BIOCHEMICAL GENETICS OF CERTAIN SPECIES OF THE BLACKBIRD FAMILY ICTERIDAE

THESIS

Presented to the Graduate Council of the
North Texas State University in Partial
Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

By

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December, 1974

Smith, Jackson K., Biochemical Genetics of Certain Species of the Blackbird Family Icteridae. Master of Science (Biology), December, 1974, 65 pp., 13 tables, 12 figures, bibliography, 50 titles.

Starch gel electrophoresis was used to compare 14 proteins encoded by 15 loci for seven species of the family Icteridae. A close genetic relationship among these species was classified into three groups. The Agelaiine group contained Agelaius phoeniceus, Sturnella magna, and S. neglecta. The Quiscaline group contained Euphagus cyanocephalus, Cassidix mexicanus, and Quiscalus quiscula. Molothrus ater, the most divergent, was placed in a separate group. Divergence times for the seven species were compared to the literature. Heterozygosity of the seven populations of the two species of Sturnella were compared to determine factors influencing their divergence. Two factors proposed were heterosis in S. neglecta and possible hybridization between S. neglecta and S. magna.

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CHAPTER I

INTRODUCTION

The Avian Family Icteridae (Troupials or New World Blackbirds) is a group of sparrow to crow-sized songbirds that occur only in the Western Hemisphere. It is comprised of 36 genera including 100 extant species (Storer 1960), of which 10 genera and 22 species occur north of Mexico (Bent 1965). The family is a distinct taxon of birds, easily separated from other families, and is characterized by having plumage of uniform black (often irridescent) or bold combinations of black, brown, buff, orange, crimson, yellow, and sometimes white. The bill is conical in shape, varying from short and massive to moderately long and slender, with nostrils always exposed. The wings have nine primary flight feathers, characteristic of New World passerine birds (Van Tyne and Berger 1959). Because of the generalized habits of the ancestry of the Icteridae, the species within the family have invaded nearly all food niches occupied by passerines within their range (Beecher 1951). Thus, this family offers the evolutionist an excellent group for modeling evolution within a large taxonomic unit of birds.

Most studies of classification of birds have been restricted to the use of coloration of plumage or a few

morphological characters such as bill shape and skull, foot, wing, and syrinx structure. Beecher (1953) proposed the use of jaw and skull musculature and bill shape to determine the phylogenetic relationships of birds. Beecher (1951) also established a phylogeny for the genera within the Family Icteridae. He suggested that the genus Molothrus (Cowbirds) was the most primitive and probably gave rise to three lines: the Agelaiine (Blackbirds), Quiscaline (Grackles), and Cacique groups. The Agelaiine line is composed of the genus Agelaius (Redwings) which gave rise to the genera Sturnella (Meadowlarks) and Icterus (Orioles). The Quiscaline line is composed of the genus Gnorimospar which gave rise to Dives which, in turn, gave rise to Euphagus (Blackbirds), Cassidix (Boat-tail Grackles), and Quiscalus (Common Grackles). The Cacique line includes a group of South American blackbirds and, therefore, will not be treated in this study.

Beecher showed that the Cowbird, Molothrus ater, was probably the ancestor for all species of the Icteridae because it has a generalized seed-cracking bill and jaw musculature that indicates common ancestry with the finches. The bill of Agelaius (Agelaiine line) is adapted for an insectivorous diet, and the muscle mass of the jaw is reduced because of the loss of seed eating activity. The bill of Sturnella has become adapted for insect gathering, and the muscle structure is modified for a gaping function. Beecher (1951) showed a departure from seed cracking to insect gathering in the bill

and a corresponding change in the muscle structure in Cassidix and Quiscalus (Quiscaline line). He also found a trend toward a sawing function (an adaptation for opening acorns) in the Grackle bill, with this condition most advanced in Quiscalus.

Mayr (1955) criticized Beecher's classification, proposing that several of Beecher's (1951) assumptions of Icterid evolution were faulty and indicated many pitfalls in using morphology to determine phylogeny among birds. Mayr (1955) stated that morphological characters are often the result of the development of other morphological features, and that similarity of anatomical characters may also be the result of convergence. Morphological characteristics are also generally controlled by several to many genes, and this complexity often makes determination of phylogenetic relationships difficult.

Since protein similarity or diversity is a good index to gene structure of organisms as controlled by DNA, it is preferable to morphological characters in studying genetic changes in populations. Recent attention has been given to the use of electrophoretic techniques to determine allelic frequencies for populations, and these techniques have great appeal to population geneticists and evolutionists. Nei (1971) stated that since the relationship between the chemical structure of genes or DNA and the amino acid sequence of proteins has been firmly established, it is best to study gene differences by examining the amino acid sequences of proteins; however, an estimate of genetic identity can be achieved through

electrophoresis. As it has been shown that genetic loci control the structure and thus the electrophoretic mobility of enzymes and other proteins (Selander et al. 1971), it is possible to estimate genetic variation in populations and to compare the populations with respect to total genetic character. Working on the assumption that a sample of loci controlling proteins is representative of the genome (Hubby and Throckmorton 1968) it is also possible to compare the genetic similarity of related species. With an estimate of genetic similarity obtained from electrophoresis, estimates of divergence time for species can also be derived (Nei 1971).

