THE RELATIONSHIPS OF THE WOOD THRUSH (*HYLOCICHLA MUSTELINA*): SOME INDICATIONS FROM THE ELECTROPHORESIS OF BLOOD PROTEINS

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The generic relationships of the Wood Thrush (*Hylocichla mustelina*) have been called into question by the works of Dilger (1956) and Bourns (1967). The four species of North American woodland thrushes presently referred (32nd Supplement to the A.O.U. Check-list 1973) to the genus *Catharus* i.e., Hermit Thrush (*Catharus guttata*); Gray-cheeked Thrush (*C. minima*); Swainson's Thrush (*C. ustulata*); and Veery (*C. fuscescens*) were formerly placed in the genus *Hylocichla* (A.O.U. Check-list 1957) and were long believed to be the closest relatives of the Wood Thrush.

Dilger (1956) has found the Wood Thrush "a misfit in the rather homogeneous *Catharus-Hylocichla* assemblage . . . ," and recommended its retention as the sole member of the genus *Hylocichla* although he believed further investigation would place it in the genus *Turdus*. Dilger's investigations into morphology and behavior received support from Bourns' (1967) series of serological tests on muscle and blood proteins. Bourns found that the Wood Thrush not only showed a greater relationship to the American Robin (*Turdus migratorius*) than to any of the four other woodland thrushes (i.e., *Catharus*), but that the Robin-Wood Thrush relationship was closer than that seen to exist between any two of the other four species. Dorst (1950) had come to a similar conclusion based on the apparent similarity between *Hylocichla mustelina* and the Song Thrush (*Turdus musicus*, now *Turdus philomelos*). Others, most notably Ridgway (1907) and Ripley (1952), have interpreted this similarity as due to convergence.

It would appear that there are three alternative schemes to deal with the systematic problems of the Wood Thrush: (1) the Wood Thrush is a member of the enlarged genus *Catharus*; (2) the Wood Thrush is the sole member of the genus *Hylocichla*; or (3) the Wood Thrush is a member of the genus *Turdus*. At the moment, one can find some evidence to support each of these positions.

Arguments over interpretation of existing data seem less likely to aid in the selection of the most reasonable of these hypotheses than does the accumulation of new evidence. Consequently, we have examined the electrophoretic properties of several blood proteins from the involved species in an effort to clarify our understanding of relationships in this group of birds. The basic philosophy behind the use of electrophoretic data on proteins as a source of taxonomic information has been published elsewhere and seems well justified (Sibley 1960, 1962, 1970; Zuckerkandl and Pauling 1965).

MATERIALS AND METHODS

Live specimens of six species of thrushes were captured near Greensboro, North Carolina, between September 1968 and October 1969 in the following numbers: *Hylocichla mustelina*, 15; *Catharus ustulatus*, 6; *Catharus guttatus*, 10; *Catharus minimus*, 6; *Catharus fuscescens*, 8; *Turdus migratorius*, 4.

In addition, a group of frozen plasma samples were obtained from Yale University through the courtesy of Dr. Charles G. Sibley. This group contained one sample each of the following species: Fieldfare (*Turdus pilaris*), Redwing (*Turdus iliacus*), Song Thrush, Clay-colored Robin (*Turdus grayi*), Red-capped Nightingale Thrush (*Catharus frantzii*), Hermit Thrush, and Veery.

All blood samples were collected in 10% EDTA (ethylenediaminetetraacetic acid) solution from live birds by cardiac puncture. The plasma was separated from whole blood by centrifugation at approximately 650 × G and frozen immediately. The remaining red blood cells were washed three times in an isotonic saline and then lysed in three volumes of distilled water. The red blood cell fragments were removed by centrifugation at approximately 1600 × G and the supernatant (mostly) hemoglobin solution was decanted and frozen.

Electrophoresis was carried out in a vertical gel electrophoresis cell, Model EC-470, manufactured by E-C Apparatus Corporation. Operating procedures followed the E-C Technical Bulletin No. 128. All runs were made in 7% polyacrylamide gels using Tris-Na₂EDTA-Boric acid buffer, pH 8.4. The samples

were run anodally at 300 volts. The temperature of the circulating cooling water within the unit was maintained between 2 and 15°C. Gels containing plasma samples were run for 2 hr, while hemoglobin samples were run for 3 hr. Twenty microliters of sample were used in each gel slot. Bromphenol blue was added to all samples to serve as a marker during electrophoretic migration. Sucrose was added to hemoglobin samples to increase density so they would remain in the gel slots.

