	*Catharus guttatus*	*Catharus minimus*	*Hylocichla mustelina*	*Turdus migratorius*	*Sialia sialis*	*Dumetella carolinensis*	*Regulus calendula*		
PEP-1	100	100	100 (0.84) 110 (0.12) 88 (0.04)	100	100	100	90	92 (0.94) 101 (0.06)	Gone	Tris-HCl	Heart
PEP-2	100	100	100	100	100	120	2-band	110	110	Tris-HCl	Heart
PEP-3	100 (0.97) 95 (0.03)	100	100	100	100	100	100	Blur	Blur	Tris-HCl	Heart
PGD	100	100	100 (0.88) 150 (0.04) 60 (0.08)	100	80	60	100	95 (0.88) 93 (0.06) 70 (0.06)	60 (0.75) 98 (0.20) 120 (0.05)	Tris-citrate I	Heart
PGI	100 (0.94) 200 (0.06)	100 (0.90) 250 (0.10)	100	100	100 (0.80) 200 (0.20)	100	100	100 (0.94) 200 (0.06)	98	Poulik	Liver
PGM	100 (0.97) 150 (0.03)	100 (0.80) 150 (0.10) 90 (0.10)	100	100 (0.75) 150 (0.25)	100	100	140 (0.86) 90 (0.14)	140	135 (0.95) 95 (0.05)	Tris-citrate I	Muscle
PT-1	100 (0.97) 110 (0.03)	100	100	100	100	100	100	100	100	Tris-HCl	Heart
PT-2	100	100	100	100	100 (0.80) 110 (0.20)	50	100	—	150	Tris-HCl	Heart

[a] Described in Selander et al. (1971).

METHODS

Most specimens were obtained from television "tower-kills" during the Fall 1978 migration, primarily at the Tall Timbers Research Station near Tallahassee, Florida. Freshly killed specimens (less than 8 h old) were frozen and returned to the laboratory for processing. A few additional specimens were collected in Athens and Atlanta, Georgia. The following species and numbers of individuals were examined: Veery (*Catharus fuscescens*), 5; Swainson's Thrush (*Catharus ustulatus*), 17; Gray-cheeked Thrush (*Catharus minimus*), 2; Hermit Thrush (*Catharus guttatus*), 13; Wood Thrush (*Hylocichla mustelina*), 5; American Robin (*Turdus migratorius*), 5; Eastern Bluebird (*Sialia sialis*), 7; Ruby-crowned Kinglet (*Regulus calendula*), 10; Gray Catbird (*Dumetella carolinensis*), 8. No significant protein denaturation was observed in the tower-kill specimens compared to specimens collected and frozen immediately.

Extracts from heart, pectoral muscle, and liver were subjected to horizontal starch-gel electrophoresis according to standard procedures described in detail by Selander et al. (1971) and Ayala et al. (1972). A total of about 300 buffer system × protein stain × tissue combinations were attempted in a series of test gels. Combinations yielding the highest quality and clarity of banding were employed to estimate electromorph frequences in all of the thrushes. These combinations are listed in Table 1. In all cases, electromorphs of questionable mobility were run side-by-side on gels prior to their final numerical designation, which was based roughly on mobility relative to the common allele at each locus in the Swainson's Thrush [arbitrarily designated "100" (anodal migration), or "−100" (cathodal migration)].

RESULTS

GENETIC VARIABILITY

Electromorph mobilities, buffer systems, and tissue specificities for each protein are presented in Table 1. Because so little information about zymogram patterns in birds has been published, the following additional information about individual protein systems is included.

Creatine kinase.—At least two loci encoding creatine kinase in birds have been reported, one predominantly expressed in brain and heart tissue, and the other in skeletal muscle (Eppenberger et al. 1964, 1967; Fisher and Whitt 1978). In our study, the locus encoding the muscle form of creatine kinase (*CK-3*) appeared monomorphic for allele *CK-3*(100) in all species except the Ruby-crowned Kinglet, which was monomorphic for *CK-3*(200).

Zymogram patterns from heart tissue were more complex. Two distinct and clear zones of banding activity representing heart creatine kinase appeared far towards the anode. Bands in these two zones usually vary in parallel in a large number of bird species that we have examined (unpublished data). For example, species exhibiting a rapidly migrating band in the first zone of activity usually exhibit a correspondingly "fast" band in the second zone as well. Furthermore, presumed heterozygotes in one zone (which show 3-band patterns consistent with the known dimeric structure of creatine kinase) also usually appear heterozygous in the second zone of activity. Dawson et al. (1967) observed that following freezing and thawing of heart tissue, the single zone of heart creatine kinase activity is converted to two zones, presumably representing two conformational states of the same enzyme. Our data also suggest, however, that the information content in the two zones of activity is not invariably redundant. In the Eastern Bluebird, for example, while the band in the second zone of activity remains identical in mobility with those of other thrushes, the band in the first zone of activity is much slower. An analogous situation occurs in the Gray Catbird. Whether or not the two zones of activity ultimately represent products of a single genetic locus, two sets of taxonomically useful characters (labeled *CK-1* and *CK-2*) have been scored and included in our data set. For purposes of calculating heterozygosities, creatine kinase from heart was counted as encoded by a single gene.