Electrophoresis studies have been used extensively on mammals, including Dipodomys (Johnson and Selander 1971), Peromyscus polionotus (Selander et al. 1971), Peromysucs floridanus (Smith et al. 1971), Sigmodon (Johnson et al. 1972), Peromycus truei and boylei (Zimmerman et al. 1974), Thomomys (Patton et al. 1972) and Mus musculus (Selander et al. 1969). These techniques have been used only occasionally on birds and mainly for studies at the species or ordinal level. Hendrickson and Yow (1973) examined a few blood proteins, mainly hemoglobins, in an attempt to establish the taxonomic position of the Wood Thrush, Hylocichla mustelina. Brush and Power (1970) restricted their work to a comparison of hemoglobin in four populations of Brewer's Blackbird, Euphagus cyanocephalus. Nottebohm and Selander (1971) used 23 enzymes encoded by 24 structural loci to establish a

relationship between vocal dialects and allozymic variation in four populations of the Chingolo Sparrow, Zonotrichia capensis. Sibley (1967) has utilized egg albumins and hemoglobins in studying the phylogenetic relationship of birds at the ordinal level.

In this study, examples of six of the ten genera of the Family Icteridae found in the United States were examined by comparing 14 proteins encoded by 15 loci using starch-gel electrophoresis. The species included in this study were the Brown-headed Cowbird, Molothrus ater; the Red-winged Blackbird, Agelaius phoeniceus; the Eastern Meadowlark, Sturnella magna; the Western Meadowlark, Sturnella neglecta; the Brewer's Blackbird, Euphagus cyancephalus; the Boat-tailed Grackle, Cassidix mexicanus; and the Common Grackle, Quiscalus quiscula. These species provided the opportunity to study the interspecific and intergeneric relationships of the two North American Icterid lines established by Beecher (1951). The objectives of this study were to determine the levels of genetic similarity within each species, to establish the time of divergence of these species based on genetic data, and to determine the factors contributing to variation within the seven species.

CHAPTER II

MATERIALS AND METHODS

Specimens (n = 215) representing seven species of the Family Icteridae were collected by shotgun and identified by standard methods using coloration, bill shape, and tail structure. Approximately 1.0 cc of blood was removed immediately after collection by heart puncture using a 20 gauge hypodermic needle and a 3-cc syringe. Half of the blood was placed in a 10 x 75 mm culture tube containing 4% sodium citrate solution to prevent clotting. The other half of the blood sample was placed in a 6 x 50 mm culture tube for removal of serum components. The birds and blood samples were placed on ice until they were returned to the laboratory where the liver was removed and placed in two volumes of buffer (0.1 M tris, 0.001 M EDTA, pH adjusted to 7.0 with hydrochloric acid) and frozen for later use.

Preparation of Tissue Extracts

The hemoglobin and erythrocyte proteins were prepared by centrifuging the blood in 4% citrate at 1000 x g for 3 minutes. The erythrocytes were washed three times in buffered saline and hemolysed following the third washing by adding an equal volume of distilled water. The sample was then stored at -20°C until used. Serum samples were prepared by

centrifuging the blood in a 6 x 50 mm culture tube at 1000 x g for 10 minutes. The serum was removed with a pipette and used immediately or stored at -20°C for several months. No differences were found in the protein patterns from fresh or frozen sera.

Tissue extracts were prepared by homogenizing the liver in buffer for approximately one minute in a 7 ml glass tissue grinder (cooled in ice) and centrifuged at 1000 x g for 10 minutes. The supernant was removed and used immediately or stored at -20°C for up to two months.

Electrophoresis Apparatus and Techniques

Horizontal starch gel electrophoresis (Smithies 1955) was used to separate all samples. Gel molds were similar to those used by Kilpatrick (1973) consisting of a glass plate (6" x 8" x 1/2") and four plexiglass strips, two (1/4" x 3/4" x 7 3/4") and two (1/4" x 3/4" x 4 1/2"), held in place by petroleum jelly. The liquid gel was poured into the mold and covered with a plexiglass plate.

All gels were prepared by suspending 18 g of hydrolysed starch (Connaught National Laboratories, Univeristy of Toronto, Toronto, Canada) in 50 ml of buffer. One hundred ml of buffer was heated to boiling in a 1000-ml boiling flask. The starch suspension was then poured into the heated buffer, shaken virogously for one minute, and degassed with an aspirator. After degassing, the clear liquid gel was poured into the mold,

covered with a plexiglass plate and allowed to cool at room temperature for at least two hours.