Gels containing hemoglobin samples were stained with Amido Black 10B and washed repeatedly with a mixture of methanol, water, and acetic acid in the ratio 5:5:1. Gels containing plasma samples were stained for lactic dehydrogenase activity, using the method of Boutwell and Chapman (1966).

A standard sample was used as a reference point on all gels so that migrating distance could be correlated among gels. Material from a captive Ring-billed Gull (*Larus delawarensis*) was used as this standard.

The patterns on the gels were traced and reproduced on graph paper. The fastest portion of the pattern of the standard was used as a reference point. Reference values (R_f values) of proteins were obtained by dividing the distance of the protein from the application point by the distance of the standard from the application point and multiplying by one hundred.

RESULTS

HEMOGLOBINS

Electrophoresis of hemoglobins (or perhaps more accurately, red blood cell lysate proteins) from these species resulted in two very dense protein bands. Sibley et al. (1968) and Baker et al. (1966) have also reported the presence of two bands from the red blood cell material in the avian species which they have studied by starch gel electrophoresis. Figure 1 is a population-range diagram for both "hemoglobin" bands drawn according to the method described in Mayr et al. (1953). For each species, the horizontal line indicates the total range of variation in the sample; the broad portion of the line, one standard deviation on each side of the mean; and the vertical line is the mean.
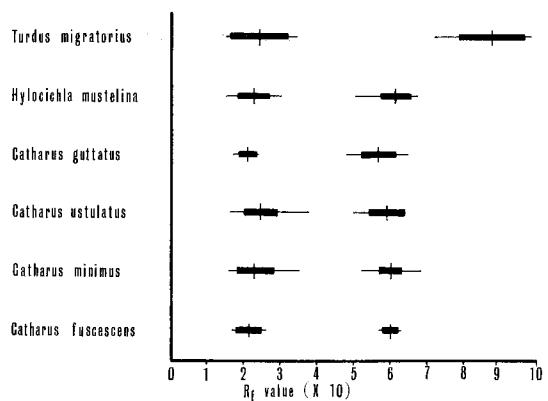
The slower of the two components appears

FIGURE 1. Population-range diagram of "hemoglobin" bands from six species of thrushes. For each species, the horizontal line indicates the total range of variation in the sample; the broad horizontal line, one standard deviation on each side of the mean; the vertical line is the mean.

inseparable among the six species examined. The range of the six means covers four R_f units. The data for the slow components are summarized in table 1. The faster component shows more variation. These data are summarized in table 2. *Turdus migratorius* has the fastest moving band, with a mean mobility of almost 86 R_f units (85.91). This component in the *Catharus* and *Hylocichla* species is slower, with mean mobilities ranging from 56.6 to 60.8 R_f units (see fig. 1).

From inspection of the gels, there appear to be no important differences among the slow components for any of the six species; however, among the fast components that of *Turdus migratorius* appears distinct.

To test these observations, an analysis of variance test using a single criterion of classification for any number of groups with unequal replications was used to test for a significance of difference among the means (Steel and Torrie 1960:112–114). The results of this test are shown in table 3. The means

TABLE 1. Data for slower hemoglobin component.

Species	No. of specimens	No. of patterns	Sum	$\bar{x}$	s	$s_{\bar{x}}$
Larus delawarensis	1	9	361.0	40.11	5.80	1.93
Turdus migratorius	4	6	143.5	23.91	7.54	3.08
Hylocichla mustelina	13	18	403.0	22.39	4.22	0.99
Catharus guttata	9	10	212.0	21.20	2.69	0.85
Catharus ustulata	6	10	246.0	24.60	4.80	2.30
Catharus minima	5	10	227.0	22.70	4.86	2.50
Catharus fuscescens	8	9	193.5	21.50	3.72	1.13

$\bar{x}$ = mean.
s = standard deviation.
$s_{\bar{x}}$ = standard error of the mean.

TABLE 2. Data for faster hemoglobin component.

Species	No. of specimens	No. of patterns	Sum	$\bar{x}$	s	$s_{\bar{x}}$
Larus delawarensis	1	9	833.0	92.55	2.17	0.72
Turdus migratorius	4	6	515.5	85.91	8.91	3.63
Hylocichla mustelina	13	18	1094.5	60.80	4.40	1.06
Catharus guttata	9	10	566.0	56.60	4.80	1.51
Catharus ustulata	6	10	589.0	58.90	4.86	1.53
Catharus minima	5	10	596.0	59.60	3.72	1.17
Catharus fuscescens	8	9	540.5	60.05	2.35	0.78

$\bar{x}$ = mean.
s = standard deviation.
$s_{\bar{x}}$ = standard error of the mean.

of the slow components show no significant differences, but the means of the fast components do show a significant difference. Since we suspected that Turdus migratorius caused this difference, we ran an analysis of variance using only the Hylocichla and Catharus species. This test showed no significant difference among the means of these species.