Esterases.—Products of a liver-specific esterase locus (*EST-1*) migrate rapidly toward the anode on tris-HCl gels. Products of a heart-predominant esterase gene (*EST-3*) migrate cathodally on the same gels. A third discreet zone of banding activity migrates more slowly in the anodal direction, occurs in both heart and liver tissue, and resolves best in heart extracts. This third zone of activity is usually characterized by a multiband zymogram phenotype, in many cases species-specific. We cannot be certain whether or not a given phenotype represents products of more than one gene, but in any event it provides a taxonomically useful character labeled *EST-2*. Because *EST-2* phenotypes consisted of two or more bands, each was labeled by letter (Table 1). A total of eight *EST-2* phenotypes was observed in the

thrushes and allies. The phenotypes were counted as products of a single locus for purposes of calculating genetic distances but were not included in heterozygosity estimates.

Glutamate oxalate transaminases.—Two GOT systems, presumably representing the anodally migrating soluble (*GOT-1*) and cathodally migrating mitochondrial (*GOT-2*) forms, band very sharply. Heterozygotes at *GOT-1* exhibited three bands.

α-Glycerophosphate dehydrogenases.—Products of *GPD-1* stain very lightly. Because *GPD-1* could not be scored reliably in two species (American Robin and Ruby-crowned Kinglet), it was not included in the cladistic analysis. Products of *GPD-2* stain more intensely. Heterozygotes exhibited a clear three-band pattern consistent with the known dimeric structure of GPD.

Hemoglobin.—Zymograms showed two bands, one anodal and one cathodal, but only the cathodally migrating band was consistently intense enough to permit reliable scoring. It was counted as a single locus for purposes of calculating distances and heterozygosities.

Isocitrate dehydrogenases.—Presumed heterozygotes at each of two loci (*IDH-1* and *IDH-2*) exhibited three-band zymogram phenotypes, indicating that the active protein products are dimers.

Lactate dehydrogenases.—Enzymes encoded by two loci (*LDH-1* and *LDH-2*) were consistently scorable. Individuals exhibited five-band phenotypes, representing the two intra-locus homotetramers and three inter-locus heterotetramers characteristic of most vertebrates.

Malate dehydrogenases.—Products of two loci (*MDH-1,* supernatant, and *MDH-2,* mitochondrial) banded with exceptional clarity. All species were monomorphic for electromorphs with identical mobility at each of the two loci. *MDH-1* is known to be very conservative throughout birds (Kitto and Wilson 1966, Karig and Wilson 1971).

Malic enzymes.—Several zones of activity, each exhibiting partial tissue specificity, were observed, but only two could be scored reliably. The *ME-1* system is highly polymorphic. The *ME-2* system also appears on gels stained for creatine kinase and 6-phosphogluconate dehydrogenase.

Nucleoside phosphorylase.—The locus encoding this enzyme (*NP*) was highly polymorphic in the thrushes, but did not resolve with sufficient clarity to be scored in all species. Hence it is not included in the cladistic analysis but is included in phenetic analyses and in heterozygosity estimates for those species in which scoring was possible. Heterozygotes appeared to exhibit four bands, consistent with the interpretation that the enzyme is a trimer.

Peptidases.—Three distinct zones of activity, presumably representing products of three loci (*PEP-1, PEP-2, PEP-3*), appeared on gels stained with substrate phenylalanyl-tyrosine. The clarity of banding was improved considerably by use of an agar overlay staining procedure described by Brewer and Sing (1970).

6-Phosphogluconate dehydrogenase.—This is one of the consistently most polymorphic of enzyme systems routinely assayed in birds (Cooper et al. 1969, Nottebohm and Selander 1972, Smith and Zimmerman 1976). In this study, we have distinguished a total of nine electromorphs. Because PGD is a dimeric molecule, heterozygotes show three bands.

Phosphoglucose isomerase.—The enzyme encoded by the *PGI* locus stains intensely on gels. Heterozygotes have a third band of intermediate mobility between the homodimeric allelic products.

Phosphoglucomutase.—Although two zones of activity typically appeared, only the less anodal, more intensely staining system (labelled *PGM*) could be consistently scored. PGM is monomeric in structure, and heterozygotes exhibit two bands.

Proteins.—Two general, nonenzymatic protein systems (*PT-1* and *PT-2*) were scored.

