Relationships of Some Families of the Suborder Passeres (Songbirds) as Indicated by Comparisons of Tissue Proteins

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Ornithologists agree that any classification of birds that attempts to reflect relationship and phylogeny presents many uncertainties. This is particularly true in regard to the Order Passeriformes, which includes more living species than all other orders combined, and of which but few fossil forms are known. Within this order the greatest taxonomic problem is posed by those species assigned to the Suborder Passeres (Oscines), the true songbirds. Opinions vary widely as to the relationships of the families in this suborder and, therefore, schemes of classification. This is because these birds form a morphologically homogeneous group, and because their slight anatomical differences have been variously interpreted by different workers. Although studies of physiology, life history, and behavior patterns (in addition to the more conventional studies of morphology) have yielded useful and important data, there remains no clear understanding of the relationships of many groups; and even when data from new and little explored fields are obtained, there will still be arguments in this regard. This fact ought not to discourage, however, the search for other kinds of information that might be of some use in establishing a natural classification.

This paper includes the results of a study of the relationships of fifteen oscine families (Table 1) which involves comparison of saline-soluble tissue proteins by serological techniques. This work was made possible by a research grant, NSF-G2904, from the National Science Foundation.

Serological techniques have been used in the study of problems of animal relationships (with varying degrees of success) over a period of some 60 years; but not extensively in ornithology. What has been done has been briefly reviewed in an earlier paper (Stallcup, 1954) and is not repeated here.

MATERIALS AND METHODS

The precipitin reaction is the most successful of the serological techniques thus far devised for systematic comparisons. The

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reaction occurs because antigenic substances introduced into the body of an animal cause formation of antibodies which precipitate

TABLE 1. SPECIES INVESTIGATED (names are those of the A.O.U. Check-list, 5th edition)

	Symbols used in figures.
Order Piciformes	
Colaptes auratus (Linnaeus): Yellow-shafted Flicker.	Co
Centurus carolinus (Linnaeus): Red-bellied Woodpecker.	Ce
Order Passeriformes	
Family Corvidae	
Cyanocitta cristata (Linnaeus): Blue Jay.	Cy
Family Paridae	
Parus carolinensis Audubon: Carolina Chickadee.	Pc
Parus bicolor Linnaeus: Tufted Titmouse.	Pb
Family Troglodytidae	
Thryothorus ludovicianus (Latham): Carolina Wren.	Th
Family Mimidae	
Mimus polyglottos (Linnaeus): Mockingbird.	Mi
Toxostoma rufum (Linnaeus): Brown Thrasher.	To
Family Turdidae	
Turdus migratorius Linnaeus: Robin.	Tu
Family Sylviidae	
Regulus calendula (Linnaeus): Ruby-crowned Kinglet.	Re
Family Bombycillidae	
Bombycilla cedrorum Vieillot: Cedar Waxwing.	Bo
Family Laniidae	
Lanius ludovicianus Linnaeus: Loggerhead Shrike.	La
Family Sturnidae	
Sturnus vulgaris Linnaeus: Starling.	St
Family Vireonidae	
Vireo olivaceus (Linnaeus): Red-eyed Vireo.	Vi
Family Parulidae	
Dendroica coronata (Linnaeus): Myrtle Warbler.	De
Family Ploceidae	
Passer domesticus (Linnaeus): House Sparrow.	Pa
Family Icteridae	
Agelaius phoeniceus (Linnaeus): Redwinged Blackbird.	Ag
Family Thraupidae	
Piranga rubra (Linnaeus): Summer Tanager.	Pi
Family Fringillidae	
Richmondena cardinalis (Linnaeus): Cardinal.	Ri
Spiza americana (Gmelin): Dickcissel.	Sp

the antigens when the two are mixed. The antisera produced show quantitative specificities in their actions; therefore, when an antiserum containing precipitins is mixed with each of several antigens, the reaction involving the homologous antigen (that used in the production of the antiserum) is greater than those reactions involving the heterologous antigens (antigens other than those used in the production of the antiserum). The magnitudes of the reactions vary according to the degrees of similarity of these antigens to the homologous one.

The method of precipitin testing follows that outlined by Leone (1949) and modified by Stallcup. Although most previous work-

ers in comparative serology (in which precipitin tests were used) employed whole sera as antigens, Martin and Leone (1952) and Stallcup showed that tissue extracts are satisfactory as antigens, and that serological differentiation can be obtained with these extracts and the antiserum to them. As the smallness of the birds tested in the present work made it impracticable to obtain enough whole sera for experiment, saline extracts of the tissues were used.

Birds were obtained by shooting, and wrapped as soon as possible separately and carefully in aluminum foil or in plastic bags to prevent dehydration of tissues. The specimens were frozen and kept so until the time when the extracts were made.

When an extract was to be prepared, a specimen was allowed to thaw but not to become warm. Muscle tissue was taken from the thoracic region and any fat carefully removed. The tissue was then placed in a Waring Blendor with 0.85 per cent aqueous NaCl buffered with phosphate to a pH of 7.0. For each gram of tissue to be extracted, 20 ml. of saline was the amount used. The tissues were ground in the blendor until the mixture appeared homogeneous. It was then transferred to a flask and allowed to stand at 2° C. for 120 hours, with periodic shaking. At the end of this time, the tissue residues were removed by centrifugation in a refrigerated centrifuge. Formalin was added to the extract in the amount necessary to make the final dilution 0.4 per cent. This formolization seems necessary to inhibit the action of autolytic enzymes during the time necessary to complete the investigations. While formalin caused a slight denaturation of some of the proteins in the extract, this effect was complete within 48 hours. At the end of this period the denatured materials were removed, the extracts clarified by centrifugation at high speed (approximately 17,000 g), and bottled and stored at 2 degrees C. until needed. The amount of protein for each extract was determined by the Biuret method with a Bausch and Lomb Spectronic 20 Colorimeter.