PLASMA LACTIC DEHYDROGENASES

Lactic dehydrogenase staining revealed five distinct bands for each of the species. Lactic dehydrogenase can occur in five forms or isozymes in the organs of most vertebrates and has been described in other avian species (Vessell and Brody 1964; Cahn et al. 1962; Zinkham et al. 1969).

The slowest or fifth band ranged from 4 to 11 R_f units from the application point in the Hylocichla and Catharus species, while this band ranged from 3 to 5 units in the species of Turdus. The fourth band in the Hylocichla and Catharus species ranged from 12 to 20 units and from 8 to 10 units in the Turdus species. The third band had a range from

TABLE 3. Comparison of hemoglobin means, analysis of variance.

Comparisons	Level of significance	Critical region	Computed F	Accept H_o[a] Reject H_o[b]
Hemoglobin Slow band All species	0.01	F > 3.37	F = 0.54	Accept H_o
Hemoglobin Fast band All species	0.01	F > 3.37	F = 33.66	Reject H_o
Hemoglobin Fast band Hylocichla and Catharus species	0.01	F > 3.72	F = 1.65	Accept H_o

[a] H_o = Null hypothesis. All means are equal.
[b] Accept alternative hypothesis. At least two means are not equal.

19 to 30 units in the Hylocichla and Catharus species and from 12 to 15 units in the Turdus species. The range for the second band was 25 to 39 for Hylocichla and Catharus and 16 to 22 for Turdus. The first or fastest band ranged from 32 to 46 units for Hylocichla and Catharus and from 20 to 28 units for Turdus. Only the fifth band showed any overlap in range for the two groups. The slower moving LDH bands seemed to be equally spaced between the application point and the fastest moving LDH band and hence their mobilities are functions of the fastest moving band. Therefore, the LDH patterns were considered as only one character complex and only the measurements taken on the fastest band were used in statistical comparisons.

A population-range diagram for the fastest band of each species is shown in figure 2. The data for the fastest band are summarized in table 4.

The patterns of the Hylocichla and Catharus species were inseparable from each other, but they were quite different from the patterns
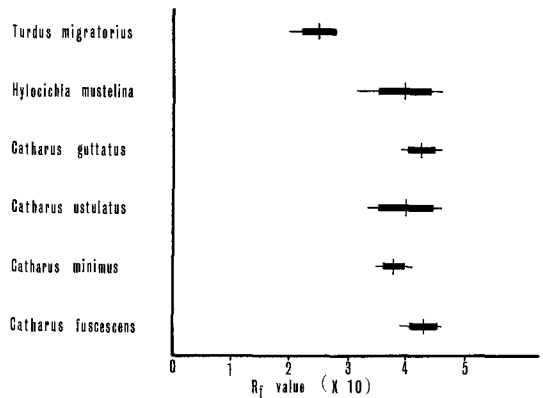

FIGURE 2. Population-range diagram of fastest lactic dehydrogenase bands from six species of thrushes. For each species, the horizontal line indicates the total range of variation in the sample; the broad horizontal line, one standard deviation on each side of the mean; the vertical line is the mean.

TABLE 4.   Data for fastest LDH component.

Species	No. of specimens	No. of patterns	Sum	$\bar{x}$	s	$s_{\bar{x}}$
Turdus species	8	10	250	25.0	2.81	0.88
Hylocichla mustelina	13	13	516	39.69	4.49	1.24
Catharus guttata	9	15	638	42.53	2.21	0.57
Catharus ustulata	6	6	241	40.16	4.74	1.93
Catharus minima	5	6	228	38.0	1.82	0.80
Catharus fuscescens	8	9	388	43.11	2.33	0.77
Catharus frantzii	1	1	37.0	37.00	–	–

$\bar{x}$ = mean.
s = standard deviation.
$s_{\bar{x}}$ = standard error of the mean.

of the *Turdus* species. The one specimen of *Catharus frantzii* produced a faint pattern, but it resembled the patterns of the other *Catharus* species. The data from this *Catharus* specimen were included although we recognize the limitations of examining only one specimen of a species. As only one specimen of each of the European and Central American *Turdus* species was available, the data from these species were combined with those from the specimens of *Turdus migratorius*.