Our sample sizes were generally too small to permit meaningful comparisons of observed heterozygote proportions at individual loci to proportions expected under Hardy-Weinberg equilibrium for randomly mating populations. Nonetheless, we can provide rough estimates of mean heterozygosities (the proportion of individuals heterozygous per locus, averaged across all assayed loci) for each species. Single-locus heterozygosities were calculated in two ways (Table 2): first, as the ratio of the counted number of heterozygotes divided by the number of individuals; and second, as the same ratio, but with numerator equal to the number of heterozygotes expected for the allele frequencies in Table 1, assuming Hardy-Weinberg equilibria. Estimates obtained by both methods agree fairly well, when averaged across loci. Expected heterozygosities range from a low of 0.016 (*Catharus minimus*) to a high of 0.065 (*Hylocichla mustelina*), with mean $\bar{H}$ across species equal to 0.043. Overall levels

TABLE 2. Mean (±SE) percentage of individuals heterozygous per locus in some North American thrushes and allies.

Species	Heterozygosity	
	Counted	Expected
Catharus ustulatus	0.053 ± 0.026	0.052 ± 0.024
Catharus fuscescens	0.056 ± 0.025	0.052 ± 0.022
Catharus guttatus	0.047 ± 0.018	0.048 ± 0.018
Catharus minimus	0.021 ± 0.021	0.016 ± 0.016
Hylocichla mustelina	0.104 ± 0.053	0.065 ± 0.031
Turdus migratorius	0.042 ± 0.029	0.033 ± 0.023
Sialia sialis	0.063 ± 0.034	0.048 ± 0.025
Dumetella carolinensis	0.034 ± 0.015	0.030 ± 0.015
Regulus calendula	0.048 ± 0.027	0.040 ± 0.022
$\bar{H}$	0.052	0.043

of electrophoretically observed genetic variability in the thrushes and relatives ($\bar{H}$'s $\simeq$ 0.05) are typical of values previously reported in other birds (Corbin et al. 1974, Nottebohm and Selander 1972, Barrowclough and Corbin 1978), and for vertebrates in general (Selander and Kaufman 1973, Nevo 1978).

GENETIC DIFFERENTIATION

The electromorph frequencies of Table 1 were converted to estimates of overall genetic similarity ($\bar{I}$) and distance ($\bar{D}$) between species using Nei's (1972) formulas. $\bar{I}$ values can range from 0 (when populations share no electromorphs) to 1 (when electromorphs are present in identical frequencies). $\bar{D}$ values ($\bar{D} = -\ln \bar{I}$) can be interpreted as estimates of the mean number of electrophoretically detectable codon substitutions per locus accumulated since the time of separation of two populations or species. The resulting matrix of similarity and distance values between species is presented in Table 3. Observed $\bar{I}$ values ranged from a low of 0.266 (*Regulus calendula* versus *Sialia sialis*) to a high of 0.990 (*Catharus ustulatus* versus *C. fuscescens*). The similarity values in Table 3 were clustered into a dendrogram using the unweighted pair-group method of analysis with arithmetic means (UPGMA), discussed by Sneath and Sokal (1973) (Fig. 1). This is a *phenetic approach* to data analysis, as information from each taxonomic character (products of a locus in this case) is weighted equally in the overall evaluation of similarity.

Raw data (in the form of presence or absence of individual electromorphs in a sample) were also subjected to a *cladistic* analysis following the general conceptual approach of Hennig (1966). In constructing cladograms for the thrushes (an example of which is shown in Fig. 2), we have followed the methodological procedure outlined by Patton et al. (1980, see also Bonde 1977). The following concepts and terms are relevant. Characters ancestral (plesiomorphic) to a group of organisms under study are disregarded in establishing evolutionary geneology within the group, as they can be distributed to various group members in indeterminate fashion through evolutionary time (they may nonetheless be important sources of information that set a group apart from other more distant groups). One approach to determination of plesiomorphic characters is through use of "outgroup" comparisons. Electromorphs shared by taxa known by other criteria to lie outside the group itself, with one or more taxa within the group, are considered primitive. In this study we have employed Gray Catbirds and Ruby-crowned Kinglets as outgroup taxa with respect to the thrushes.

TABLE 3. Genetic distances (above diagonal) and similarities (below diagonal) between species of thrushes and allies, based upon electromorph frequencies at 25–27 loci and calculated according to Nei's (1972) formulas.

	Catharus ustulatus	*Catharus fuscescens*	*Catharus guttatus*	*Catharus minimus*	*Hylocichla mustelina*	*Turdus migratorius*	*Sialia sialis*	*Dumetella carolinensis*	*Regulus calendula*
Catharus ustulatus	—	0.010	0.026	0.028	0.114	0.436	0.377	0.738	1.171
Catharus fuscescens	0.990	—	0.028	0.025	0.124	0.458	0.366	0.742	1.170
Catharus guttatus	0.974	0.973	—	0.028	0.118	0.438	0.366	0.732	1.165
Catharus minimus	0.972	0.975	0.973	—	0.100	0.428	0.337	0.716	1.158
Hylocichla mustelina	0.892	0.884	0.889	0.905	—	0.430	0.452	0.734	1.163
Turdus migratorius	0.646	0.633	0.645	0.652	0.650	—	0.622	0.881	1.106
Sialia sialis	0.686	0.693	0.694	0.714	0.636	0.539	—	0.741	1.325
Dumetella carolinensis	0.478	0.476	0.481	0.489	0.480	0.414	0.477	—	0.957
Regulus calendula	0.310	0.310	0.312	0.314	0.312	0.331	0.266	0.384	—