To increase the effectiveness of the extracts as antigens, each extract was mixed with two adjuvants, paraffin oil and Arlacel A (Atlas Powder Company, Wilmington, Delaware), as follows: 8.5 volumes of paraffin oil, 1.5 volumes of Arlacel A, and 10 volumes of the antigenic substance. The adjuvants were mixed

before the antigen was added. This mixture was agitated until an emulsion was formed.

All antisera were produced in laboratory rabbits. Each rabbit received two series of subcutaneous injections, each injection being administered on alternate days and doubling in amount: 1.0 ml., 2.0 ml., 4.0 ml., and 8.0 ml. The two series of injections were separated by an interval of eight days. On the eighth day after the last injection of the second series, the rabbit was completely exsanguinated by cardiac puncture. The whole blood was placed in clean test tubes and allowed to clot. It was allowed to stand at 2 degrees C. for 12 to 18 hours so that most of the serum would be expressed from the clot. The serum was then decanted, centrifuged to remove all blood cells, sterilized by filtration through a Seitz filter, bottled in sterile vials, and stored at 2° C. until used.

In each series of precipitin tests the amount of antiserum was held constant, and the amount of antigen was varied. The volume for each antigen dilution was always 1.7 ml., and to this was added 0.3 ml. of antiserum to make up a volume of 2.0 ml. Antigens were diluted with 0.85 per cent phosphate-buffered saline to which had been added 'Merthiolate' in a final dilution of 1:10,000 to prevent bacterial growth in the antigen-antiserum mixtures. Tests were run in standard Kolmer test tube racks, each test consisting of 12 tubes. Since the protein concentrations in each antigen were known, it was possible to place in the first tube of each series of tests the same proportions of protein to saline. Each successive tube contained a protein dilution one-half the concentration of the preceding tube. Both antiserum controls and antigen controls were maintained with each test to determine the turbidities inherent in these solutions. These control turbidities were deducted from the total turbidity developed in each reaction tube, the resultant turbidity then being considered as that which was caused by the interaction of antigens and antibodies. The turbidities were allowed to develop over a period of 8 to 10 hours. The Libby (1938) Photronreflectometer was used to measure the turbidities developed in the reaction tubes. I am indebted to Dr. Charles A. Leone of the University of Kansas for the loan of the Photronreflectometer.

Since the number of antigens that could be tested with one antiserum was limited by the amount of antiserum to 11 or 12, the species under investigation were separated into two groups (Tables 2 & 3), and these were studied separately. In order that some correlation could be made of the data obtained from the studies of the two groups, several of the same or closely related species were used in both series of tests.

TABLE 2. RESULTS OF PRECIPITIN TESTS WITH SPECIES OF GROUP 1. Each column of species represents one test of the antigens with the antiserum to the species listed first. Numerals are per cent values, the homologous reaction being considered 100 per cent.

TURDUS	100	BOMBYCILLA	100	DENDROICA	100
Sturnus	92	Parus bicolor	83	Spiza	87
Dendroica	86	Spiza	77	Agelaius	85
Parus bicolor	84	Dendroica	76	Turdus	82
Cyanocitta	80	Thryothorus	72	Bombycilla	81
Bombycilla	79	Sturnus	62	Cyanocitta	79
Thryothorus	79	Colaptes	61	Parus bicolor	79
Spiza	77	Turdus	60	Toxostoma	76
Toxostoma	75	Cyanocitta	57	Thryothorus	75
Colaptes	62	Toxostoma	56	Sturnus	72
Agelaius	62	Agelaius	50	Colaptes	57
Lanius	50	Lanius	47	Lanius	56
STURNUS	100	TOXOSTOMA	100	THRYOTHORUS	100
Toxostoma	92	Sturnus	91	Bombycilla	85
Spiza	90	Cyanocitta	81	Parus bicolor	83
Parus bicolor	90	Turdus	81	Spiza	80
Cyanocitta	88	Parus bicolor	79	Dendroica	79
Turdus	86	Bombycilla	79	Cyanocitta	72
Dendroica	84	Spiza	78	Toxostoma	71
Thryothorus	82	Dendroica	74	Turdus	67
Bombycilla	81	Agelaius	73	Sturnus	64
Agelaius	79	Thryothorus	71	Agelaius	63
Lanius	68	Lanius	65	Colaptes	59
Colaptes	67	Colaptes	59	Lanius	57
AGELAIUS	100	PARUS BICOLOR	100	SPIZA	100
Spiza	94	Cyanocitta	82	Dendroica	79
Sturnus	88	Bombycilla	80	Parus bicolor	76
Parus bicolor	85	Turdus	78	Sturnus	74
Dendroica	80	Dendroica	77	Agelaius	69
Cyanocitta	79	Thryothorus	76	Thryothorus	69
Toxostoma	74	Toxostoma	73	Turdus	61
Turdus	72	Spiza	73	Bombycilla	60
Thryothorus	71	Agelaius	70	Cyanocitta	58
Colaptes	67	Sturnus	70	Colaptes	56
Bombycilla	63	Lanius	57	Toxostoma	55
Lanius	50	Colaptes	56	Lanius	35
CYANOCITTA	100	LANIUS	100	COLAPTES	100
Parus bicolor	91	Cyanocitta	95	Bombycilla	56
Toxostoma	86	Toxostoma	83	Parus bicolor	52
Thryothorus	86	Turdus	75	Dendroica	49
Bombycilla	85	Parus bicolor	72	Thryothorus	49
Sturnus	85	Sturnus	72	Cyanocitta	48
Spiza	82	Agelaius	72	Spiza	47
Dendroica	81	Spiza	67	Toxostoma	46
Turdus	77	Thryothorus	63	Sturnus	43
Agelaius	77	Dendroica	62	Turdus	42
Colaptes	74	Bombycilla	62	Agelaius	39
Lanius	68	Colaptes	49	Lanius	38

Each antiserum was tested with each of the antigens prepared from the birds of one group. For each antigen-antiserum series the corrected values for the turbidities obtained were added. The total of each was converted to a percentage value, that of the homo-

logous reaction being considered 100 per cent. For each test, then, the species were listed in order of total magnitude of reaction (Tables 2 & 3). The first species listed in each case is the species for which the antiserum was prepared (the homologous reaction); thus, the value of 100 per cent for this species. The other species are listed in descending order of reaction with the antiserum. Both the rank of the heterologous reaction in relation to the homologous one and the value of the reaction were used in attempting to establish serological relationship.