The same analysis of variance procedure was used to test for a significant difference among the means. The results are shown in table 5. There is a significant difference when all the species are compared. However, when the species of *Turdus* are removed from the analysis, the means appear as a homogeneous group.

DISCUSSION

The evidence presented in this paper indicates that the electrophoretic properties of two independent protein molecules in *Hylocichla mustelina* show no detectable differences from the same molecules found in thrushes of the genus *Catharus*, but do show marked, significant differences from these molecules found in thrushes of the genus *Turdus*. These electrophoretic data are most easily interpreted to mean that the protein molecules are more

TABLE 5.   Comparison of fastest LDH component means, analysis of variance.

Comparisons	Level of signifi-cance	Critical region	Computed F	Accept H_o[a] Reject H_o[b]
All species	0.01	F > 3.18	F = 38.64	Reject H_o
Hylocichla and *Catharus* species	0.01	F > 3.47	F = 3.38	Accept H_o

[a] H_o = Null hypothesis. All means are equal.
[b] Accept alternative hypothesis. At least two means are not equal.

similar in their primary structure (i.e., amino acid sequence) among *Hylocichla* and *Catharus* species than they are among *Hylocichla* and *Turdus* species. Because of the relationship between protein structure and deoxyribonucleic acid (DNA) structure, it can be inferred that there exists a greater degree of genetic similarity between species of *Hylocichla* and *Catharus* than between the species of *Hylocichla* and *Turdus*. The most probable conclusion to be drawn is that *Hylocichla mustelina* is more closely related to the species of *Catharus* than to the species of *Turdus*.

The underlying assumptions relative to the evidence used in this study concern the differences in mobility rates caused by differences in electrical charges on the total molecule. Such differences in net charge are caused by the substitution of an amino acid with a given electrical potential by another amino acid with a different potential. If homologous proteins from a series of closely related species are examined and no detectable differences are noted, we assume that the proteins examined are extremely similar. We would expect to find an increased number of amino acid substitutions, with a correlated change in net charge and electrophoretic mobility in more distantly related species as a result of normal genetic divergence. Homologous proteins with different mobility rates are unquestionably structurally different molecules.

However, proteins with the same net charge and mobility may not be structurally identical. This situation, known as electrophoretic coincidence, has been discussed by Sibley (1970) and is not likely to present serious problems when comparisons are made between homologous proteins of closely related species whose propinquity has been determined by independent means.

The possibility of nearly identical protein structures among two groups of organisms of different ancestry due to extremely strong and similar selective pressures on the functional

properties of the protein molecules (convergent evolution on a molecular scale) does exist as a theoretical consideration. However, this also appears to be an improbable interpretation of the data as has been thoroughly explained by others (Zuckerkandl and Pauling 1965; Sibley 1970).

The original question as to the generic allocation of the Wood Thrush, while somewhat simplified, still remains. The results of this study argue against the inclusion of the Wood Thrush in the genus *Turdus*. There remain only the possibilities of classifying this species as a *Catharus* or a *Hylocichla*.

The alternative one chooses is largely dependent upon how important one considers the behavioral differences noted by Dilger (1956). The serological evidence of Bourns (1967) seems to us to be of little significance because of the low levels of reactivity found between the species of known close relationship in the genus *Catharus*. In view of the relative plasticity of behavioral characters, and the high degree of uncertainty in correlating behavioral differences to amounts of genetic change, we are inclined to weigh behavioral differences very lightly. Consequently, it is our feeling that the Wood Thrush should be retained in the same genus as the other species of North American woodland thrushes and finding no evidence contrary to the opinion of Ripley (1952), we recommend the elimination of the generic name *Hylocichla* and use of the name *Catharus*.

SUMMARY

An electrophoretic comparison of blood proteins was undertaken to clarify the relationships of the Wood Thrush (*Hylocichla mustelina*). The patterns obtained from lactic dehydrogenase isozymes and hemoglobin in polyacrylamide gels were almost identical for *H. mustelina* and the species of New World woodland thrushes. The patterns of *Turdus* species were quite similar to one another, but distinctly different from patterns of specimens classified as either *Hylocichla* or *Catharus*. It is concluded, on the basis of this study, that the Wood Thrush cannot be included in the genus *Turdus*, but is a member of the genus *Catharus*. The genus *Hylocichla* should be eliminated and considered a synonym of *Catharus*.

ACKNOWLEDGMENTS

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