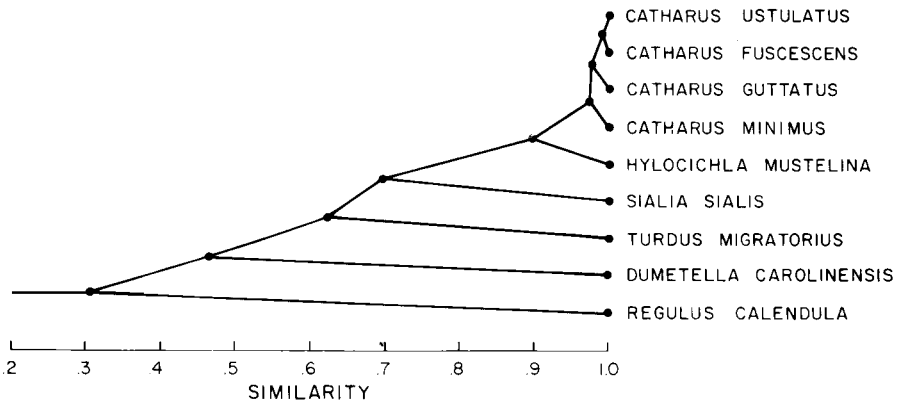

Fig. 1. Phenogram of North American thrushes and allies based on unweighted pair-group clustering using arithmetic means of similarity coefficients derived from electromorph frequencies at 25–27 loci.

Similarly, electromorphs unique (autapomorphic) to a taxon, or to a clade for which plesiomorphs are unknown, do not permit designation of cladistic affinity. Only shared-derived (synapomorphic) electromorphs, for which ancestral states can be inferred, establish branching points of phylogenetic trees. Designation according to these criteria of the 101 electromorphs observed in this study, and their placement along the cladogram of Fig. 2, are presented in Table 4. As discussed later, very few character states provide useful information in a cladistic framework, despite the large number assayed, and in at least one important instance the useful data yield alternative interpretations.

We will discuss the results of phenetic and cladistic analyses of protein differentiation in these North American thrushes and relatives. Current generic and species names employed are those listed by Morony et al. (1975).

Genus Catharus.—The four examined species of this genus, *fuscescens, minimus, ustulatus,* and *guttatus,* are remarkably similar genetically; all comparisons yield similarity values above 0.97. In no case have we observed fixed allelic differences. At the phosphoglucomutase locus, *PGM* (150) was observed as a rare electromorph

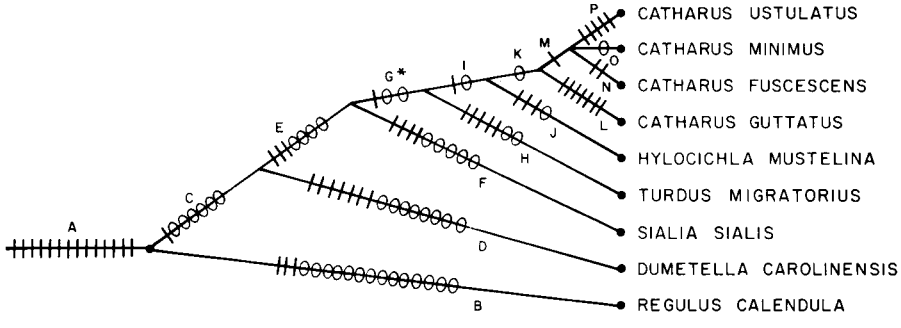

Fig. 2. One possible cladogram for North American thrushes and allies. Other cladograms consistent with the data, which involve changes in "G" in the figure, are described in Results. Circles denote electromorphs whose presumed ancestral states remain undetermined, and lines crossing branches denote derived electromorphs, some of which aid in defining various clades. See Table 4 for a description of electromorphs along all branches of the tree.

TABLE 4. Electromorphs observed in North American thrushes and allies. Letters refer to the stem and branches of Fig. 2. Line **A** lists electromorphs considered plesiomorphic (ancestral) for all taxa examined. Successive lines list apomorphic (derived) electromorphs, some of which aid in defining the respective clades in Fig. 2. Electromorphs in brackets are those considered autapomorphic (unique) to a single observed taxon or clade, but whose presumed ancestral electromorphs remain undetermined.

A) *PGD*(60); *PEP-2*(110); *IDH-1*(100); *IDH-2*(−120); *ME-2*(−100); *EST-3*(−100); *PT-1*(100); *GOT-1*(100); *LDH-2*(−100); *MDH-1*(100); *MDH-2*(−100); *GOT-2*(−100); *PEP-3*(blur); *GPD-2*(−200).

