APPLICATIONS OF ELECTROPHORETIC DATA IN AVIAN SYSTEMATICS

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ABSTRACT.—This paper is a survey of applications of electrophoretic techniques in ornithology, with an emphasis on post-1970 publications. The majority of electrophoretic studies of birds have been limited in a variety of ways. Many have dealt with "domesticated" species or have been limited to the examination of blood and/or egg-white proteins. Problems in comparing results from different studies have arisen because of: (1) dissimilar electrophoretic techniques; (2) varying numbers of taxa; (3) nonstandardized enzyme and locus nomenclature; and, especially, (4) different methods of data analysis. These methodological problems must be addressed in order to broaden the utility of electrophoretic data in avian systematics. I suggest that the enzyme names recognized by the International Union of Biochemistry be used exclusively and that a standardized locus nomenclature, comparable with that used in other vertebrate classes, be developed. The predominating use of allozyme characters can be supplemented by "isozyme characters" (e.g. different numbers of genes, heteropolymer assembly, and regulation of expression *sensu* Buth in press), which possibly could be applied to a determination of systematic relationships of higher-level taxonomic ranks. Allozyme and/or isozyme data should be retained in particulate form (i.e. not summarized as genetic distances). The use of outgroups to assign evolutionary direction is encouraged. *Received 2 May 1983, accepted 19 April 1984.*

THE evolutionary relationships among birds have long been a matter of interest. Among vertebrates, birds seem to constitute a rather homogeneous assemblage, a situation that has contributed to the difficulty of establishing detailed systematic relationships. In attempting to determine these relationships, avian systematists have relied upon a number of taxonomic characteristics. Most have used morphological features (e.g. intestinal convolutions, musculature, nasal bones, and palate type), although some have used ecological, ethological, geographical, or physiological attributes (Van Tyne and Berger 1976). With the application of biochemical techniques to systematic analyses, a new suite of characters became available. Techniques that have used biochemical characters and that have been applied in ornithological studies include DNA-DNA hybridization (Sibley and Ahlquist 1980), immunoelectrophoresis (Ryttman et al. 1980), micro-complement fixation (Ho et al. 1976), and electrophoresis (studies reviewed herein). Historically, electrophoresis has been the most important in terms of numbers of studies using biochemical techniques to solve problems in avian biology. Electrophoresis continues to be a cost-effective method of collecting large amounts of data and providing alternative data sets for comparison with results obtained through other biochemical techniques.

Electrophoresis is a useful tool in examining a variety of genetic and developmental phenomena. In avian biology, it has been used to obtain quantitative estimates of genetic variation in natural populations, to study the patterns of gene activation, and to examine the genetic control of various proteins. Another important application of these data has been as a systematic tool. It was noted quite early that biochemical data would be useful in systematic studies (e.g. McCabe and Deutsch 1952, Sibley 1960, Gysels 1963), but "... even by 1970, a major review of electrophoretic literature included almost no discussion of uses of electrophoretic data in systematics, other than for description and identification of species" (Avise 1974: 465). Even now, there are relatively few comprehensive studies using this data base to elucidate systematic relationships among birds, particularly at higher taxonomic ranks (but see Sibley 1970 and Sibley and Ahlquist 1972 for exceptions).

Electrophoretic data provide alternative suites of characters that can be used to discern systematic relationships among birds. Initially, it was thought that biochemical (molecular) characters were more conservative than other types

of characters and therefore had greater utility. That is, it was thought that these characters would reflect the evolutionary history of the group under investigation, despite possible adaptive changes or convergences (Sibley 1965). Avise (1974) stated that electrophoretic characters were more precise and objective than other types of characters. Although it has been shown that these are not necessarily valid attributes of biochemical characters (Selander 1971, Wiley 1981), these characters are still useful in systematic studies. Some advantages to the use of electrophoretic data include high inheritance of characters, the expression of which is usually not subjected to environmental effects, and codominant phenotypic expression (Whitt 1983), which permits an accurate estimation of the allelic and genotypic composition of the sample.

Although it was initially believed that electrophoretic techniques would resolve many systematic problems, a perusal of the avian literature indicates that this has not been the case. If past studies are examined, however, problems can be evaluated and mistakes rectified so that the full potential of this technique can be realized. In this paper, I will discuss problematic areas identified in past electrophoretic studies and make suggestions to insure that future investigators will be better able to apply this technique to problems of avian systematics. Barrowclough (1983) and Corbin (1983) have recently reviewed ornithological papers employing electrophoresis (and other biochemical techniques) in microevolutionary and certain systematic studies. This review will be limited to papers in which electrophoretic data have been applied to avian systematics or in which such data have contributed to systematic applications. I will further restrict my comments to papers published between 1970 and 1984; for a review of earlier papers see Sibley et al. (1974).

HISTORICAL REVIEW

The work of Landsteiner et al. (1938), in which the egg albumin of six species was compared, was the first reported application of electrophoretic techniques to an avian study. During the next two decades, there were at least two dozen electrophoretic studies of various avian proteins, the majority of which were aimed at determining the electrophoretic constituents of blood or egg-white proteins. In the 1960's, the number of electrophoretic studies increased dramatically (Fig. 1). This increase can be attributed partially to the development of starch-gel supporting medium, combined with the use of histochemical stains to locate the positions of enzymatic proteins (Avise 1974). During the 1970's, further improvements in biochemical techniques and new methods of data analysis resulted in an increased application of electrophoresis in ornithology. I will present a synopsis of these studies after arbitrarily dividing them into three overlapping categories: biochemical genetics, population genetics, and systematics.

Biochemical genetics.—Many studies have dealt with avian biochemical genetics, and the results have supplied empirical support for certain assumptions necessary in the application of electrophoretic data to systematics. For example, it is usually assumed that the gene products examined via electrophoresis (i.e. electromorphs) are controlled by codominant alleles at a single structural gene locus, an assumption confirmed by numerous studies. Variation in conalbumin has been found to be controlled by two codominant alleles at an autosomal locus in the Black-tailed Gull (*Larus crassirostris*, Kimura 1972), the Black-crowned Night-Heron (*Nycticorax nycticorax*, Kimura and Isogai 1973), the Japanese Quail (*Coturnix japonica*, Kimura et al. 1977), and in domesticated ducks (*Anas platyrhynchos*, Przytulski and Csuka 1980). Pre-conalbumin in domesticated ducks (Przytulski and Csuka 1980) and albumin in Willow Ptarmigan (*Lagopus l. scoticus*, Henderson 1976a) were also found to be controlled by codominant expression of alleles at a single locus. As with those using egg-white proteins, many of the investigators employing blood as a tissue source have determined the genetic control of the various loci. Variation in several esterases has been found to be controlled by codominant alleles at autosomal loci in the Ringed Turtle-Dove (*Streptopelia risoria*, Bohem and Irwin 1971), the chicken (*Gallus gallus*, Tanabe and Ise 1972), the Blue Grouse (*Dendragapus obscurus*, Redfield 1973a), and in the Willow Ptarmigan (Henderson 1976b). In other tissues, the following systems were found to be expressed by codominant alleles: alkaline phosphatase (Maeda et al. 1972), adenosine deaminase (Grunder and Hollands 1977), albumin (Lucotte et al. 1978), and transferrin (Montag and Dahlgren 1973, Przytulski and Csuka 1979).