TABLE 3. RESULTS OF PRECIPITIN TESTS WITH SPECIES OF GROUP 2. Each column of species represents one test of the antigens with the antiserum to the species listed first. Numerals are per cent values, the homologous reaction being considered 100 per cent.

TURDUS	100	BOMBYCILLA	100	VIREO	100
Parus caro.	67	Parus caro.	86	Parus caro.	82
Mimus	66	Turdus	86	Turdus	80
Bombycilla	61	Mimus	82	Passer	80
Richmondena	57	Passer	72	Bombycilla	78
Passer	57	Richmondena	64	Piranga	73
Piranga	53	Vireo	62	Richmondena	72
Vireo	48	Piranga	62	Mimus	71
Lanius	45	Lanius	58	Centurus	64
Centurus	40	Centurus	51	Lanius	61
				Regulus	51
PASSER	100	MIMUS	100	PIRANGA	100
Parus caro.	83	Turdus	81	Richmondena	100
Turdus	81	Parus caro.	80	Turdus	99
Bombycilla	79	Bombycilla	78	Parus caro.	94
Mimus	78	Passer	71	Passer	90
Piranga	78	Richmondena	65	Mimus	87
Richmondena	77	Vireo	64	Bombycilla	78
Vireo	74	Piranga	63	Vireo	65
Lanius	60	Lanius	60	Lanius	60
Centurus	58	Centurus	48	Centurus	46
Regulus	54	Regulus	47		
REGULUS	100	PARUS CARO.	100	RICHMONDENA	100
Parus caro.	92	Turdus	79	Piranga	89
Bombycilla	89	Passer	76	Turdus	89
Passer	89	Bombycilla	74	Parus caro.	78
Mimus	86	Mimus	73	Passer	76
Vireo	85	Piranga	71	Bombycilla	76
Piranga	85	Richmondena	70	Mimus	72
Turdus	82	Lanius	54	Vireo	67
Richmondena	77	Centurus	48	Centurus	58
Lanius	72			Lanius	51
Centurus	64			Regulus	45
LANIUS	100	CENTURUS	100		
Parus caro.	98	Turdus	38		
Mimus	96	Bombycilla	38		
Passer	94	Parus caro.	38		
Turdus	94	Mimus	36		
Bombycilla	89	Vireo	36		
Piranga	85	Richmondena	33		
Richmondena	79	Passer	33		
Centurus	66	Piranga	30		
		Lanius	30		
		Regulus	25		

DISCUSSION

The rabbit is a variable to be considered in serological tests of the sort described above. Two rabbits exposed to the same antigen, under the same conditions, may produce antisera that differ greatly

in their capacities to distinguish different antigens. It is logical to assume, therefore, that two rabbits exposed to different antigens may produce antisera which also differ in this respect. This explains the unequal values of reciprocal tests shown in Tables 2 and 3. Thus, in the test which involved the antiserum to the extracts of *Spiza* (Table 2), a value of 79 per cent was obtained for *Dendroica* antigen, whereas in the test involving anti-*Dendroica* serum, a value of 87 per cent was obtained for *Spiza* antigen.

An additional point to consider in the interpretation of these tests is that the techniques used tend to separate more sharply species that are closely related, while species distantly related are not so easily separated. In other words, comparative serological studies with the photronreflectometer tend to minimize the differences between distant relatives and to exaggerate the differences between close relatives.

In analyzing the serological relationships of the species used in this study, it becomes obvious that two or more series of tests must be considered before the birds can be placed in relation to each other. For example, the data presented in Table 2 indicate that *Agelaius* and *Thryothorus* antigens show the same degree of serological correspondence to *Spiza* antiserum. This does not imply, necessarily, that *Agelaius* and *Thryothorus* are closely related. If the tests involving *Agelaius* antiserum are examined, it can be determined that *Spiza* shows much greater serological correspondence to *Agelaius* than does *Thryothorus*. By reference to other series of tests involving these three species, a more exact determination of their relationships may be obtained.

To illustrate this point by a hypothetical example, two species might seem equidistant, serologically, from a third species. Additional testing should indicate if the first two species are equidistant in the same direction or in opposite directions. A single test supplies only two dimensions of a three dimensional arrangement.

Since antisera do differ greatly in their capacities to distinguish different antigens and since, as a result, unequal values in reciprocal tests are obtained, it seems better to use as a basis for interpretation of data the relative order of reaction of antigen with a given antiserum rather than the actual turbidity values measured. For example (Table 2), the tests involving anti-*Turdus* serum in-

dicate that of the antigens tested, *Sturnus* antigen was most like the homologous (*Turdus*) antigen, *Dendroica* antigen was next, and so on. This means that of all the species used in these tests, *Sturnus* is, in its tissue proteins, more like *Turdus* than are any of the other species. This does not imply that *Sturnus* is more like *Turdus* than *Sturnus* is like other species. As a matter of fact, examination of the tests involving anti-*Sturnus* serum shows that there are several species that are, serologically, more like *Sturnus* than is *Turdus*. By reference to other tests involving these species, a better idea of their relationships may be obtained.