B) *IDH-1*(50); *PGD*(98,120); [*PT-2*(150); *PEP-1*(blank); *ME-1*(85); *CK-1*(80); *PGM-2*(95,135); *EST-1*(80,85); *PGI*(98); *EST-2*(D); *CK-2*(80); *LDH-1*(−75); *Hb*(−250); *CK-3*(200)].

C) *IDH-2*(−100); [*PGM*(140); *EST-1*(100); *PGI*(200,100); *Hb*(−100); *CK-3*(100)].

D) *PGD*(70,93,95); *IDH-1*(120); *GPD-2*(−20); *EST-3*(−125); *GOT-1*(110); [*PT-2*(blank); *ME-1*(89); *CK-1*(97); *EST-2*(E); *LDH-1*(50); *CK-2*(105); *PEP-1*(92,101)].

E) *GPD*(−100); *PEP-3*(100); *PGM*(90); [*ME-1*(100); *PT-2*(100); *PGD*(100)[a]; *CK-2*(100)].

F) *EST-3*(−75); *GOT-1*(50); *PEP-2*(2 band); *ME-1*(90); [*PEP-1*(90); *CK-1*(95); *EST-2*(C,C′); *LDH-1*(40)].

G) *PGM*(100); [*PEP-1*(100); *CK-1*(100)].

H) *PEP-2*(120); *EST-3*(−50); *EST-1*(98); *ME-1*(95); *PT-2*(50); [*LDH-1*(65); *EST-2*(G)].

I) *PEP-2*(100); [*LDH-1*(100)].

J) *PT-2*(110); *PGD*(80); *ME-1*(80); [*EST-2*(F)].

K) [*EST-2*(A)].

L) *ME-1*(175); *GPD-2*(100); *IDH-1*(150); *EST-1*(90); *PGD*(150); *PEP-1* (88,110).

M) *PGM*(150).

N) *IDH-1*(200); *PGI*(250).

O) [*EST-2*(B)].

P) *PEP-3*(95); *PT-1*(110); *ME-2*(−105); *IDH-2*(−130); *ME-1*(150).

[a] See text.

in *ustulatus, minimus,* and *fuscescens*. Because this is an apparently derived electromorph, it defines a clade distinct from *guttatus*. This conclusion is very weak, however, as *PGM* (150) may have been lost in *guttatus* or may still be present in low frequency. A number of autapomorphic electromorphs were also observed, particularly in the somewhat larger samples of *ustulatus* and *guttatus* (Table 4, Fig. 2). There is no doubt that all four species are genetically very close.

Hylocichla mustelina.—Except at *PGD, EST-2,* and *NP,* the Wood Thrush appears to share common alleles with the four *Catharus* species; mean genetic similarity with these taxa equals 0.89. This yields genetic distance estimates roughly 3–4 times as great as those observed among *Catharus* species. Nonetheless, the Wood Thrush appears phenetically far closer to *Catharus* than it does to *Turdus migratorius* ($\bar{I}$ = 0.65) or to *Sialia sialis* ($\bar{I}$ = 0.64). Furthermore, there are no observed synapomorphs that define the *Catharus* assemblage as belonging to a clade distinct from *H. mustelina* [*EST-2*(A) is unique to *Catharus,* but the presumed plesiomorph at that locus cannot be defined from our data].

Turdus migratorius and Sialia sialis.—The American Robin and Eastern Bluebird are genetically quite distinct from the *Catharus* and *Hylocichla* species examined. Much of this distinctness appears phenetic, however, and is attributable to the rather large number of electromorphs unique to each of these taxa. There is only a single synapomorphic character state [*PEP-2*(100)] that defines a *Hylocichla-Catharus* clade distinct from *Turdus* and *Sialia* [*LDH-1*(100) subsequently confirms this distinction, although by itself, it cannot define the clade, because the plesiomorph remains unknown]. The overall mean genetic similarities of *T. migratorius* and *S. sialis* with the *Hylocichla-Catharus* taxa examined are $\bar{I}$ = 0.64 and $\bar{I}$ = 0.68, respectively. Similarity between *T. migratorius* and *S. sialis* is $\bar{I}$ = 0.54.

The cladistic placement of *Turdus* and *Sialia* with respect to *Hylocichla-Catharus* cannot be unambiguously determined from our data. In the interpretation presented

in Fig. 2, *Turdus* is shown as belonging with the *Hylocichla-Catharus* clade by the shared possession with it of the synapomorph *PGM*(100). This interpretation is subsequently supported by allelic distributions at *PEP-1* and *CK-1*. On the other hand, it is just as possible that a *Sialia-Hylocichla-Catharus* clade could have been drawn by the shared possession by these taxa of a synapomorph *PGD*(100). In this case supportive evidence would come from electromorphs at *ME-1* and *PT-2*. If the former interpretation were correct, the American Robin would have had to lose PGD(100) [and *ME-1*(100) and *PT-2*(100)] after its incorporation into the clade, and if the latter interpretation were correct, the Eastern Bluebird would have had to lose *PGM*(100) [and *PEP-1*(100) and *CK-1*(100)] after its incorporation into that clade. A third and equally plausible cladogram would have pictured *Sialia* and *Turdus* branching from a common point whose ancestral lineage possessed the relevant alleles in polymorphic condition, subsequently distributed to various taxa in a cladistically uninformative manner. With our data we cannot decide among these alternative possibilities.