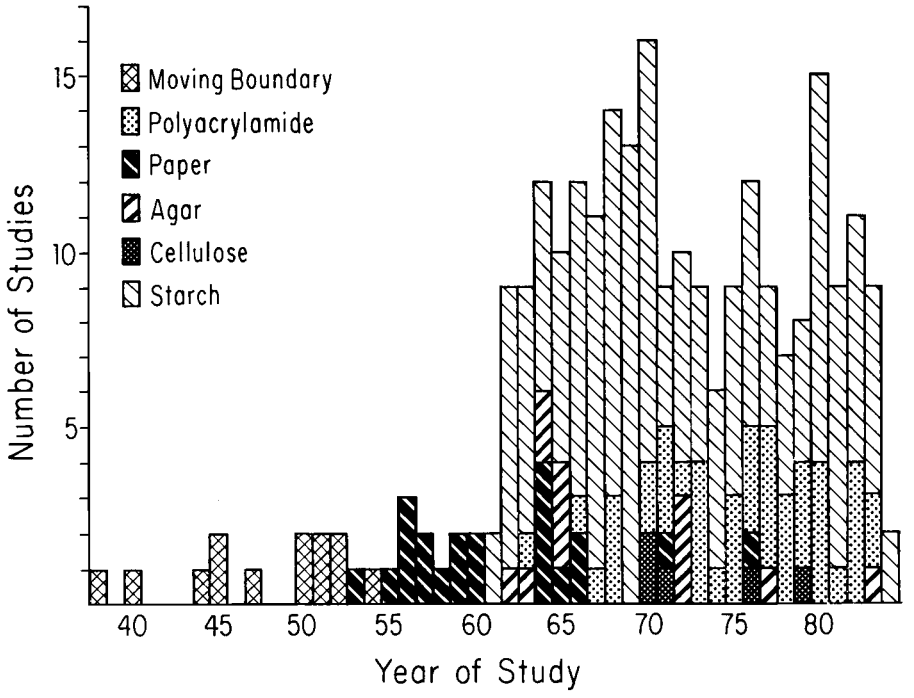

Fig. 1. Histogram depicting the changes in support media used in avian electrophoretic studies conducted from 1938 to 1984. This graph is based upon the examination of 238 papers; 258 studies are indicated, however, because some investigators employed more than one medium in a single study.

The general pattern of glucose-6-phosphate dehydrogenase in birds and other vertebrates was described by Nobrega et al. (1970). Shiraishi and Hirai (1983) have determined that mannosephosphate isomerase is controlled by autosomal codominant alleles in the Helmeted Guineafowl (*Numida meleagris*). Many of these investigators have employed mating experiments to determine the genetic basis of the electromorph patterns obtained (e.g. Maeda et al. 1972, Tanabe and Ise 1972, Przytulski and Csuka 1979, Shiraishi and Hirai 1983). Although the vast majority of these studies demonstrate that these patterns resulted from the expression of codominant alleles, some did not [e.g. esterase-4 in chickens (Tanabe and Ise 1972), prealbumin in domesticated ducks (Przytulski and Csuka 1979)]. For the purposes of data interpretation, however, it is reasonable to assume that electromorphs represent products of codominant alleles.

Patterns of gene activation have been investigated as well. The ontogeny of hemoglobins in Macaroni Penguins (*Eudyptes chrysolophus*) was studied by Shaugnessy (1970a). Brush and Scott (1972) examined developmental changes in gene expression at several loci in Red-winged Blackbirds (*Agelaius phoeniceus*). The ontogeny and activation of gene loci encoding for alcohol dehydrogenase (Le Vine and Haley 1975), 6-phosphogluconate dehydrogenase and glucose-6-phosphate dehydrogenase (Leung and Haley 1974), and lactate dehydrogenase, fructose 1,6-diphosphatase, "malic enzyme," and aspartate aminotransferase (Meyerhof and Haley 1975, 1976) have been studied in *Coturnix japonica* and *Coturnix* x *Gallus* hybrids. It was found that embryos, chicks, and adults may differentially express electrophoretically distinct forms of gene products and that enzymes are activated at different times during ontogeny. There were also indications that some maternally produced enzymes were stored in eggs (Meyerhof and Haley 1975). These findings lend credence to the conclusions of Tegelstrom et al. (1980) that, unless comparisons are made between comparable ontogenetic stages, eggs and embryos are often not the best sources of tissue.

Due to the high fat content in these embryonic tissues, resolution of electromorphs may be poor (Tegelstrom et al. 1980).

Population genetics.—During the 1970's, a large number of workers attempted to determine the amounts of genetic variation in populations and to distinguish between populations and/or subspecies (e.g. Brown et al. 1970; Shaugnessy 1970b; Redfield 1973a, b; Corbin et al. 1974; Morgan et al. 1977a, b; Guttman et al. 1980; Matson 1980; Parker et al. 1981; Ryttman and Tegelstrom 1981; Barrett and Vyse 1982). These workers used the products of relatively large numbers of loci to compute values for genetic descriptors such as percentage of polymorphic loci (P), average heterozygosity (H), and Wright's F-statistic (F) (e.g. Manwell and Baker 1975; Lucotte and Kaminski 1976a, b; Fleischer 1983; Zink and Winkler 1983). Various distance and similarity coefficients (e.g. Nei 1972, Rogers 1972) were also calculated. The results of these studies [which have been reviewed by Barrowclough (1983) and Corbin (1983)] suggest that, within species, birds are not lacking in genetic variation. Genetic distance measures, however, are generally lower among conspecific bird populations than among populations of other vertebrates (e.g. Barrowclough and Corbin 1978, Barrowclough et al. 1981, Barrowclough 1983).

Electrophoretic data have been used by others to investigate additional population-level phenomena. For example, Martin and Selander (1975), Corbin et al. (1979), Barrowclough (1980), Braun (1981), Johnson and Zink (1983), and Braun et al. (1984) have studied hybridization between species or integradation between subspecies. In general, it appears that electrophoretic data are useful in separating parental and hybrid individuals. Electrophoretic data have also been used by Johnson and Brown (1980) to investigate the breeding structure of Grey-crowned Babblers (*Pomastostomus temporalis*). Sherman (1981) encouraged the use of electrophoresis to investigate problems concerning mating systems and genealogy. Nottebohm and Selander (1972), Handford and Nottebohm (1976), and Baker (1974, 1975) used electrophoretic data in investigations of the role of song dialects in reducing gene flow between populations of *Zonotrichia capensis* and *Z. leucophrys*, respectively. Results of these studies have been equivocal. Baker and Fox (1978) examined relationships between genotypes, morphology, dominance, and mortality in captive and wild *Junco hyemalis*.