It is impossible to interpret and to picture the serological data satisfactorily in two dimensions; therefore, three-dimensional models were constructed to represent the serological relationships of the birds involved, and drawings of these models are presented with the discussions below.

For each family of oscine birds represented in this study some of the more recent ideas as to the affinities of the group have been set forth; and for each family data obtained in this study are presented. Certainly, these data do not provide answers for all of the questions pertaining to the relationships of the oscine families, nor should these data, more than those from other fields, be expected to do this. In some instances, several interpretations of these serological data are possible. Moreover, these data cannot be used to determine phyletic trends since it would be difficult to explain the results in terms of adaptation. Nor can the question as to whether a group is primitive or advanced be answered on the basis of this information. Similarity and differences in proteins of the birds tested, however, should indicate that certain groups are more or less alike, and it is suggested that these data may help clarify relationships that are not certain on the basis of observations from other fields of endeavor.

The families represented in this study are placed in the sequence suggested by Wetmore (1951). For each family, I have prepared a diagram that indicates the most likely relationships of the species studied on the basis of tissue proteins. The symbols used in the diagrams are those listed in Table 1. Since each diagram represents a three-dimensional arrangement of species, the relative distances of the species from one another are given to facilitate interpreta-

tion. The shortest distance between species in each diagram is represented as 1.0, and the other distances are listed accordingly.

Family Corvidae.—Wetmore places the corvids near the first of the list of oscine families whereas Mayr & Amadon (1951) consider this group among the most highly evolved of all birds and place these species and their supposed relatives in the terminal position. The arrangements of Delacour & Vaurie (1957) suggest that the corvids are more closely related to the families Laniidae and Sturnidae than they are to any of the other families that I studied.

Cyanocitta cristata was the corvid used for the serological investigations, and on the basis of tissue proteins seems to be more like *Parus bicolor* of the family Paridae than any of the other species studied (Fig. 1). Members of several families are more like *Cyanocitta* than is *Sturnus*, and *Cyanocitta* and *Lanius* show little serological correspondence.

Family Paridae.—Wetmore's classification and that of Mayr & Amadon imply that the members of this group are not too closely related to any of the other families that I studied. Delacour & Vaurie place this family near the family Turdidae. Beecher (1953) states that the members of this family "stem" from the family Troglodytidae "in the sequence: Troglodytidae-Certhiidae-Sittidae-Paridae."

Parus carolinensis and *Parus bicolor* were the parids used in these studies. Serologically they are most like *Cyanocitta* and *Turdus* (Figs. 2 and 3).

Family Troglodytidae.—Wetmore places this family in the sequence Troglodytidae, Mimidae, and Turdidae. Mayr & Amadon include the wrens as a subfamily in the family Muscicapidae, the latter including, also, as subfamilies the warblers (Sylviinae, including *Regulus*), the thrushes (Turdinae), and the mockers and thrashers (Miminae). Delacour & Vaurie include the wrens near the mockers and thrashers. Beecher states that the wrens serve as the stem group for the wren-creeper-nuthatch-titmouse assemblage.

Thryothorus ludovicianus was the wren used for the tests described here. *Bombycilla* and *Parus bicolor* showed greater serological correspondence to *Thryothorus* than did any of the other species studied (Fig. 4). The fact that with *Thryothorus* antiserum *Bombycilla* antigen was more reactive than the other heterologous anti-

gens does not imply, necessarily, a close relationship between these two species. An examination of the data from tests involving *Bombycilla* antiserum (Fig. 7) reveals that several other species are more closely related to *Bombycilla* than is *Thryothorus*. *Thryothorus* does not resemble closely the members tested of the families Mimidae, Turdidae, and Regulidae. *Parus bicolor* seems more like *Thryothorus* than do members of these three families.

Family Mimidae.—In Wetmore's classification this family is placed between the families Troglodytidae and Turdidae. Mayr & Amadon place the mockers and thrashers in the subfamily Miminae along with the subfamilies Sylviinae, Turdinae, and Troglodytinae. Delacour & Vaurie place the mimids next to the wrens, and Beecher includes the mimids in the family Turdidae with the thrushes.

The mimids used in the serological tests were *Mimus polyglottos* and *Toxostoma rufum*. Although these two species were tested with different groups of birds, in so far as the data overlap, the results are approximately the same. Of the species tested with *Toxostoma*, *Sturnus* showed the closest serological correspondence; next, in order, were *Cyanocitta*, *Turdus*, and *Parus bicolor*. Of the species tested the *Mimus*, *Turdus* showed the greatest correspondence, and *Parus carolinensis* was second. Neither *Sturnus* nor a corvid was tested with *Mimus* (Fig. 5). On the basis of the serological data there is some justification for assuming relationship between the mimids and the thrushes, although the serological resemblance of the species tested of these two families is not as great as that between *Toxostoma* and *Sturnus*.

Family Turdidae.—In Wetmore's classification this family is placed in the sequence Troglodytidae, Mimidae, Turdidae. Mayr & Amadon designate this group a subfamily along with the Sylviinae, Miminae, and Troglodytinae in the family Muscicapidae. Delacour & Vaurie include these birds as a subfamily in the family Muscicapidae along with the Sylviinae, but place the mimids in an entirely different assemblage. Beecher implies that the turdids and the mimids are related by placing both groups in the family Turdidae.

The serological data present no clear idea as to the species most like the turdid tested (*Turdus*). *Turdus* was tested with both groups of species, and attempts to correlate data from the two sources are not completely successful. The species that show great-

est serological correspondence to *Turdus* are *Sturnus, Dendroica, Parus,* and *Mimus* (Fig. 6). It would seem, therefore, that there is some serological evidence that the turdids and the mimids are related. There are other species, however, that show greater serological correspondence to *Turdus* than does *Mimus. Sturnus* is a notable example.