Dumetella carolinensis and Regulus calendula.—The Gray Catbird and Ruby-crowned Kinglet are very distinct phenetically from all examined members of the subfamily Turdinae; mean similarity values are $\bar{I} = 0.47$ and $\bar{I} = 0.31$, respectively. They are also very distinct from one another, $\bar{I} = 0.38$. Two loci (*GPD* and *PEP-3*) are useful in defining members of Turdinae as a clade distinct from *Dumetella* and *Regulus,* and several other loci subsequently support this contention (Table 4; Fig. 2). A large number of autapomorphs observed in *Dumetella* and *Regulus* contribute to their phenetic distinctness from Turdinae.

Surprisingly, the Gray Catbird appears somewhat closer phenetically to the Turdinae than does the Ruby-crowned Kinglet. Cladistically, the hemoglobin locus [exhibiting *Hb*(−250) in *Regulus* and *Hb*(−100) in *Dumetella* and examined members of Turdinae] permits the tentative designation of *Regulus* as the outgroup taxon. This interpretation is suggested by the occurrence of *Hb*(−250) in a number of representatives of Parulidae (unpubl. data), a group of warblers currently thought to be only distantly related to families represented in this study. *IDH-2*(−100) subsequently confirms a *Dumetella-Turdinae* clade, and several other electromorphs [*CK-3*(100), *EST-1*(100), *PGI*(100)] add weight to this conclusion (Fig. 2).

DISCUSSION

We have employed both phenetic and cladistic approaches to analyze electromorph distributions in several North American thrushes and relatives. There is currently a great deal of debate concerning the relative merits of these methods of data analysis (Sneath and Sokal 1973, Mickevich and Johnson 1976), but at least in this study, in broad perspective both methods yield similar summaries of probable relationships among species (Figs. 1 and 2). A particularly strong advantage of the cladistic approach is the highly testable nature of the resulting cladograms. Because discreet character states (electromorphs) are defined for all branches, points of ambiguity in the tree may be identified and advocated as important areas for further data acquisition. In our cladogram, as described above, the major area of ambiguity concerns the relative placement of *Turdus migratorius* and *Sialia sialis* to the *Hylocichla-Catharus* clade. Another advantage of the cladistic approach is that, because plesiomorphic and apomorphic character states are distinguished, relative confidence in determination of clades can be assessed by counting the number of derived char-

acters contributing to their definition. At least in this study, these advantages of cladistic analysis are counterbalanced somewhat by the fact that, although a large number of character states (101) were identified, very few contributed to a determination of the branching sequence of the cladogram.

All four species of *Catharus* (*fuscescens, minimus, ustulatus,* and *guttatus*) are nearly identical in electromorph composition at all 27 assayed loci. The Wood Thrush appears phenetically and cladistically allied to members of *Catharus*. Both the American Robin and the Eastern Bluebird lie outside the *Hylocichla-Catharus* clade and appear phenetically quite distinct from it and from each other. These conclusions correspond very closely to those presented by Gibson et al. (1976) for these taxa, based upon Principal Component analyses of 49 skeletal characteristics. They also agree with the results of Hendrickson and Yow (1973), which place *Hylocichla mustelina* much closer to *Catharus* than to *Turdus*. In this latter issue, our results conflict sharply with conclusions reached by Dilger (1956) and Bourns (1967), who suggested, on the basis of behavior traits and serological relationships, respectively, that *Hylocichla mustelina* was more closely related to *Turdus* than to *Catharus*. Gibson et al. (1976) have thoroughly discussed possible reasons for the apparent conflicting interpretations presented in the earlier papers. Particularly with the inclusion of the results from the present study, we believe data are now overwhelming in support of close evolutionary relationships between *Hylocichla mustelina* and the *Catharus* species examined.

Despite its current placement in a related family, Mimidae, the Gray Catbird appears cladistically and phenetically more closely allied to the Turdinae than does the Ruby-crowned Kinglet, a current member of Muscicapidae, subfamily Sylviidae, in the classification of Morony et al. (1975). This may not be too surprising, as Ginn (1978) states that the Mimidae have considerable affinities with thrushes (which he places in Turdidae, distinct from the predominantly Old-World family, Sylviinae, containing the Ruby-crowned Kinglet). We prefer to defer further discussion of this problem until we obtain data from more species in related families.