Systematics.—Investigators have used electrophoresis techniques at the interspecific level to study systematic relationships. Electrophoretic patterns of egg-white proteins (e.g. Ferguson 1971; Sibley 1973, 1974, 1976; Sibley and Ahlquist 1973) and blood proteins (e.g. Sibley and Hendrickson 1970, Ferguson and Bamford 1973, Hendrickson and Yow 1973, Ford et al. 1974, Harper 1978, Lopes et al. 1979, Mosher et al. 1982) have been used to make interspecific comparisons within a number of families. The most detailed application of electrophoretic data to avian systematics was provided by Sibley (1970) and Sibley and Ahlquist (1972), who hypothesized the relationships of 668 species of passerines and 816 species of nonpasserines, respectively. These relationships were expressed as a series of probability statements, a summary of which can be found in Sibley et al. (1974). It was Sibley's stated goal to assess degress of "genetic relatedness" among taxa examined and to develop a classification that would reflect degrees of genetic difference demonstrated between the taxa (Sibley 1970). It was assumed that the possession of similar patterns by taxa resulted from the genetic relatedness of the species examined. Variation in number and mobility of proteins was considered to be taxonomically significant (Sibley 1970), and taxa that exhibited similar electrophoretic patterns were deemed to be more closely related than taxa with different patterns. In these studies, relatively few characters were used (usually only general proteins), and there usually was no attempt to analyze data quantitatively.

The number of systematic studies increased with the advent of widespread allozyme electrophoresis in the latter part of the 1970's and into the 1980's. This corresponded with the shift away from using egg whites and/or blood as a tissue source and toward the incorporation of a variety of organs. Refinements in histochemical staining procedures made it possible to assay more enzymes, thereby increasing the number of possible characters in a study. Concurrently, a major change in the treatment of electrophoretic data occurred with the introduction of measures to quantify the genetic differences between populations. Nei (1972), Rogers (1972), and others developed mathematical formulae that summarize allelic frequencies

TABLE 1. Electrophoretic studies in which systematic relationships among avian taxa are depicted by means of clustering algorithms.

Study	Orientation	Coefficient[a]	Coding[b]	Clustering[c]
Smith and Zimmerman (1976)	Distance	Rogers (S)	NA	WPGMA
		Nei (I)	NA	WPGMA
Morgan et al. (1977b)	Distance	Jaccard	NA	Average linking
Barrowclough and Corbin (1978)	Distance	Rogers (D)	NA	Distance Wagner
Lopes et al. (1979)	Distance	—	NA	Pairing affinity
Avise et al. (1980a)	Distance	Nei (I)	NA	UPGMA
	Character	NA	+/−	Bonde
Avise et al. (1980b)	Distance	Nei (D)	NA	UPGMA
				FM
				Distance Wagner
Avise et al. (1980c)	Distance	Nei (D)	NA	UPGMA
Yang and Patton (1981)	Distance	Rogers (S)	NA	UPGMA
		Rogers (D)	NA	FM
				Distance Wagner
Avise et al. (1982)	Distance	Nei (D)	NA	UPGMA
Mosher et al. (1982)	Distance	Jaccard	NA	Average linking
Zink (1982)	Distance	Nei (D)	NA	UPGMA
		Rogers (D)		WPGMA
				FM
				Distance Wagner
Gutiérrez et al. (1983)	Distance	Rogers (D)	NA	UPGMA
				WPGMA
				FM
				Distance Wagner
Johnson and Zink (1983)	Distance	Rogers (D)	NA	UPGMA
				WPGMA
				FM
				Distance Wagner

[a] Nei (I) = genetic identity; Nei (D) = genetic distance (Nei 1972). Rogers (S) = genetic similarity; Rogers (D) = genetic distance (Rogers 1972).

[b] NA = Not Applicable; +/− = scored as presence/absence of alleles.

[c] UPGMA = unweighted pair-group method with arithmetic averages; WPGMA = weighted pair-group method with arithmetic averages (Sneath and Sokal 1973); FM = Fitch and Margoliash (1967); Distance Wagner = Farris (1972); for other methods, refer to the specific study.

across loci as a measure of the amount of difference or similarity among taxa. Biological significance was ascribed to electrophoretic data under the assumption that these distances (as measured by Nei's coefficient) actually reflected the number of codon differences per locus (Nei 1972). The distance data could be clustered to produce dendrograms depicting relationships. The most popular methods used were the UPGMA phenetic clustering technique (see Sneath and Sokal 1973) and, to a much lesser degree, methods of constructing phylogenetic trees such as the Fitch-Margoliash method (Fitch and Margoliash 1967) and the distance Wagner procedure (Farris 1972) (Table 1).

In several studies, clustering techniques have been employed. Smith and Zimmerman (1976) examined six icterid genera and, from allelic frequency data, calculated both Nei's and Rogers' coefficients of genetic similarity. Using these coefficients, they constructed phenograms depicting relationships of genera within the family. Similar types of data were collected and analyzed by means of phenetic and distance-oriented cladistic methods. A Wagner network produced by Barrowclough and Corbin (1978) depicted relationships among three genera of parulid warblers. The relationships of turdids, emberizids, parulids, mimids, and vireonids were examined by Avise and colleagues (Avise et al. 1980a, b, c, 1982). Yang and Patton (1981) discussed the relationships of 11 species of Galápagos finches, whereas Gutiérrez et al. (1983) examined 10 species of galliform birds. Zink (1982) compared morphological and genic variation in an attempt to describe the relationships among four emberizid species. Finally, Johnson and Zink (1983)

cladistically and phenetically examined electrophoretic data from four species of sapsuckers in the genus *Sphyrapicus*.

Trees based on electrophoretic data, when compared with trees constructed from other data bases (e.g. morphology, behavior, ecology), have varied in agreement. In studies by Smith and Zimmerman (1976) and Yang and Patton (1981), biochemical results were concordant with those based on more traditional data sets. In other situations, however (e.g. Zink 1982, Gutiérrez et al. 1983, Johnson and Zink 1983), the systematic relationships from electrophoretic data did not necessarily agree with those previously reported. In most of the above studies, it was found that both phenetic and cladistic types of data analysis yielded similar patterns of relationships in the taxa examined. Most authors seemed to agree that the cladistic method of data analysis was, at least theoretically, the method of choice. Although these methods represent improvements over those used in the past, there are further modifications that will increase the utility of these data in the study of evolutionary relationships. These suggested improvements will be discussed below.