After the gels had cooled the plexiglass plate and long plexiglass strips were removed. The gels were cut parallel to and 2.0 cm from one of the short sides of the gel to form an insertion line for samples. The gels for hemoglobin, GOT, MDH, and LDH were cut in the center with the insertion line parallel to the short side, and the smaller portion of the gel was gently pushed back. Samples to be analyzed were absorbed onto No. 3 filter paper (4 mm x 5 mm) with the exception of the albumin samples, which were absorbed onto No. 1 filter paper, and blotted dry. The small pieces of filter paper were placed approximately 3 mm apart against the exposed cut surface of the long side of the gel, and the smaller portion of the gel was pushed carefully back to contact the larger portion. A piece of Sarah Wrap (Dow Chemical Company) was used to cover the surface of the gel during electrophoresis (Kristjannson 1963).

The electrophoresis chamber consisted of a plexiglass tray (16" x 14 1/2" x 3 1/2") divided into three compartments. The two outer (electrode) compartments (16" x 4"), each containing a No. 22 platinum wire 12" in length, were filled with electrode buffer. The gel, supported on the glass plate, was placed across the central compartment (6 1/2" x 16" x 2 1/4"), and sponge cloths (8" x 5 1/2" x 1/4") were used as bridges between the gel and the electrode buffer. A glass plate was

placed on top of the sponge cloth bridges to cover the gel and hold the bridges in place. Power was supplied by a Gelman Electrophoresis Power Supply Model 38206 or Heathkit 1P17 H.V. Power Supply. Electrophoresis was performed in a controlled temperature chamber at 0°C to 2°C.

Buffer Systems

Six buffer systems were used to separate the different proteins used in this study. Hemoglobins and erythrocyte Esterase-1 were separated in a discontinuous buffer system consisting of 0.01 M tris-hydrochloric acid gel buffer, pH 8.5 and a 0.3 M sodium borate electrode buffer, pH 8.2. Separation was achieved at 250 V for 1 to 1 1/2 h (Selander et al. 1971).

The electrode buffer for the separation of 6-Phosphogluconate dehydrogenase and Esterase-6 from the hemolysate consisted of 0.1 M tris-0.1 M maleic acid-0.01 M EDTA-0.01 M magnesium chloride, pH 7.4. The gel buffer was comprised of a 1:9 dilution of the electrode buffer with deionized water. The best separation was obtained with a potential of 100V for 6 hours for 6-PGD and 2.5 hours for Esterase-6 (modified from Selander et al. 1971).

Albumin was separated from serum in a discontinuous buffer system of a 0.004 M citric acid gel buffer, pH adjusted to 6.0 with 0.1 M tris, and a 0.3 M sodium borate electrode buffer, pH 6.5. Separation was achieved by a potential of 300 V until

the borate boundary migrated 8 cm (approximately 4 to 6 h) (Jensen and Rassmussen 1971).

Transferrin from serum and tissue esterases from liver extracts were obtained from a gel buffer of a 1:9 mixture of: (A) a 0.03 M lithium hydroxide - 0.19 M boric acid solution, pH 8.1, and (B) a 0.05 M tris - 0.008 M citric acid solution, pH 8.4. The electrode buffer was stock solution A. Gels were run at 350 V and 25 MA for 3 hours (Selander et al. 1971).

Glutamate oxaloacetic transaminases were electrophoresed from liver in a continuous buffer system. The gel buffer consisted of 22.89 mM tris - 5.22 mM citric acid, pH 8.0, and the electrode buffer was composed of 0.687 M tris - 0.157 M citric acid, pH 8.0. Optimum separation was achieved at 100 V for 6 hours (modified from Selander et al. 1971).

Malate dehydrogenases and Lactate dehydrogenases were obtained from liver in a continuous system consisting of 0.008 M tris - 0.003 M citric acid gel buffer, pH 6.7. The electrode buffer was 0.223 M tris - 0.086 M citric acid, pH 6.3. A potential of 170 V for 4 hours was required to separate these proteins (modified from Selander et al. 1971).

Staining and Identification of Proteins

Gels were prepared for staining by removing the short plexiglass strips from the ends of the gel, separating the gel, and removing the filter paper strips. Two plexiglass strips, 1/8 in thick, were placed on the glass plate on both sides of the gel. The gel cutter (a 22-gauge piece of piano

wire stretched tightly between two ends of a frame) were guided along the plexiglass strips slicing the gel. The two halves were then separated and placed on a glass or plexiglass plate with the cut surface exposed for staining.

The general protein stain used for hemoglobin, albumin, and transferrin consisted of a 2% solution of Buffalo Black NBR (naphthol blue black) in a 5:5:1 mixture of methanol, water, and glacial acetic acid. Gels were placed in a pan with the stain and allowed to set for 20 minutes at 20°C.

Transferrins were identified by staining with a mixture of 300 ml deionized water, 3 g hydroxylamine hydrochloride, 8.1 g sodium acetate trihydrated, 4.5 ml glacial acetic acid, 1.5 g nitroso-R (1 nitroso-2 napthol-3,6-disulfonic acid disodium salt) for 15 minutes and counterstained with 2% Buffalo Black (Jensen 1970).