We have employed two approaches to data analysis that are considerably different in their underlying philosophy but that nonetheless lead to rather similar conclusions when applied to our data set. Although myriad other approaches to phylogenetic data analysis are now available, we believe it would be fruitless to analyze exhaustively and overinterpret this existing data set on thrushes. In a particularly insightful review of various approaches to molecular data acquisition and analysis, Throckmorton (1978) emphasizes the indeterminancy inherent in molecular evolution, due to "persistent heterozygosity, haphazard retention of ancestral alleles among descendant species, convergent mutation, and parallel generation of similar genotypes." These contingencies place restrictions on any method of phylogenetic analysis, and, indeed, are certainly not limited to molecular information.

Any final taxonomic or systematic interpretations to be derived from these data must be made with full appreciation of comparable results previously obtained with other organisms. In the 15 yrs since multi-locus electrophoretic approaches first became available to evolutionists, literally hundreds of comparisons among congeneric and confamilial pairs of species have been reported, using sets of loci comparable to those employed in this study. In mammals and other tetrapods, fishes, and in a number of invertebrate groups, the overwhelming conclusion is that even very closely related congeners differ considerably in allelic composition at one-third to one-half or more of their loci, and genetic similarities are typically 0.75 and lower

(Avise 1974, 1976; Ayala 1975). Exceptional cases do exist in which genetic similarities between species fall within the range typically reported for conspecific populations ($\bar{I} \simeq 0.85$–1.00), but these are relatively rare (e.g. Avise et al. 1975). For Passeriformes, however, a very different picture is beginning to emerge. In Icteridae (Smith and Zimmerman 1976, Corbin et al. 1979), Parulidae (Barrowclough and Corbin 1978), Sturnidae (Corbin et al. 1974), Hirundinidae (Martin and Selander 1975), and now in Muscicapidae (present study), comparisons among congeners have almost invariably yielded genetic similarities greater than 0.85, and species placed in confamilial genera have often exhibited similarities greater than 0.80 (see review in Barrowclough and Corbin 1978).

Thus, when evaluated against a scale provided by most other vertebrates and invertebrates, the differences among the *Catharus* species and *Hylocichla mustelina* seem trivial indeed, certainly not sufficient to warrant placement in different genera. By these same criteria, *Sialia* and *Turdus* might well be considered more divergent congeners as well. But when evaluated against the developing scale provided by other birds, *Hylocichla* and *Catharus* might logically retain generic status, and *Sialia* and *Turdus* would appear to be highly divergent genera from them. The assignment of higher taxonomic categories for any group of organisms certainly entails that somewhat arbitrary decisions be made. A desirable goal in the past has been consistency of criteria in assignment of categories within any group (such as a class or phylum) of organisms. With the increasing availability of molecular genetic data, which can often provide a common scale of comparison among as well as within higher categories, new challenges and opportunities exist for developing a common-base taxonomy and systematics.

ACKNOWLEDGMENTS

We wish to express our deep appreciation for the hospitality given us by members of the Tall Timbers Research Station. Special thanks are due Mr. Robert Crawford for aid in obtaining Florida samples and to Richard Parks for supplying additional specimens from Atlanta. Bob Chapman graciously assisted with the data analysis. Work was supported by a grant from the Penrose Fund of the American Philosophical Society and by NSF grant DEB 7814195.

LITERATURE CITED

AVISE, J. C. 1974. Systematic value of electrophoretic data. Syst. Zool. 23: 465–481.

———. 1976. Genetic differentiation during speciation. Pp. 106–122 *in* Molecular evolution (F. J. Ayala, Ed.). Sunderland, Massachusetts, Sinauer.

———, J. J. SMITH, & F. J. AYALA. 1975. Adaptive differentiation with little genic change between two native California minnows. Evolution 29: 411–426.

AYALA, F. J. 1975. Genetic differentiation during the speciation process. Evol. Biol. 8: 1–78.

———, J. R. POWELL, M. L. TRACEY, C. A. MOURÃO, & S. PÉREZ-SALAS. 1972. Enzyme variability in the *Drosophila willistoni* group. IV. Genetic variation in natural populations of *Drosophila willistoni*. Genetics 70: 113–139.

BARROWCLOUGH, G. F., & K. W. CORBIN. 1978. Genetic variation and differentiation in the Parulidae. Auk 95: 691–702.

BONDE, N. 1977. Cladistic classification as applied to vertebrates. Pp. 741–804 *in* Major patterns in vertebrate evolution (M. K. Hecht, P. C. Goodbody, and B. M. Hecht, Eds.). New York, Plenum Press.

BOURNS, T. K. R. 1967. Serological relationships among some North American thrushes. Can. J. Zool. 45: 97–99.

BREWER, G. J., & C. F. SING. 1970. An introduction to isozyme techniques. New York, Academic Press.

COOPER, D. W., M. R. IRWIN, & W. H. STONE. 1969. Inherited variation in the dehydrogenases of doves (*Streptopelia*). I. Studies on 6-phosphogluconate dehydrogenase. Genetics 62: 597–606.