OBSERVATIONS AND CONCLUSIONS

As evidenced by the foregoing discussion, electrophoresis has had a wide range of applications in ornithology. The use of this technique has been limited as a tool in systematics, however. It is generally accepted that electrophoretic data are useful at or below the generic level (e.g. Feduccia 1970) but of questionable utility in solving higher-level systematic problems (Prager et al. 1976, Bush and Kitto 1978). As noted by Buth (in press), the basis of this limitation is that only allozymes [i.e. alternative forms of an enzyme produced by different alleles at a locus (Prakash et al. 1969)] were analyzed in these studies. With higher-level taxonomic categories, allozyme characters begin to demonstrate divergence at all loci. This eventually renders the taxa totally different and leaves nothing shared among the taxa with which to assess relationships. Therefore, these data have been applied only to lower taxonomic levels, an unnecessary limitation on the use of "electrophoretic data." If the distinction between allozymes and isozymes (i.e. alternative forms of an enzyme that are the gene products of separate loci) is made, then it is possible to obtain additional information from electrophoretic data (e.g. heteropolymer formation, number of structural genes controlling multilocus systems, tissue-specific expression) for use in higher-level taxonomic studies (Buth 1981, in press). As noted by Whitt (1983: 20), the "tissue and developmental patterns of isozyme locus expression are often characteristic of a species, genus, or *higher taxon* . . ." (emphasis mine). Making this distinction between allozyme and isozyme data has been useful in solving systematic problems in fishes (Buth et al. 1980) and lizards (Murphy et al. 1983). Recognition of this distinction may increase the range of applications of electrophoretic data in avian systematics. How the distinction between allozyme and isozyme characters can be made is explained below. Other problems have also limited the application of this technique as a systematic tool. These include problems with the number of taxa examined, electrophoretic techniques employed, tissues used, nomenclature used, and methods of data analysis.

Taxa.—Of 225 avian electrophoretic papers published since 1938, 105 (47%) concentrated on nine taxa (*Meleagris gallopavo, Gallus gallus, Columba livia, Streptopelia* spp., *Coturnix japonica, Chrysolophus* spp., *Phasianus colchicus, Cairina moschata, Anas platyrhynchos*) as the primary subjects of investigation. These "domesticated" species have worked well for those investigating problems concerning the modes of inheritance of a particular gene product or the chemical structure of a particular protein. With the increased interest in population genetics and evolution, however, it became necessary to examine natural populations. Yet, even though Sibley (1970) and Sibley and Ahlquist (1972) examined egg-white proteins from 1,484 species of birds and Kuroda et al. (1982) and Kakizawa et al. (1982) examined malate dehydrogenase in 285 species, the number of bird species that have been examined for allozyme variation remains proportionately small. Since 1970, 141 species representing 7 orders and 20 families of birds (Morony et al. 1975, A.O.U. 1983) have been the subjects of electrophoretic studies involving 10 or more loci. If this technique is to continue to have an impact on avian systematics, it must be applied to natural populations from a wide variety of taxa.

Techniques.—A major problem in comparing the results of these various studies is due to the

fact that a number of different electrophoretic techniques have been used. For example, of 125 post-1970 papers examined, 81 (65%) have used starch-gel electrophoresis (including the majority of systematic studies). The remaining studies employed polyacrylamide (21%), agar (2%), cellulose (1%), or paper (1%), or some combination of these (10%) as the support medium. Although the relative advantages and disadvantages of each technique are reported elsewhere (Brewer 1970, Smith 1976, Ferguson 1980), it is important to realize that these different techniques produce different results, which are not necessarily comparable. For example, acrylamide disc electrophoresis "does not permit careful comparative studies or the detection of subtle differences in migration which may arise from genetic variation or other sources" (Brewer 1970: 48). Furthermore, caution must be observed even whem comparing results obtained with the same technique. Brush (1979) used cellulose-acetate electrophoresis to examine egg-white proteins from 63 species of birds representing seven orders. He found that different electrophoretic conditions may affect results and warned that this should be accounted for during comparisons of results from different studies. Aquadro and Avise (1982) reexamined six enzymes in nine species of thrushes and their relatives in order to assess the effect of varying electrophoretic conditions on the results obtained. They used nine buffers with varying ionic concentrations, pH values, and running times. Although their taxonomic conclusions remained essentially the same, they were able to resolve eight additional electrophoretic variants. When compared with the work of Tegelstrom et al. (1980), Zink and Winkler (1983) sharpened resolution and detected more variation in the gulls they examined by using alternative buffer systems. These examples serve to emphasize the need for screening multiple buffer systems for a determination of optimal electrophoretic conditions in order to resolve the maximum amount of variation.

Tissues.—The distinction between allozyme and isozyme characters has not been recognized in avian systematic analyses. Distinguishing between allozymes and isozymes has been complicated because of the paucity of tissues examined in past studies. In some studies conducted in the 1970's, egg white and blood (i.e. hemoglobin, plasma and serum) were used as a tissue source. Blood was a favorite tissue for electrophoretic work, because it was readily obtainable, and its collection did not necessitate sacrifice of the specimen. Other tissues were also examined. Brush (1976) and Knox (1980) had some limited success in obtaining taxonomically useful characters in feather proteins. Frenkel and Gillespie (1979) used beak proteins to determine taxonomic relationships and concluded that, even with some limitations, electrophoresis of beak proteins provided more information than electrophoresis of feather proteins. In most studies, the general protein stains employed could detect the presence of 6–12 gene products (e.g. lysozyme, ovoalbumin, transferrin), but only the overall pattern (of differences in mobilities and numbers of proteins) was considered to be of systematic importance. With the advent of allozyme electrophoresis, the number of characters available for study increased. Yet, recent studies have also been limited in numbers of tissues examined. The four most commonly examined tissues are heart, kidney, liver, and muscle, and in many studies only one or two of these are examined (e.g. Tegelstrom et al. 1980). Although examination of only one or two tissues may be necessary or even desirable in some instances, such restrictions may result in potential loss of characters. For example, Marsden and May (1984) found that feather pulp yielded more gene products than blood but fewer than internal organs. Whitt (1983: 28) has stated ". . . the tissue patterns of expression of a given set of isozymes can differ from one taxonomic group to another. . . . In such cases, these different tissue expressions can be used to understand the evolutionary and systematic relationships among species and higher taxa." Tissue-specific expression has been employed as an isozyme character by workers using lactate dehydrogenase of fishes (Shaklee and Whitt 1981), creatine kinase of amphibians and reptiles (Buth et al. in press), and NADP-dependent malate dehydrogenase ("malic enzyme") of larids and passerids (Matson unpubl. data). If isozyme characters are to be resolved and applied to systematic studies, more tissues must be routinely examined.

Increasing the number of tissues surveyed will also aid in determining the homologies of loci between taxa. "Because of the substantial differences among isozymes, and the relatively conserved tissue-specific expressions, we can

readily determine orthologous isozymes in different species, i.e., which isozyme loci of one species corresponds to which isozyme loci of another species . . ." (Whitt 1983: 20). Knowing locus homology is essential to the establishment of a standardized nomenclature and to the determination of polarity.

Nomenclature.—Comparisons of results are complicated by the lack of a standardized system of enzyme and locus nomenclature in avian studies. Independent investigators have often developed their own systems of nomenclature. This practice has resulted in a multiplicity of names for homologous enzymes and genes in different species. As noted by Buth (1983: 394): ". . . many biologists fail to keep abreast of . . . changes in enzyme nomenclature, many are unaware of the multiplicity of names, their synonyms or even published recommendations for their use! . . . The real need for improvement lies in the area of information content, i.e., improving the designations for identifying relationships among loci . . . so more accurate statements regarding homologies can be made." To avoid confusion, enzyme names should be those recognized by the International Union of Biochemistry (1979) and should be accompanied by the Enzyme Commission (E.C.) number (a practice that is on the increase). Buth's (1983) suggestions for stabilizing locus nomenclature in the ichthyological literature can and should be applied to avian studies as well. These simple steps would facilitate the comparison of results from different studies.