Glutamate oxaloacetic transaminases were stained for 15 to 30 minutes at 37°C in a mixture of 0.5 mg pyridoxal-5'-phosphate, 20 mg a-aspartic acid, 100 mg a-ketoglutaric acid, and 150 mg Fast Blue RR salt in 50 ml 0.2 M tris-hydrochloric acid buffer (pH 8.0). The stain was prepared prior to use and applied immediately (Delorenzo and Ruddle 1970).

Lactate dehydrogenases were identified by staining in a mixture of 30 ml deionized water, 20 ml 0.2 M tris-hydrochloric acid buffer (pH 8.0), 9 ml 0.5 M sodium DL-lactate, 20 mg b-diphosphopyride nucleotide, 10 mg MTT tetrazolium and 8 mg of phenazine methosulfate. The stain was applied

to the gel for 1 to 2 hours in the dark at 37°C (modified from Markert and Massaro 1966).

Malate dehydrogenases were detected with a mixture of 30 ml of deionized water, 20 ml 0.2 M tris-hydrochloric acid buffer (pH 8.0), 5 ml 2.0 M malate solution (Ph adjusted to 7.0 with concd sodium hydroxide), 20 mg of triphosphopyridine nucleotide and 10 mg b-diphosphopyridine nucleotide, 20 mg MTT tetrazolium, and 5 mg phenazine methosulfate. The gels were incubated in the dark at 37°C for 45 minutes to 2 hours (Shows and Ruddle 1968).

The tissue esterase stain was composed of 1 ml 0.2 M monobasic sodium phosphate, 1 ml of 0.2 M dibasic sodium phosphate, 47 ml of deionized water, 25 mg Fast Blue RR salt and 1 ml of a solution of 1 g of a-naphthyl propionate in 10 ml of acetone. The gels were incubated for 10 to 30 minutes at 37°C. The hemolysate esterase stain was comprised of 2.4 ml of 0.2 M monobasic sodium phosphate, 6 ml 0.2 M diabasic sodium phosphate, 20 ml deionized water, 25 mg Fast Garnet GBC Salt, and 1 ml of a solution of 1 g a-naphthyl propionate in 100 ml of acetone. These gels were covered with No. 1 chromatography paper, the stain was spread over the paper, and the gel was incubated at 37°C for 1 to 2 hours.

The stain for 6-Phosphogluconate dehydrogenase consisted of 7 ml 0.2 M tris-hydrochloric acid buffer (pH 8.0), 7 ml 0.1 M magnesium chloride, 3 ml of deionized water, 20 mg of barium-6-phosphate gluconic acid, 1 mg of triphosphopyridine

nucleotide, 4 mg of MTT tetrazolium, and 1 mg of phenazine methosulfate. The gels were painted with the stain and incubated in the dark for 1 to 2 hours.

Gels that were stained with naphthol blue black were destained with repeated washings in a 5:5:1 solution of methanol, water, and glacial acetic acid. All gels were fixed for a minimum of 24 hours and stored in Sarah Wrap under refrigeration until scored.

Gels were scored according to the distance of migration of the proteins from the origin, with each protein band representing a different allele. In most cases homozygous individuals appeared as a single band and heterozygous individuals appeared as double or triple bands depending on the protein. The bands migrating the farthest from the origin were scored as a and each consecutive shorter migrating band with consecutive letters of the alphabet.

Calculation of Gene Frequency and Divergence Time

Gene frequencies were calculated by counting the number of like alleles for each species at a given locus and dividing by the total number of alleles at that locus. Since heterozygous individuals could be identified, all alleles could be coded, and direct allelic frequencies could be calculated.

The species and genera were analyzed on the basis of genetic similarity of the 15 loci examined by using Roger's (1972) coefficient of genetic similarity (S). The genetic

similarity between two populations was calculated by summing the probabilities of drawing identical genotypes from the two populations for each genotype of the locus and dividing by the sum of the probabilities of drawing identical genotypes from the same population on two successive independent draws from each genotype of the locus as shown in the formula:

$$S_R = 1 - \frac{1}{L} \sum_{i=1}^{L} \left[1/2 \sum_{j=1}^{A} (P_{ijx} - P_{ijy})^2 \right] 1/2$$

where L is the number of loci, A_i is the number of alleles at the i^{th} locus and P_{ijx} and P_{ijy} are the frequencies of the j^{th} allele at the i^{th} locus in populations x and y respectively.

Genetic similarity, distance, and divergence time were calculated for paired combinations of all species using Nei's coefficient (1971). The normalized identify of genes between two populations (species) designated X and Y with respect to each locus is defined as:

$$I_j = j_{xy} / \sqrt{j_x j_y}$$

where j_x is the probability of identify of two randomly chosen genes in population X, while j_y is the probability of identity of randomly chosen genes in population Y, and j_{xy} is the probability of identity of a gene from X and a gene from Y.

The normalized identity of genes between X and Y with respect to all loci is defined as:

$$I = J_{XY}/\sqrt{J_X J_Y}$$

where J_X, J_Y, and J_{XY} are the arithmetic means of j_x, j_y, and j_{xy}, respectively, over all loci, including monomorphic loci.