Family Sylviidae.—Wetmore places the kinglets in a separate family Regulidae and lists this family near the families Mimidae and Turdidae. As stated above, Mayr & Amadon and Delacour & Vaurie assign this group to the family Muscicapidae. Beecher considers this group the stem group for a superfamily Sylvioidea that includes several families members of which were used in this study.

There was antigen enough to conduct only six sets of precipitin tests with *Regulus.* If the data obtained by use of anti-*Regulus* serum are examined, it appears that *Parus carolinensis, Bombycilla,* and *Passer* show the greatest serological correspondence with this species. When the data from reciprocal tests are examined, however, it is clear that in every instance *Regulus* showed least serological correspondence to the homologous antigen. Thus, it would seem that *Regulus* is quite different in regard to tissue proteins from all the other species tested (Fig. 13).

Family Bombycillidae.—None of the classifications cited in the preceding paragraphs lists this family near any of the families studied in this work.

In each series of serological tests *Parus* (*carolinensis* in one and *bicolor* in the other) showed the greatest serological correspondence to *Bombycilla* (Fig. 7). Of the other species tested *Turdus, Mimus,* and *Passer,* in the order listed showed some resemblance to the waxwing.

Family Laniidae.—Wetmore, Mayr & Amadon, and Beecher list this family near none of those studied here. Delacour & Vaurie place this family near the starlings and the corvids but imply no close relationship.

Lanius, the species used in both series of serological tests, showed the greatest serological correspondence to *Cyanocitta,* but the relationship is not a close one. With the exception of *Regulus* and the piciform species used, *Lanius* seems, serologically, to be least like any of the other passeriform species studied (Fig. 13).

Family Sturnidae.—In Wetmore's classification this family is not placed near any of the families studied by me. Mayr & Amadon suggest that the families Sturnidae and Ploceidae might be related. Delacour & Vaurie include the sturnids in a general group with the families Laniidae and Corvidae but imply no close relationship. Beecher states that the members of family Sturnidae show strong affinities to the thrushes in plumage and internal structure.

Serologically, *Sturnus* seems to be most like *Toxostoma.* Although *Passer* was not tested in the same series with *Sturnus,* comparisons of data indicate that there is a possible relationship between these two species (Figs. 8 and 10). Although tests with *Turdus* antiserum indicate that *Sturnus* showed the greatest serological correspondence to *Turdus* (Table 2), tests with *Sturnus* antiserum show that the relationship is not great (Table 2 and Fig. 8).

Family Vireonidae.—In Wetmore's list the vireos are placed nearer the family Parulidae than any of the other groups studied here. Mayr & Amadon state that the vireos may constitute the most primitive family of the vireo-tanager-finch assemblage and that the wood warblers (Parulinae) are most like the vireos. Delacour & Vaurie imply, also, that the vireos are related to the nine-primaried oscines and to the family Ploceidae as well. Beecher states that on anatomical grounds the vireos appear to have given rise to the wood warblers and to the tanagers. Tordoff (1954) lists the vireos as a separate family, related to other nine-primaried oscines.

On the basis of serological data, *Vireo olivaceus* does not seem particularly close to any of the species tested. *Vireo* is set apart from the main cluster of species but is not as distant from this cluster as are *Regulus* and *Lanius* (Fig. 13). It is unfortunate that *Vireo* and *Dendroica* were not used in the same series of tests. When data from these series are compared, however, it can be seen that *Dendroica* shows great serological correspondence to the other nine-primaried oscines (Fig. 9), whereas *Vireo* does not (Table 3). Species that show greatest serological similarity to *Vireo* are *Parus carolinensis, Turdus,* and *Passer,* but the data in hand suggest no close relationship of *Vireo* to these species.

Family Parulidae.—Wetmore lists this group as a family in the sequence Parulidae, Ploceidae, Icteridae. Mayr & Amadon place the wood warblers in a subfamily in the family Thraupidae which in-

cludes, also, the tanagers and cardinal grosbeaks as separate subfamilies. Delacour & Vaurie imply, also, a close relationship of the wood warblers, cardinal grosbeaks, and tanagers. Beecher maintains that the parulids evolved directly from the vireos and give rise indirectly to the icterids. Tordoff agrees that the wood warblers are related to the rest of the nine-primaried oscine assemblage.

The data from the serological investigations show that *Dendroica* is more like *Spiza* than any of the other species tested (Fig. 9 and Table 2). *Dendroica* and *Agelaius* show great serological correspondence, also, although there are several uncertainties involved with the placement of these species in relation to each other. Certainly, these data support those classifications that imply close relationship between the wood warblers and the cardinal grosbeaks. No close relationship of *Dendroica* to *Passer* is indicated.

Famliy Ploceidae.—In Wetmore's classification the ploceids are placed in the sequence Parulidae, Ploceidae, Icteridae. Mayr & Amadon suggest a relationship between the families Ploceidae and Sturnidae. Delacour & Vaurie place the ploceids in an assemblage with the finches, tanagers, wood warblers, vireos, and blackbirds. Beecher and Tordoff suggest no particular affinities of this group to any of the other families studied here.

Passer showed greater serological similarity to *Parus carolinensis* and *Turdus,* respectively, than to any of the other species studied. *Passer* was not tested in the same series with *Sturnus,* but comparisons of data indicate that there might be serological correspondence here. Whether one might be more like the other, than either is to some other species, it would not be possible to determine from the data available. Certainly, *Passer* seems more closely related to several of the other species tested than to any of the nine-primaried oscines (Fig. 10).

Family Icteridae.—In Wetmore's list of families the blackbirds are placed just after the ploceids. Mayr & Amadon group the icterids with the other nine-primaried oscines. Delacour & Vaurie follow this arrangement but include, also, the ploceids. Beecher states that the icterids are evolved from the parulids through the emberizines, and Tordoff includes this family as one of the nine-primaried group.