CORBIN, K. W., C. G. SIBLEY, A. FERGUSON, A. C. WILSON, A. H. BRUSH, & J. E. AHLQUIST. 1974. Genetic polymorphism in New Guinea starlings of the genus *Aplonis*. Condor 76: 307–318.

———, ———, & ———. 1979. Genic changes associated with the establishment of sympatry in orioles of the genus *Icterus*. Evolution 33: 624–633.

DAWSON, D. M., H. M. EPPENBERGER, & N. O. KAPLAN. 1967. The comparative enzymology of creatine kinases. II. Physical and chemical properties. J. Biol. Chem. 25: 210–217.

DILGER, W. C. 1956. Relationships of the thrush genera *Catharus* and *Hylocichla*. Syst. Zool. 5: 174–182.

EPPENBERGER, H. M., M. EPPENBERGER, R. RICHTERICH, & H. AEBI. 1964. The ontogeny of creatine kinase isozymes. Devel. Biol. 10: 1–16.

———, D. M. DAWSON, & N. O. KAPLAN. 1967. The comparative enzymology of creatine kinases. I. Isolation and characterization from chicken and rabbit tissues. J. Biol. Chem. 242: 204–209.

FISHER, S. E., & G. S. WHITT. 1978. Evolution of isozyme loci and their differential tissue expression. Creatine kinase as a model system. J. Molec. Evol. 12: 25–55.

GIBSON, A. R., M. A. GATES, & R. ZACH. 1976. Phenetic affinities of the Wood Thrush, *Hylocichla mustelina* (Aves: Turdinae). Can. J. Zool. 54: 1679–1687.

GINN, H. B. 1978. Mockingbirds and thrashers. Pp. 205–206 *in* Bird families of the world (C. J. O. Harrison, Ed.). New York, Abrams Inc.

HENDRICKSON, H. T., & M. YOW. 1973. The relationships of the Wood Thrush (*Hylocichla mustelina*): some indications from the electrophoresis of blood proteins. Condor 75: 301–305.

HENNIG, W. 1966. Phylogenetic systematics. Chicago, Univ. Illinois Press.

KARIG, L. M., & A. C. WILSON. 1971. Genetic variation in supernatant malate dehydrogenase of birds and reptiles. Biochem. Genet. 5: 211–221.

KITTO, G. B., & A. C. WILSON. 1966. Evolution of malate dehydrogenase in birds. Science 153: 1408–1410.

MARTIN, R. F., & R. K. SELANDER. 1975. Morphological and biochemical evidence of hybridization between Cave and Barn swallows. Condor 77: 362–364.

MICKEVICH, M. F., & M. S. JOHNSON. 1976. Congruence between morphological and allozyme data in evolutionary inference and character evolution. Syst. Zool. 25: 260–270.

MORONY, J. J., W. J. BOCK, & J. FARRAND, JR. 1975. Reference list of the birds of the world. New York, Spec. Publ. Amer. Mus. Nat. Hist.

NEI, M. 1972. Genetic distance between populations. Amer. Natur. 106: 283–292.

NEVO, E. 1978. Genetic variation in natural populations: patterns and theory. Theor. Popul. Biol. 13: 121–177.

NOTTEBOHM, F., & R. K. SELANDER. 1972. Vocal dialects and gene frequencies in the chingolo sparrow (*Zonotrichia capensis*). Condor 74: 137–143.

PATTON, J. C., R. J. BAKER, & J. C. AVISE. 1980. Biochemical evolution in *Peromyscine* rodents. *In* Mammalian population genetics (J. Joule and M. H. Smith, Eds.). Athens, Georgia, Univ. Georgia Press in press.

POWELL, J. R. 1975. Protein variation in natural populations of animals. Evol. Biol. 8: 79–119.

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

———, & D. W. KAUFMAN. 1973. Genic variability and strategies of adaptation in animals. Proc. Natl. Acad. Sci. USA 70: 1875–1877.

SIBLEY, C. G. 1970. A comparative study of the egg-white proteins of passerine birds. Peabody Mus. Nat. Hist. Bull. 32.

———, & J. E. AHLQUIST. 1972. A comparative study of the egg-white proteins of non-passerine birds. Peabody Mus. Nat. Hist. Bull. 39.

SMITH, J. K., & E. G. ZIMMERMAN. 1976. Biochemical genetics and evolution of North American blackbirds, family Icteridae. Comp. Biochem. Physiol. 53B: 319–324.

SNEATH, P. H., & R. R. SOKAL. 1973. Numerical taxonomy. San Francisco, W. H. Freeman and Company.

THROCKMORTON, L. H. 1978. Molecular phylogenetics. Pp. 221–239 *in* Biosystematics in agriculture (R. H. Foote, L. Knutson, and P. L. Lentz, Eds.). New York, John Wiley and Sons.