For use in calculating divergence time it is more convenient to use the geometric mean. Therefore, the genetic distance between X and Y is then defined as:

$$D = -\log_e I \quad .$$

The divergence time was calculated using the formula:

$$t = D/(2cn_T\lambda_a)$$

where D is the geometric mean of genetic similarity, c is a constant .04, n_T is the average number of amino acids per protein, and λ_a is the average rate of amino acid substitutions per site per year.

From a matrix of coefficients of genetic similarity (S and I) for species of Icteridae, cluster analysis was performed by the weighted pair group method of Sokal and Sneath (1963), and a dendrogram was constructed from the calculated similarities.

Mean number of alleles was determined for breeding populations of Sturnella magna and S. neglecta by calculating the total number of alleles at a given locus for all populations and dividing by the number of populations for each species. The affective number of alleles (N_e) was then

calculated as the reciprocal of the sum of the squares of the frequencies of all alleles in a population as shown by the formula:

$$N_e = 1/(p^2 + q^2)$$

where p and q are the frequencies of the alleles for the population.

Proportion of loci polymorphic per population was calculated by dividing the number of polymorphic loci in a population by the total number of loci. Proportion of loci polymorphic per individual was determined by counting the number of heterozygotes of a population at each locus, dividing by the number of individuals in the population, and summing these values for all loci.

CHAPTER III

RESULTS

Fourteen proteins controlled by fifteen loci were identified for 215 birds representing seven species of the family Icteridae. Five proteins, encoded by five loci, were monomorphic among all the species studied. No more than six alleles were found segregating at any of the nine remaining loci. Since most studies have shown that no significant sexual differences in frequencies of alleles for populations of over seven occur (Johnson and Selander 1971, Johnson et al. 1971, Selander and Yang 1969, 1970), no attempt was made to determine sexual differences in allelic frequencies for the species in this study.

Proteins Scored

Hemoglobin

All seven species were monomorphic for hemoglobin showing one anodally migrating and one cathodally migrating band.

Glutumate Oxaloacetic Transaminase

GOT-1, migrating anodally, was essentially monomorphic showing only one variant in the 215 specimens examined. GOT-2 migrated cathodally and was monomorphic for all specimens examined.

Malate Dehydrogenase

MDH-1, a dimer (Selander et al. 1971), was an anodally migrating protein that was basically monomorphic in all species. One specimen of Agelaius phoeniceus was heterozygous showing the three banded pattern. MDH-2, migrating cathodally, was monomorphic in all species.

Lactate Dehydrogenase

Lactate dehydrogenase polypeptides produced by the two loci combine to form a tetramere producing a five-banded electrophoretic pattern in birds similar to those formed in most other vertebrates (Markert 1968). Variation in lactate dehydrogenase in the Icteridae from liver extract was found to be controlled by two loci designated LDH-1 and LDH-2 (Fig. 1).

The LDH-1 system, migrating anodally, was monomorphic for all species but Sturnella magna which showed one heterozygous individual with a faster migrating, multibanded system and C. mexicanus which had one heterozygote with a slower migrating c allele.

The LDH-2 locus, with two alleles segregating and migrating cathodally, showed a faster migrating a band and a slower migrating b band. The a allele was predominant in all species and monomorphic in Sturnella neglecta, Euphagus cyanocephalus, and Quiscalus quiscula (Table 3). Cassidix mexicanus showed one heterozygous individual, all other specimens being fixed for the a allele. S. magna, showing no heterozygotes, was