Systematic methods.—The most serious difficulty in comparing the results of previous avian studies is the result of different philosophies used in making systematic decisions. Phenetic techniques have been most commonly used for analysis of electrophoretic data, although more recent studies have employed cladistic methods. Cladistic analyses assess relationships that are based on the possession of synapomorphic (shared, evolutionarily derived) characters rather than on overall similarity (as in phenetic analyses). The possession of synapomorphies characterizes holophyletic groups (*sensu* Holmes 1980) that are thought to be descended from a common ancestor and therefore genealogically related. Although the theoretical advantages of cladistic analysis have been well documented (e.g. Wiley 1981), its influence has been slow to affect avian systematics (Cracraft 1981). The first avian electrophoretic paper to present results that had been analyzed cladistically was that of Barrowclough and Corbin (1978). Since then, cladistic methods have been employed more often (Table 1).

Even with this shift in systematic approaches to the use of cladistic methods, problems still remain with the treatment of data. In most systematic studies, the loci were not treated as characters, but, rather, electromorphs were converted into the allelic frequencies used to compute various distance coefficients. The distance coefficients were then analyzed cladistically. Farris (1981), however, raised serious objections to the use of *any* sort of distance data for determination of phylogenetic relationships of taxa (cf. Felsenstein 1984). Farris argued that the use of Nei's distance is not valid, because this measure does not satisfy the triangle inequality. [The triangle inequality states that, when the distances between three taxa (A,B,C) are compared, the following mathematical relationship must hold: distance (A,C) $\leq$ distance (A,B) + distance (B,C).] Nonmetricity makes it impossible to interpret the network branch lengths in a phylogenetically meaningful way. For distance coefficients that are metric (e.g. Rogers' distance), the results are no better but for different reasons. On a tree constructed using any distance coefficient, Farris demonstrated that the common ancestor of three taxa cannot exist on any branch that connects any two of the three taxa. This is clearly impossible, and it lead Farris (1981: 18) to conclude that metrics ". . . no more yield physically interpretable branch lengths than do nonmetrics . . . ." He suggested analyzing electrophoretic data by using the electromorphs directly and discarding the use of frequency data. Farris (1981: 22) further stated that ". . . there is not much comparative information in the frequencies beyond simple presences . . ." and that "direct phylogenetic analysis of alleles as characters, moreover, avoids the information loss that attends reducing character data to distances."

Some problems with distance data can be avoided by the use of character/state data. This type of data has an advantage over distance data in that homoplasious steps may be identified, and branch order and length have biological interpretation (Buth in press). Few students of birds have attempted to analyze electrophoretic data cladistically by means of characters

and character states. In one study (Avise et al. 1980a), alleles were treated as characters, the presence/absence of a given allele being considered the character state. This "independent alleles" method of encoding data, however, has recently been critically examined by Mickevich and Mitter (1981), who suggested alternative methods for encoding electrophoretic data. In the new interpretation, the locus *per se* is considered to be the character and the allelic composition of a locus to be the character state. This recognition of characters and character states in electrophoretic data is biologically more meaningful (after all, some allele *must* occupy a given locus). Although Zink (1982) and Gutiérrez et al. (1983) mention encoding data in this manner, detailed results using this coding method have yet to be reported in any avian study.

Problems also exist in linking characters into an evolutionary sequence when two or more states are recognized. Although several ordering methods have been developed by Mickevich and Mitter (1981, 1983), this area of systematics remains a problem (Buth in press). Nevertheless, use of character-state data deserves consideration and may be thought of as an alternative to distance coefficients.

A problem related to the ordering of character states is the establishment of evolutionary polarity. Part of the reason that loci were not treated directly as characters was because many investigators thought it difficult to establish polarity of biochemical characters (e.g. Brush 1979). There is no reason why, given a sufficient number of characters and more than one potential outgroup, that outgroup methods of inferring evolutionary polarity would not be applicable in avian electrophoretic studies. Baverstock et al. (1979) have shown that standard outgroup methods are suitable for establishing such polarity. A herpetological study by Murphy et al. (1983) demonstrated the successful use of the outgroup method for allozyme data. If the outgroup method cannot be applied, other methods for determining polarity exist (Crisci and Stuessy 1980, Stevens 1980). For isozymes (but not allozymes), heteropolymer assembly (i.e. the interaction of different enzyme subunits) in multimeric enzymes can be used as both an isozyme character and a method of determining polarity. The restriction of heteropolymer formation has been used as a derived character uniting taxa (e.g. Gorman 1971, Buth et al. 1980). The ability of multimeric enzymes to form heteropolymers is assumed to represent the primitive condition (Buth in press). Thus, while outgroup analysis is the method of choice in inferring polarity, alternatives are available.

As Markert (1983: 16) noted: "All problems in biology involving *genes* and *enzymes* are candidates for the application of our technique and our understanding of isozymes. The research frontier is expanding, not shrinking, and isozymes are certain to make basic contributions to the solution of problems in many areas of biology simply because they are a fundamental and pervasive manifestation of the structure and function of organisms." Electrophoresis has played an important role in ornithology during the past 46 yr. If former inadequacies in data gathering and data analysis are addressed, electrophoresis may prove even more useful in the future. This can be assured by sampling a wider array of taxa and tissues and by using isozyme as well as allozyme data. Investigators should encode data by using the locus as the character, retain data in particulate form, and use shared derived characters to infer phylogeny. Only then will the full potential of this technique be realized in avian systematics.

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Literature Cited

American Ornithologists' Union. 1983. Check-list of North American birds, sixth ed. Washington, D.C., American Ornithologists' Union.

Aquadro, C. F., & J. C. Avise. 1982. Evolutionary genetics of birds. VI. A reexamination of protein divergence using varied electrophoretic conditions. Evolution 36: 1003–1019.

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

———, J. C. PATTON, & C. F. AQUADRO. 1980a. Evolutionary genetics of birds. I. Relationships among North American thrushes and allies. Auk 97: 135-147.

———, ———, & ———. 1980b. Evolutionary genetics in birds. II. Conservative protein evolution in North American sparrows and relatives. Syst. Zool. 29: 323-334.

———, ———, & ———. 1980c. Evolutionary genetics of birds. J. Hered. 71: 303-310.

———, C. F. AQUADRO, & J. C. PATTON. 1982. Evolutionary genetics of birds. V. Genetic distances within Mimidae (mimic thrushes) and Vireonidae (vireos). Biochem. Genet. 20: 95-104.

BAKER, M. C. 1974. Genetic structure of two populations of White-crowned Sparrows with different song dialects. Condor 76: 351-356.

———. 1975. Song dialects and genetic differences in White-crowned Sparrows (*Zonotrichia leucophrys*). Evolution 29: 226-241.