The serological data indicate that *Agelaius* is more like *Spiza* than

any of the other species tested (Fig. 11). Although *Agelaius* and *Passer* were not used in the same series of tests, comparisons of the data available indicate that there is no close relationship between these species. The placement of the icterids near the other nine-primaried groups seems justified on the basis of the present work.

Family Thraupidae.—All of the classifications cited in the preceding paragraphs include this family with the other groups of nine-primaried oscines.

Certainly, the data obtained in these investigations indicate that this arrangement is justified. Serologically, *Piranga* is very similar to *Richmondena* (Fig. 12).

Family Fringillidae.—All the authors whose papers are cited above include this family with the other families of nine-primaried oscines.

The serological relationships of *Spiza* (Figs. 9 and 11) and of *Richmondena* (Figs. 10 and 12) seem to justify the placement of this group in the classifications cited.

For comparative purposes, two species of the Order Piciformes were tested along with the passeriform birds. *Colaptes auratus* was used in one test series (Table 2) and *Centurus carolinus,* in the other (Table 3). An examination of the data presented in these two tables reveals that these two species show little serological resemblance to the species of passeriform birds. With the exceptions of *Regulus, Lanius,* and *Vireo* the passeriform species constitute a rather homogeneous assemblage, and *Colaptes* and *Centurus,* certainly, lie outside this assemblage. In Figure 13, an attempt has been made to show the serological relationships of the two piciform species and those of *Regulus, Lanius,* and *Vireo* to the main assemblage of passeriform species.

Recapitulation of what has been said in the preceding accounts of the families is hardly necessary. In each account ideas of relationship based on serological data are compared with ideas held by current authorities in the field of avian taxonomy. The implications of data obtained from tests involving *Regulus, Lanius,* and *Vireo,* however, seem worthy of special note. These species seem to be set quite apart, serologically, from the other oscine species; this is particularly true of *Regulus* and *Lanius.* Although no suggestions as to their proper positions can be made on the basis of my data, I hope

the information given may stimulate more work in this direction.

It should be restated and emphasized that no attempt is made here to classify the species involved on a serological basis, nor is there any suggestion that the data presented be used to the exclusion of those from other fields of study in trying to establish a clearer idea as to the natural relationships of the oscine families. Such an attempt would, indeed, be foolish since relatively few families have been studied, and since only one or two species in each family were used. Such are the limitations of work of this sort.

Since, however, no clear understanding of the relationships of many families in this suborder exists, it is suggested that "no stone be left unturned" in an attempt to gather new information that might be useful in this regard. With this thought in mind, these investigations have been carried out. In some cases the data from the serological tests support the placement of families in one or more of the current classifications, while in other instances, the data suggest new patterns of relationship. In some cases, the data shed no light on the subject of relationship.

The results of the investigations reported here emphasize the homogeneity of the oscine birds. In such an assemblage the possibilities of relationship of one group to another are numerous. These data will, perhaps, suggest to others new ideas of relationship, and thus stimulate research in other fields of taxonomic endeavor.

SUMMARY

This paper reports the results of a study of the biochemical relationships of members of fifteen families of oscine birds, a study that involves comparisons of saline-soluble proteins of skeletal muscle by the use of serological techniques. These proteins, carefully processed, were used as antigens. Antisera were produced in rabbits. The method of testing involved turbidometric analyses of precipitin reactions.

Utilizing the relative orders of reaction of the antigens with the antisera, models were constructed in an attempt to represent the serological relationships of one species to those that appeared to be most similar. For each of the families represented in this study, a diagram of the appropriate model is presented, and the implications of relationship based on data from the serological tests compared with those based on work carried out in other fields of endeavor.

Two species of the order Piciformes were investigated for purposes of comparison.

The results of this study emphasize the homogeneity of the oscine birds. In some instances the data support the placement of families in one or more of the current classifications; in other instances, new patterns of relationship are suggested. *Regulus* and *Lanius* to a great extent and *Vireo* to a lesser extent seem unlike, serologically, the other oscine species tested. It is hoped that this information will stimulate more work in regard to the families to which these species are assigned.

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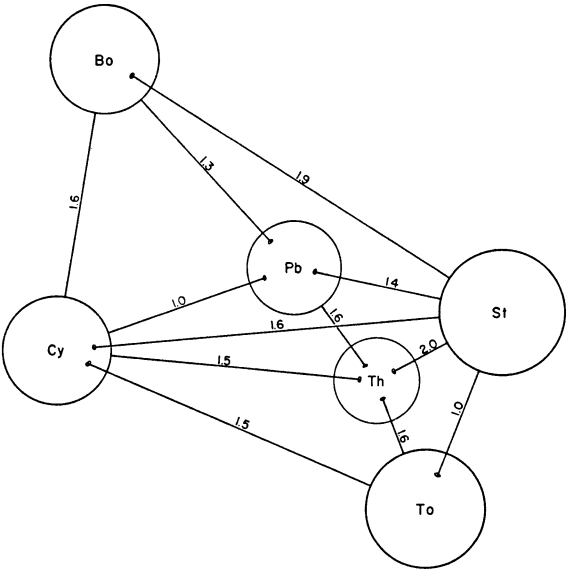

Fig. 1. Serological relationship of *Cyanocitta* to other species of oscine birds.