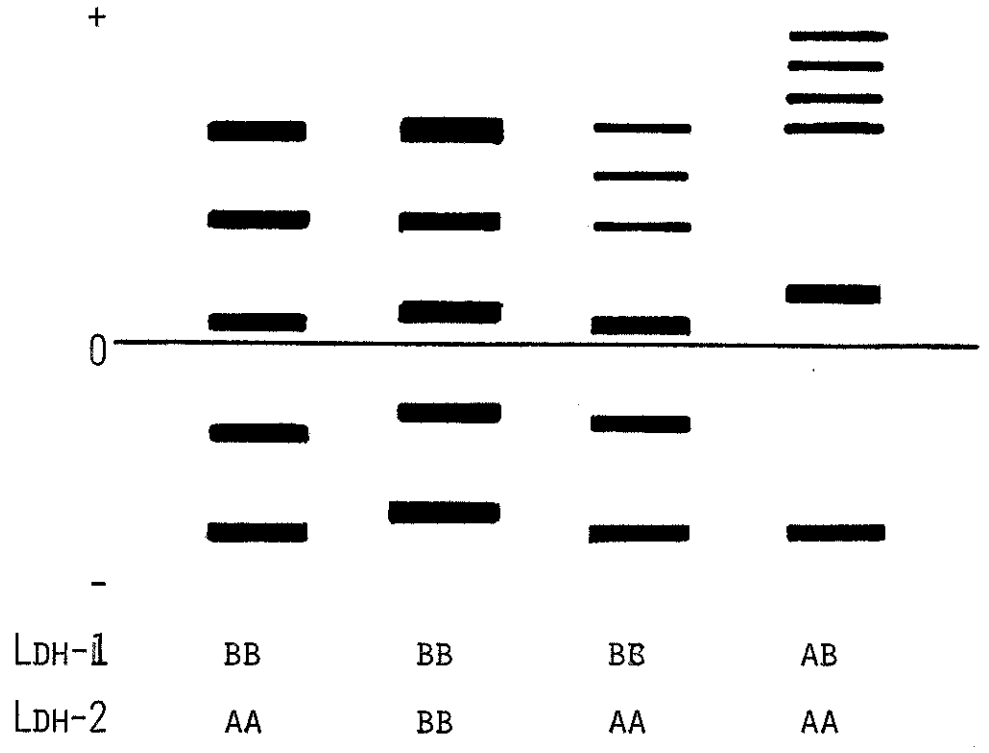

Fig. 1--Variation in lactate dehydrogenase (LDH-1 and LDH-2).

polymorphic with the b allele occurring at a frequency of 0.03. Agelaius pheoniceus showed the b allele at a frequency of 0.04, with no heterozygotes occurring in the population. Molothrus ater also had no heterozygotes with the b allele occurring at a frequency of 0.12.

6-Phosphogluconate Dehydrogenase

This enzyme is a dimer, heterozygotes forming three-banded patterns. Five alleles were present in the species in this study (Fig. 2). The predominant allele for the Agelaiine group was the e allele which was monomorphic in S. neglecta (Table 1). The e allele occurred at a frequency of 0.94 in both S. magna and A. phoeniceus. A. phoeniceus showed slower migrating c and d alleles, both occurring at a frequency of 0.03. S. magna had a d allele present only in the heterozygous form at a frequency of 0.06.

M. ater had two alleles present, all occurring in the homozygous condition. The b allele was predomonant with an a allele occurring at a frequency of 0.05. The predominant allele for the Quiscaline group was the d allele, being monomorphic in E. cyanocephalus and C. mexicanus. Quiscalus quiscula showed a homozygous e allele at a frequency of 0.15.

Albumin

The seven species in this study showed a double banded pattern for albumin in the homozygous form, a light fast band, which was probably a prealbumin, and a heavier slow band (Owen

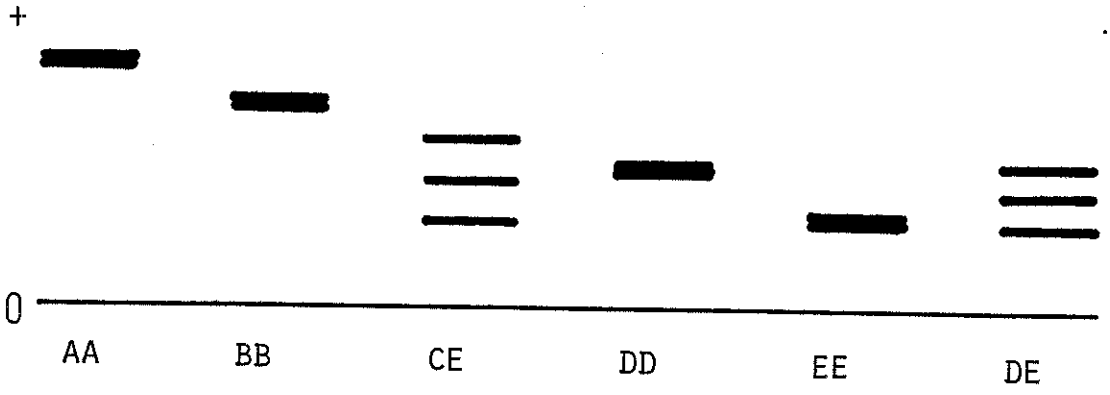

Fig. 2--Variation in 6-phosphogluconate dehydrogenase.

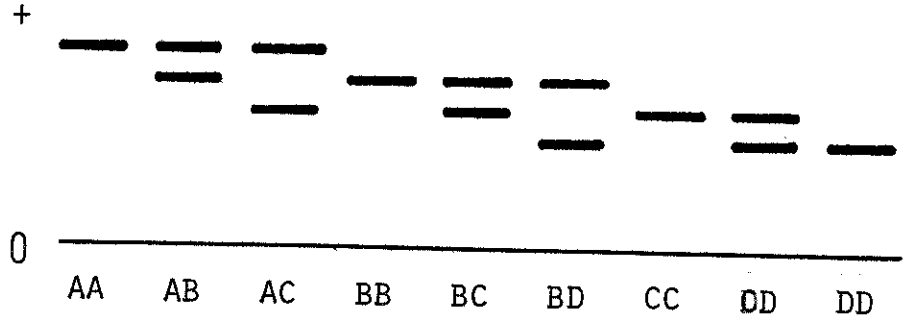

Fig. 3--Variation in hemolysate esterase (Es.-1).

Table 1. Allelic frequency and genetic variability of the 6-phosphogluconate dehydrogenase loci of seven species of the family Icteridae.