———, & S. F. FOX. 1978. Dominance, survival, and enzyme polymorphism in Dark-eyed Juncos, *Junco hyemalis*. Evolution 32: 697-711.

BARRETT, V. A., & E. R. VYSE. 1982. Comparative genetics of three Trumpeter Swan populations. Auk 99: 103-108.

BARROWCLOUGH, G. F. 1980. Genetic and phenotypic differentiation in a wood warbler (genus *Dendroica*) hybrid zone. Auk 97: 655-668.

———. 1983. Biochemical studies of microevolutionary processes. Pp. 223-261 *in* Perspectives in ornithology (A. H. Brush and G. A. Clark, Eds.). New York, Cambridge Univ. Press.

———, & K. W. CORBIN. 1978. Genetic variation and differentiation in the Parulidae. Auk 95: 691-702.

———, ———, & R. M. ZINK. 1981. Genetic differentiation in the Procellariiformes. Comp. Biochem. Physiol. 69B: 629-632.

BAVERSTOCK, P. R., S. R. COLE, B. J. RICHARDSON, & C. H. S. WATTS. 1979. Electrophoresis and cladistics. Syst. Zool. 28: 214-219.

BOHEM, L. G., & M. R. IRWIN. 1971. Cellular esterases of the ringneck dove. J. Hered. 62: 44-45.

BRAUN, M. J. 1981. Hybridization and high protein variability in finches of the genus *Pipilo*. Isozyme Bull. 14: 90.

BRAUN, D., G. B. KITTO, & M. J. BRAUN. 1984. Molecular population genetics of tufted and black-crested forms of *Parus bicolor*. Auk 101: 170-173.

BREWER, G. J. 1970. An introduction to isozyme techniques. New York, Academic Press.

BROWN, I. R. F., W. H. BANNISTER, & C. DELUCCA. 1970. A comparison of Maltese and Sicilian sparrow haemoglobins. Comp. Biochem. Physiol. 34: 557-562.

BRUSH, A. H. 1976. Waterfowl feather proteins: analysis of use in taxonomic studies. J. Zool. 179: 467-498.

———. 1979. Comparison of egg-white proteins: effect of electrophoretic conditions. Biochem. Syst. Ecol. 7: 155-165.

———, & A. F. SCOTT. 1972. Development of protein polymorphisms in Redwing Blackbirds. J. Embryol. Exp. Morphol. 28: 481-489.

BUSH, G. L., & G. B. KITTO. 1978. Applications of genetics to insect systematics and analysis of species differences. Pp. 89-118 *in* Biosystematics in agriculture (J. A. Romberger, Ed.). New York, John Wiley and Sons.

BUTH, D. G. 1981. Cladistic treatment of isozyme (rather than allozyme) data. Presentation at the Willi Hennig Society, 2nd Annual Meeting. Ann Arbor, Michigan.

———. 1983. Duplicate genes in fishes: origins, distributions, phyletic consequences and locus nomenclature. Pp. 381-400 *in* Isozymes: current topics in biological and medical research, vol. 10 (M. C. Rattazzi, J. G. Scandalios, and G. S. Whitt, Eds.). New York, Alan R. Liss, Inc.

———. In press. This application of electrophoretic data in systematic studies. Ann. Rev. Ecol. Syst.

———, B. M. BURR, & J. R. SCHENCK. 1980. Electrophoretic evidence for relationships and differentiation among members of the percid subgenus *Microperca*. Biochem. Syst. Ecol. 8: 297-304.

———, R. W. MURPHY, M. M. MIYAMOTO, & C. S. LIEB. In press. Creatine kinases of amphibians and reptiles: evolutionary and systematic aspects of gene expression. Copeia.

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.

CORBIN, K. W. 1983. Genetic structure and avian systematics. Pp. 211-244 *in* Current ornithology, vol. 1. (R. F. Johnston, Ed.). New York, Plenum Press.

———, C. G. SIBLEY, & A. FERGUSON. 1979. Genic changes associated with the establishment of sympatry in orioles of the genus *Icterus*. Evolution 33: 624-633.

CRACRAFT, J. 1981. Toward a phylogenetic classification of the recent birds of the world (class Aves). Auk 98: 681-714.

CRISCI, J. V., & T. F. STUESSY. 1980. Determining primitive character states for phylogenetic reconstructions. Syst. Bot. 5: 12-135.

FARRIS, J. S. 1972. Estimating phylogenetic trees from distance matrices. Amer. Natur. 106: 645-668.

———. 1981. Distance data in phylogenetic analysis. Pp. 3-23 *in* Advances in cladistics (V. A. Funk and D. R. Brooks, Eds.). New York, New York Botanical Gardens.

FEDUCCIA, J. A. 1970. Variation in avian plasma proteins. Condor 72: 498-499.

FELSENSTEIN, J. 1984. Distance methods for inferring phylogenies: a justification. Evolution 38: 16-24.

FERGUSON, A. 1971. Geographic and species variation in transferrin and ovotransferrin polymorphism in the Columbidae. Comp. Biochem. Physiol. 38B: 477-486.

———. 1980. Biochemical systematics and evolution. New York, John Wiley and Sons.

———, & D. R. BAMFORD. 1973. An electrophoretic study of the plasma and egg-white proteins of *Columba* and *Streptopelia*. Comp. Biochem. Physiol. 44B: 803-817.

FITCH, W. M., & E. MARGOLIASH. 1967. Construction of phylogenetic trees. Science 155: 279-284.

FLEISCHER, R. C. 1983. A comparison of theoretical and electrophoretic assessments of genetic structure in populations of the House Sparrow (*Passer domesticus*). Evolution 37: 1001-1009.

FORD, H. A., A. W. EWING, & D. T. PARKIN. 1974. Blood proteins in Darwin's finches. Comp. Biochem. Physiol. 47B: 369-375.

FRENKEL, M. J., & J. M. GILLESPIE. 1979. Proteins of beaks: possible use in taxonomy of birds. Australian J. Zool. 27: 443-452.

GORMAN, G. C. 1971. Evolutionary genetics of island lizard populations. Year Book Amer. Phil. Soc.: 318-319.

GRUNDER, A. A., & K. G. HOLLANDS. 1977. Inheritance of electrophoretic variants of adenosine deaminase in chickens and turkeys. Can. J. Genet. Cytol. 19: 574 (abstr.).

GUTIÉRREZ, R. J., R. M. ZINK, & S. Y. YANG. 1983. Genic variation, systematic, and biogeographic relationships of some galliform birds. Auk 100: 33-47.

GUTTMAN, S. I., G. A. GRAU, & A. A. KARLIN. 1980. Genetic variation in Lake Erie (USA) Great Blue Herons (*Ardea herodias*). Comp. Biochem. Physiol. 66B: 167-169.

GYSELS, H. 1963. New biochemical techniques applied to avian systematics. Experientia 19: 107-108.

HANDFORD, P., & F. NOTTEBOHM. 1976. Allozymic and morphological variation in population samples of Rufous-collared Sparrow, *Zonotrichia capensis*, in relation to vocal dialects. Evolution 30: 802-817.