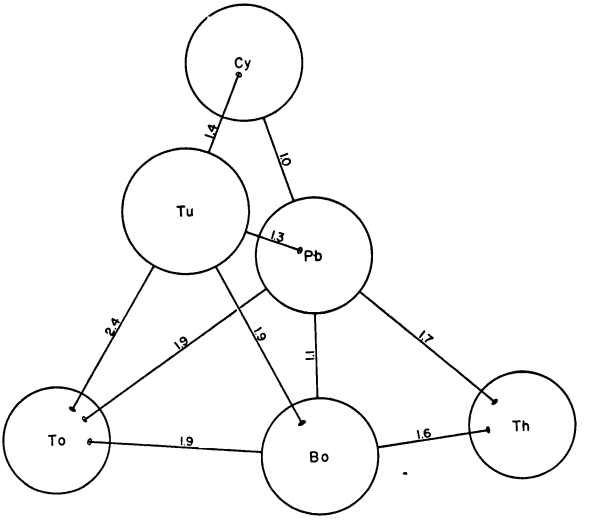

Fig. 2. Serological relationship of *Parus bicolor* to other species of oscine birds.

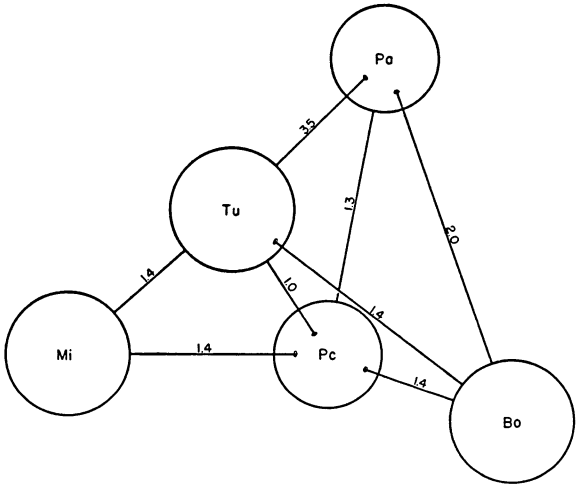

Fig. 3. Serological relationship of *Parus carolinensis* to other species of oscine birds.

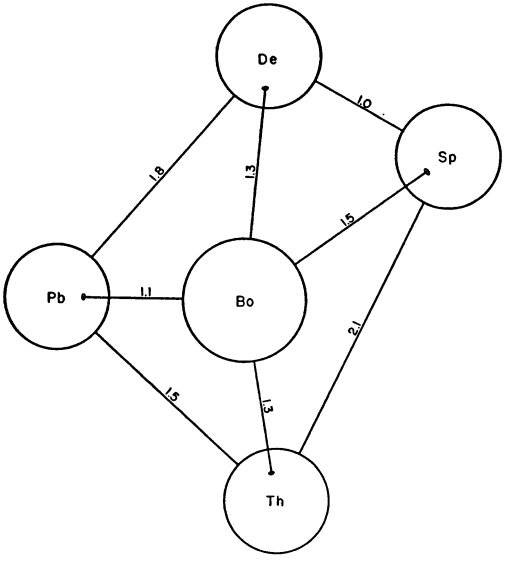

Fig. 4. Serological relationship of *Thryothorus* to other species of oscine birds.

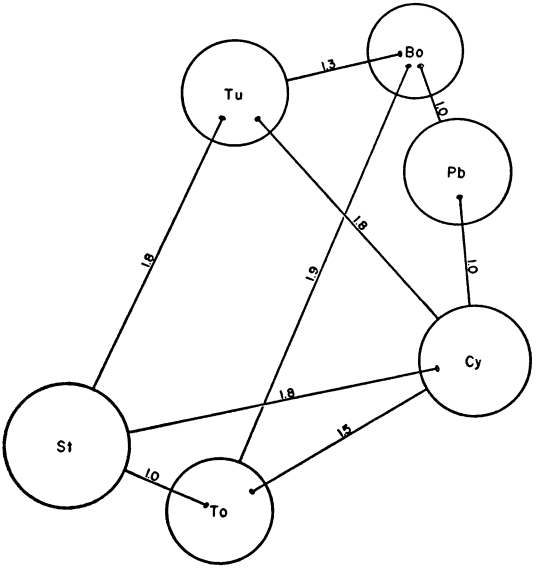

Fig. 5. Serological relationship of *Toxostoma* to other species of oscine birds.

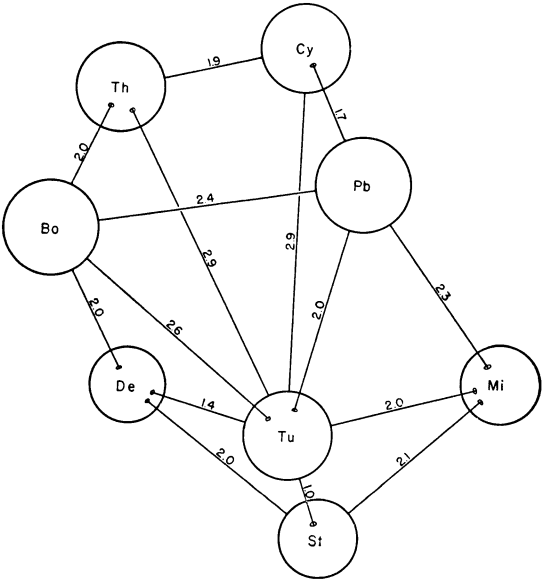

Fig. 6. Serological relationship of *Turdus* to other species of oscine birds.

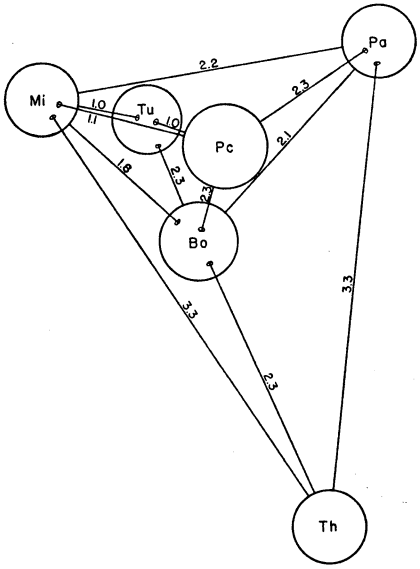

Fig. 7. Serological relationship of *Bombycilla* to other species of oscine birds.