	Loci				
	a	b	c	d	e
Sturnella magna	. .	. .	. .	0.06	0.94
Sturnella neglecta	. .	. .	. .	. .	1.00
Agelaius phoeniceus	. .	. .	0.03	0.03	0.94
Molothrus ater	0.05	0.95	. .	. .	. .
Euphagus cyanocephalus	. .	. .	. .	1.00	. .
Cassidix mexicanus	. .	. .	. .	1.00	. .
Quiscalus quiscula	. .	. .	. .	0.85	0.15

and Bennett 1972) (Fig. 4). Heterozygotes formed a triple banded pattern. Five alleles were present with no more than two alleles present in a single species. The b allele was predominant in the Agelaiine group (Table 2). The d allele was present at a frequency of 0.07 in S. magna and 0.06 in S. neglecta and A. phoeniceus. M. ater showed a frequency of 0.11 for the a allele and 0.89 for the c allele. The predominant allele for the Quiscaline group was d, which was monomorphic in C. mexicanus and Q. quiscula. E. cyanocephalus was homozygous for the d allele in all but one specimen which was heterozygous for the d and e alleles.

Transferrin

Three alleles were identified in transferrin for the seven species (Fig. 5). The b allele was most common in all species, being monomorphic in S. neglecta and E. cyanocephalus (Table 3). The c and a alleles were present in M. ater at a frequency of 0.08. Thus, the b allele occurred at a frequency of 0.84 in this species. The frequency of the a allele in the other species was 0.03 for S. magna, 0.47 for A. phoeniceus, 0.19 for C. mexicanus, and 0.15 for Q. quiscula.

Esterase

Considerable variation was observed in the esterases, and these proteins proved to be the most polymorphic systems both between and within the species. Five esterase systems were included in this study.

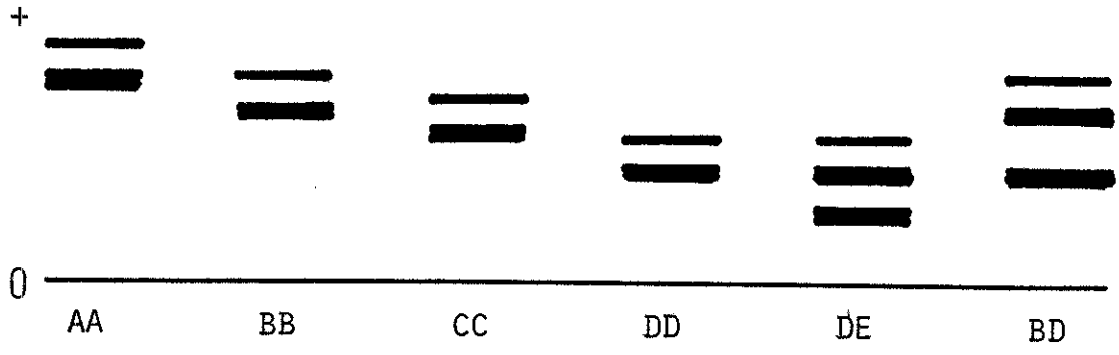

Fig. 4--Variation in serum ablumin.

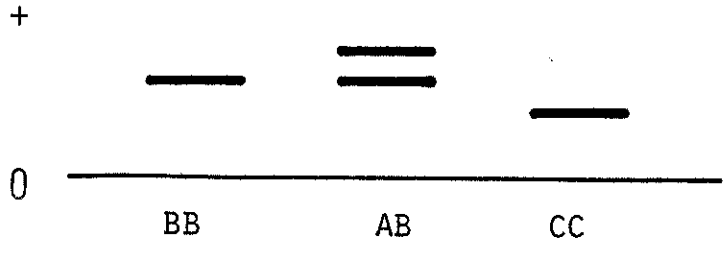

Fig. 5--Variation in transferrin.

Table 2. Allelic frequency and genetic variation of the albumin loci of seven species of the family Icteridae.

	Loci				
	a	b	c	d	e
Sturnella magna	. .	0.93	. .	0.07	. .
Sturnella neglecta	. .	0.94	. .	0.06	. .
Agelaius pheoniceus	. .	0.94	. .	0.06	. .
Molothrus ater	0.11	. .	0.89	. .	. .
Euphagus cyanocephalus	. .	. .	. .	0.95	0.05
Cassidix mexicanus	. .	. .	. .	1.00	. .
Quiscalus quiscula	. .	. .	. .	1.00	. .

Table 3. Allelic frequencies and genetic variation of the transferrin and lactate dehydrogenase loci of seven species of the family Icteridae.

	Loci				
	Transferrin			LDH-2	
	a	b	c	a	b
Sturnella magna	0.03	0.97	. .	0.97	0.03
Sturnella neglecta	. .	1.00	. .	1.00	. .
Agelaius phoeniceus	0.47	0.53	. .	0.96	0.04
Molothrus ater	0.08	0.84	0.08	0.88	0.12
Euphagus cyanocephalus	. .	1.00	. .	1.00	. .
Cassidix mexicanus	0.19	0.81	. .	0.93	0.07
Quiscalus quiscula	0.15	0.85	. .	1.00	. .