HARPER, P. C. 1978. The plasma proteins of some albatrosses and petrels as an index of relationship in the Procellariiformes. New Zealand J. Zool. 5: 509-549.

HENDERSON, B. A. 1976a. Protein polymorphism in the egg albumen of Red Grouse (*L. lagopus scoticus*). Heredity 36: 413-415.

———. 1976b. Black bands on a starch gel stained for serum esterases in Red Grouse. J. Hered. 67: 254-255.

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.

HO, C. Y. K., E. M. PRAGER, A. C. WILSON, D. T. OSUGA, & R. E. FEENEY. 1976. Penguin evolution: protein comparisons demonstrate phylogenetic relationship to flying aquatic birds. J. Molec. Evol. 8: 271-282.

HOLMES, E. B. 1980. Reconsideration of some systematic concepts and terms. Evol. Theory 5: 35-87.

INTERNATIONAL UNION OF BIOCHEMISTRY. 1979. Enzyme nomenclature, 1978. New York, Academic Press.

JOHNSON, M. S., & J. L. BROWN. 1980. Genetic variation among trait groups and apparent absence of close inbreeding in Grey-crowned Babblers. Behav. Ecol. Sociobiol. 7: 93-98.

JOHNSON, N. K., & R. M. ZINK. 1983. Speciation in sapsuckers (*Sphyrapicus*): I. Genetic differentiation. Auk 100: 871-884.

KAKIZAWA, R., N. KURODA, & S. UTIDA. 1982. Evolution of mitochondrial malate dehydrogenase in birds (II). J. Yamashina Inst. Ornithol. 14: 131-136.

KIMURA, M. 1972. An intraspecific variation of the egg-white protein of the black-tailed gull (*Larus crassirostris*). Japanese J. Genet. 47: 301-304.

———, & I. ISOGAI. 1973. Electrophoretic variation of egg white proteins of the night heron (*Nycticorax nycticorax*). Res. Bull. Fac. Agri., Gifu Univ. 34: 353-359. (In Japanese.)

———, S. ITO, Y. MORISHIMA, & I. ISOAGI. 1977. Conalbumin electrophoretic pattern of the quail *Coturnix coturnix japonica*. Japanese J. Genet. 52: 251-255.

KNOX, A. G. 1980. Feather protein as a source of avian taxonomic information. Comp. Biochem. Physiol. 65B: 45-54.

KURODA, N., R. KAKIZAWA, H. HORI, Y. OSAKA, N. USUDA, & S. UTIDA. 1982. Evolution of mitochondrial malate dehydrogenase in birds. J. Yamashina Inst. Ornithol. 14: 1-15.

LANDSTEINER, K., L. G. LONGSWORTH, & J. VAN DER SCHEER. 1938. Electrophoretic experiments with egg albumins and hemoglobins. Science 88: 83-85.

LEUNG, E., & L. E. HALEY. 1974. The ontogeny of phosphogluconate dehydrogenase and glucose-6-phosphate dehydrogenase in Japanese quails and chicken-quail hybrids. Biochem. Genet. 11: 221-230.

LE VINE, J. P., & L. E. HALEY. 1975. Gene activation of alcohol dehydrogenase in Japanese quail and chicken-quail hybrid embryos. Biochem. Genet. 12: 435-441.

LOPES, C. R., E. J. DE LUCCA, A. M. DE ANDRADE, & C. M. J. FAULIN. 1979. A phylogenetic interpretation of chromosomal and electrophoretic data in Columbiformes. Cytologica 44: 39-48.

LUCOTTE, G., & M. KAMINSKI. 1976a. Polychromatisme et polymorphisme biochimique chez cinq espèces de phaisanides. Biochem. Syst. Ecol. 4: 69-71.

——— & ———. 1976b. Polymorphisme biochimique chez le faisan commun (*Phasianus colchicus*). Biochem. Syst. Ecol. 4: 223-226.

———, ———, & A. PERRAMON. 1978. Heterosis in the codominance model: electrophoretic studies of proteins in the chicken-quail hybrid. Comp. Biochem. Physiol. 60B: 169-171.

MAEDA, Y., T. HASHIGUCHI, & M. TAKETOMI. 1972. Genetical studies on serum alkaline phosphatase isozyme in the Japanese quail. Japanese J. Genet. 47: 165-170.

MANWELL, C., & C. M. A. BAKER. 1975. Molecular genetics of avian proteins XIII. Protein polymorphism in three species of Australian passerines. Australian J. Biol. Sci. 28: 545-557.

MARKERT, C. L. 1983. Isozymes: conceptual history and biological significance. Pp. 1-17 *in* Isozymes: current topics in biological and medical research, vol. 7 (M. C. Rattazzi, J. G. Scandalios, and G. S. Whitt, Eds.). New York, Alan R. Liss, Inc.

MARSDEN, J. E., & B. MAY. 1984. Feather pulp: a nondestructive sampling technique for electrophoretic studies of birds. Auk 101: 173-175.

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

MATSON, R. H. 1980. Genetic variation in two insular populations of the house finch, *Carpodacus mexicanus* (Aves: Fringillidae). Unpublished M.S. thesis. Long Beach, California, California State Univ.

MCCABE, R. A., & H. F. DEUTSCH. 1952. The relationships of certain birds as indicated by their egg-white proteins. Auk 69: 1-18.

MEYERHOF, P. G. & L. E. HALEY. 1975. Ontogeny of lactate dehydrogenase isozymes in chicken-quail hybrid embryos. Biochem. Genet. 13: 7-18.

———, & ———. 1976. Initial expression of the genes for fructose 1-6 diphosphatase, malic enzyme, and aspartate aminotransferase in Japanese quail and chicken-quail hybrid embryos. Biochem, Genet. 13: 457-470.

MICKEVICH, M. F., & C. MITTER. 1981. Treating polymorphic characters in systematics: a phylogenetic treatment of electrophoretic data. Pp. 45-58 *in* Advances in cladistics (V. A. Funk and D. R. Brooks, Eds.). New York, New York Botanical Gardens.

———, & ———. 1983. Evolutionary patterns in allozyme data: a systematic approach. Pp. 169-176 *in* Advances in cladistics, vol. 2 (N. I. Platnick and V. A. Funk, Eds.). New York, Columbia Univ. Press.

MONTAG, D. G., & R. B. DAHLGREN. 1973. Transferrin polymorphism in the Ring-necked Pheasant. Auk 90: 318-323.

MORGAN, R. P., S. T. SULKIN, & S. B. BLOCK. 1977a. Serum proteins of Sandhill Cranes and Bald Eagles. Comp. Biochem. Physiol. 57B: 297-298.

———, ———, & C. J. HENNY. 1977b. Serum proteins of Canada Goose (*Branta canadensis*) subspecies. Condor 79: 275-278.

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

MOSHER, J. A., R. P. MORGAN, II, W. L. GOODFELLOW, JR., E. A. HAUG, & T. C. ERDMAN. 1982. Serum proteins of selected falconiformes and strigiformes. Biochem. Syst. Ecol. 10: 373-376.