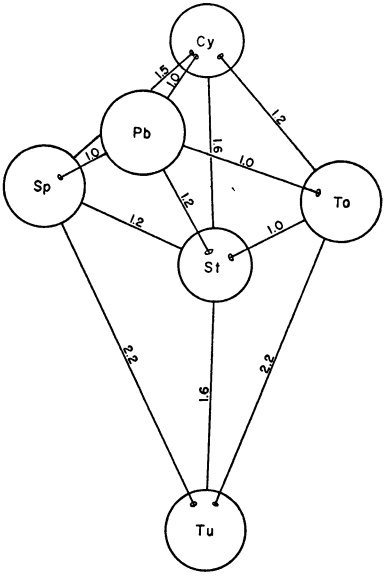

Fig. 8. Serological relationship of *Sturnus* to other species of oscine birds.

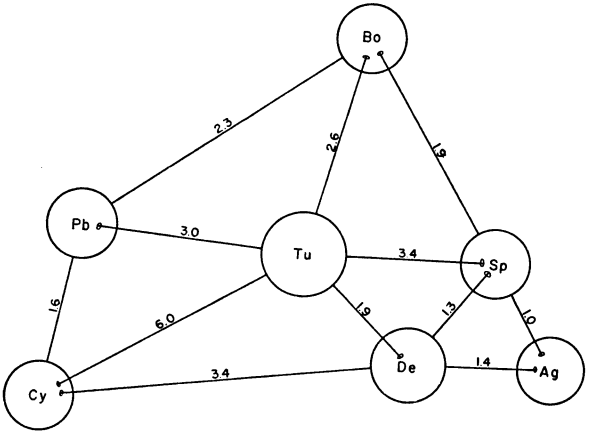

Fig. 9. Serological relationship of *Dendroica* to other species of oscine birds.

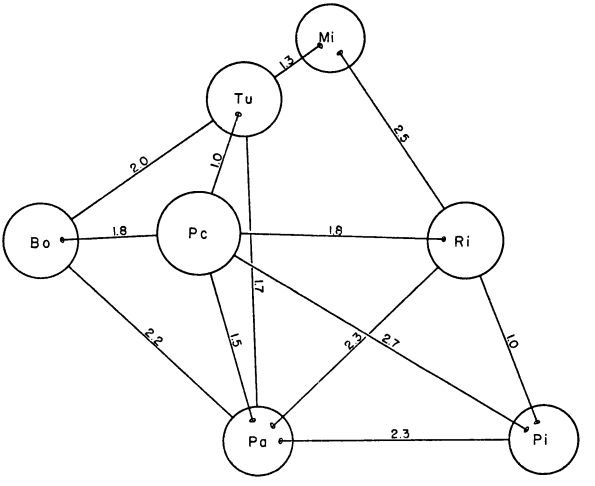

Fig. 10. Serological relationship of *Passer* to other species of oscine birds.

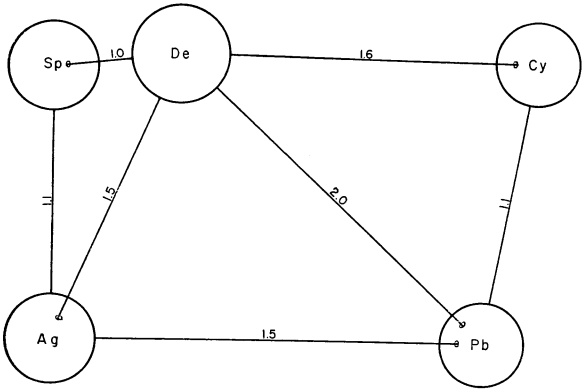

Fig. 11. Serological relationship of *Agelaius* to other species of oscine birds.

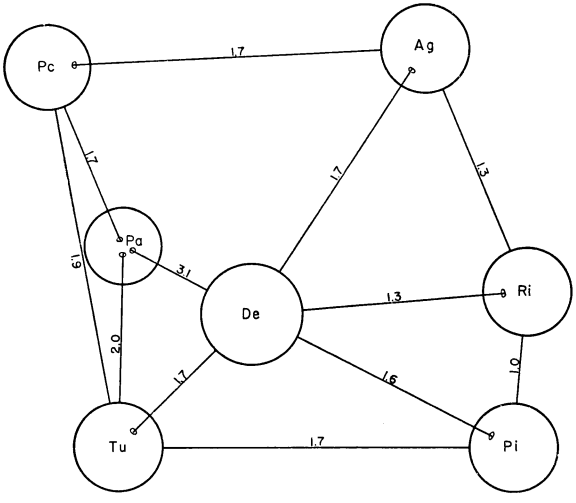

Fig. 12. Serological relationships of *Piranga* and *Richmondena* to other species of oscine birds.

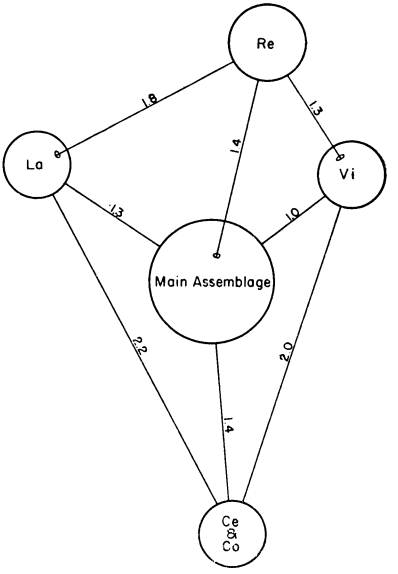

Fig 13. Serological relationship of *Colaptes, Centurus, Regulus, Lanius,* and *Vireo* to the main assemblage of oscine birds.