MURPHY, R. W., W. E. COOPER, & W. S. RICHARDSON. 1983. Phylogenetic relationships of the North American five-lined skinks, genus *Eumeces* (Sauria: Scincidae). Herpetologia 39: 200-211.

NEI, M. 1972. Genetic distances between populations. Amer. Natur. 106: 283-292.

NOBREGA, F. G., J. C. C. MAIA, W. COLLI, & P. H. SALDANHA. 1970. Heterogeneity of erythrocyte glucose-6-phosphate dehydrogenase (G6PD, E.C. 1.1.1.49) activity and electrophoretic patterns among representatives of different classes of vertebrates. Comp. Biochem. Physiol. 33: 191-199.

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

PARKER, L. E., E. G. BOLEN, & R. J. BAKER. 1981. Genetic variation in a winter population of Mallard ducks. Southwestern Natur. 26: 425-428.

PRAGER, E. M., A. C. WILSON, D. T. OSUGA, & R. E. FEENEY. 1976. Evolution of flightless land birds on southern continents: transferrin comparison shows monophyletic origin of ratites. J. Molec. Evol. 8: 282-294.

PRAKASH, S., R. C. LEWONTIN, & J. L. HUBBY. 1969. A molecular approach to the study of genic heterozygosity in natural populations. IV. Patterns of genic variation in central, marginal and isolated populations of *Drosophila pseudoobscura*. Genetics 61: 841-858.

PRZYTULSKI, T., & J. CSUKA. 1979. Electrophoretic characterization of serum protein and enzyme in the interspecific hybrids of ducks. Comp. Biochem. Physiol. 62B: 249-250.

———, & ———. 1980. Further polymorphic proteins in egg white in the inter-genetic hybridization of ducks. Comp. Biochem. Physiol. 67B: 711-714.

REDFIELD, J. A. 1973a. Demography and genetics in colonizing populations of Blue Grouse (*Dendragapus obscurus*). Evolution 27: 576-592.

———. 1973b. The use of incomplete family data in the analysis of genetics and selection at the

Ng locus in Blue Grouse (*Dendragapus obscurus*). Heredity 31: 35–42.

ROGERS, J. S. 1972. Measures of genetic similarity and genetic distance. Univ. Texas Publ. 7213: 145–153.

RYTTMAN, H., & H. TEGELSTROM. 1981. Low degree of isozyme variation within and between Herring Gull (*Larus argentatus*), Lesser Black-backed Gull (*Larus fuscus*), and their British and Swedish subspecies. Hereditas 94: 161–164.

———, ———, & H. JANSSON. 1980. Immunoelectrophoretical comparison of sera from Herring Gull (*Larus argentatus*) and Lesser Black-backed Gull (*Larus fuscus*). Hereditas 92: 113–116.

SELANDER, R. K. 1971. Systematics and speciation in birds. Pp. 57–147 *in* Avian biology (D. S. Farner and J. R. King, Eds.). New York, Academic Press.

SHACKLEE, J. B., & G. S. WHITT. 1981. Lactate dehydrogenase isozymes of gadiform fishes: divergent patterns of gene expression indicate heterogeneous taxon. Copeia 1981: 563–578.

SHAUGNESSY, P. D. 1970a. Ontogeny of haemoglobin in the Royal Penguin *Eudyptes chrysolophus schlegeli*. J. Embryo. Exp. Morphol. 24: 425–428.

———. 1970b. Serum proteins of two sibling species of the giant petrel (*Macronetes* spp.). Comp. Biochem. Physiol. 33: 721–723.

SHERMAN, P. W. 1981. Electrophoresis and avian genealogical analyses. Auk 98: 419–422.

SHIRAISHI, K., & Y. HIRAI. 1983. Genetic variants of liver and pancreas mannosephosphate isomerase in the Guinea Fowl. Japanese J. Genet. 58: 373–375.

SIBLEY, C. G. 1960. The electrophoretic patterns of avian egg-white as taxonomic characters. Ibis 102: 215–259.

———. 1965. Molecular systematics: new techniques applied to old problems. Oiseau 35: 112–124.

———. 1970. A comparative study of the egg-white proteins of passerine birds. Peabody Mus. Nat. Hist. Bull. 32: 1–131.

———. 1973. The relationships of the silky flycatchers. Auk 90: 394–410.

———. 1974. The relationships of the lyrebirds. Emu 74: 65–78.

———. 1976. Protein evidence of the relationships of some Australian passerine birds. Proc. 16th Intern. Ornithol. Congr.: 557–570.

———, & J. E. AHLQUIST. 1972. A comparative study of the egg-white proteins of non-passerine birds. Peabody Mus. Nat. Hist. Bull. 39: 1–276.

———, & ———. 1973. The relationships of the hoazin. Auk 90: 1–13.

———, & ———. 1980. The relationships of "primitive insect eaters" (Aves: Passeriformes) as indicated by DNA × DNA hybridization. Proc. 17th Intern. Ornithol. Congr.: 1215–1220.

———, & H. T. HENDRICKSON. 1970. A comparative electrophoretic study of avian plasma proteins. Condor 72: 43–49.

———, K. W. CORBIN, J. E. AHLQUIST, & A. FERGUSON. 1974. Birds. Pp. 89–176 *in* Biochemical and immunological taxonomy of animals (A. Wright, Ed.). New York, Academic Press.

SMITH, I. 1976. Chromatographic and electrophoretic techniques, vol. 2, fourth ed. Chicago, William Heinemann Medical Books Publ.

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. A., & R. R. SOKAL. 1973. Numerical taxonomy. San Francisco, W. H. Freeman and Co.

STEVENS, P. F. 1980. Evolutionary polarity of character states. Ann. Rev. Ecol. Syst. 11: 333–358.

TANABE, Y. & T. ISE. 1972. Inheritance of electrophoretic variants of liver esterases in the chicken. Japanese J. Genet. 47: 257–263.

TEGELSTROM, H., H. JANSSON, & H. RYTTMAN. 1980. Isozyme differences among three related *Larus* species (Aves). Hereditas 92: 117–122.

VAN TYNE, J., & A. J. BERGER. 1976. Fundamentals of ornithology, second ed. New York, John Wiley and Sons.

WHITT, G. S. 1983. Isozymes as probes and participants in developmental and evolutionary genetics. Pp. 1–40 *in* Isozymes: current topics in biological and medical research, vol. 7 (M. C. Rattazzi, J. G. Scandalios, and G. S. Whitt, Eds). New York, Alan R. Liss, Inc.

WILEY, E. O. 1981. Phylogenetics: the theory and practice of phylogenetic systematics. New York, John Wiley and Sons.

YANG, S. Y., & J. L. PATTON. 1981. Genic variability and differentiation in the Galapagos finches. Auk 98: 230–242.

ZINK, R. M. 1982. Patterns of genic and morphologic variation among sparrows in the genera *Zonotrichia, Melospiza, Junco,* and *Passerela*. Auk 99: 632–649.

———, & D. W. WINKLER. 1983. Genetic and morphological similarity of two California gull populations with different life history traits. Biochem. Syst. Ecol. 11: 397–403.