Esterase-1.--This erythrocyte esterase appeared to have four alleles segregating from a single locus. Heterozygotes showed a double banded pattern suggesting this protein was a monomer (Fig. 3). The c allele was present in all species at different frequencies. The b allele was the predominant allele in all species except the two species of Sturnella where it was absent (Table 4). The d allele was the most common allele in both species of Sturnella with the c allele present only in the heterozygous state. The frequencies of the d allele were 0.67 in S. magna and 0.79 in S. neglecta. The c allele was present at a frequency of 0.31 and the a allele at a frequency of 0.02 in S. magna. The c allele was present at a frequency of 0.21 in S. neglecta.

A. phoeniceus showed three alleles, the b allele at a frequency of 0.56, the c allele at a frequency of 0.35, and the d allele at a frequency of 0.09. Three alleles were also present in M. ater, the a and c alleles each at frequencies of 0.15 and the b allele at a frequency of 0.60.

E. cyanocephalus and Q. quiscula showed only b and c alleles. The frequency of the b allele, which was predominant, was 0.83 in E. cyanocephalus and 0.65 in Q. quiscula. Three alleles were present in C. mexicanus, the b allele at a frequency of 0.38 and the c and d alleles each at a frequency of 0.31.

Table 4. Allelic frequency and genetic variability of the esterase-1 loci of seven species of the family Icteridae.

	Loci			
	a	b	c	d
Sturnella magna	0.02	. .	0.31	0.67
Sturnella neglecta	. .	. .	0.21	0.79
Agelaius phoeniceus	. .	0.56	0.35	0.09
Molothrus ater	0.15	0.60	0.15	. .
Euphagus cyanocephalus	. .	0.83	0.17	. .
Cassidix mexicanus	. .	0.38	0.31	0.31
Quiscalus quiscula	. .	0.65	0.35	. .

Esterase-2.--Esterase-2 was the fastest migrating band from liver extract and stained lighter than the other tissue esterases (Fig. 6). The a allele was monomorphic in M. ater and all members of the Quiscaline group. Four alleles were present in S. magna and S. neglecta (Table 5). The frequencies for the alleles in S. magna were 0.16 for the a allele, 0.31 for the b allele, 0.44 for the c allele, and 0.09 for the d allele. S. neglecta showed frequencies of 0.12 for the a allele, 0.19 for the b allele, 0.46 for the c allele and 0.23 for the d allele. The predominant allele in A. phoeniceus was a, with a frequency of 0.88. The b allele occurred at a frequency of 0.08 and the c allele occurred at a frequency of 0.04 in this species.

Esterases 3 and 4.--Esterases-3 and 4 were darkly staining tissue esterases obtained from liver. Esterase-3 was the faster, anodally migrating band and appeared as single or double bands, suggesting that it is a monomer (Fig. 7). All species were polymorphic, having two alleles in M. ater and the members of the Quiscaline group and as many as five alleles in members of the Agelaiine group (Table 6). The predominant allele for all species except Sturnella was the a allele, which occurred at a frequency of 0.67 in A. phoeniceus, 0.88 in M. ater, 0.89 in E. cyanocephalus, 0.81 in C. mexicanus, and 0.92 in Q. quiscula. The other allele in M. ater, E. cyanocephalus, and C. mexicanus was the b allele while the rate c allele occurred at a frequency of 0.08 in Q. quiscula.

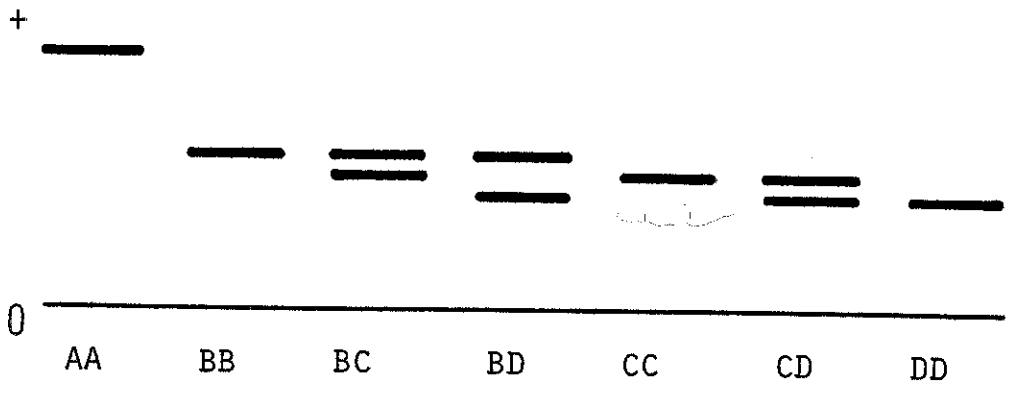

Fig. 6 -- Variation in tissue esterase (Es-2).

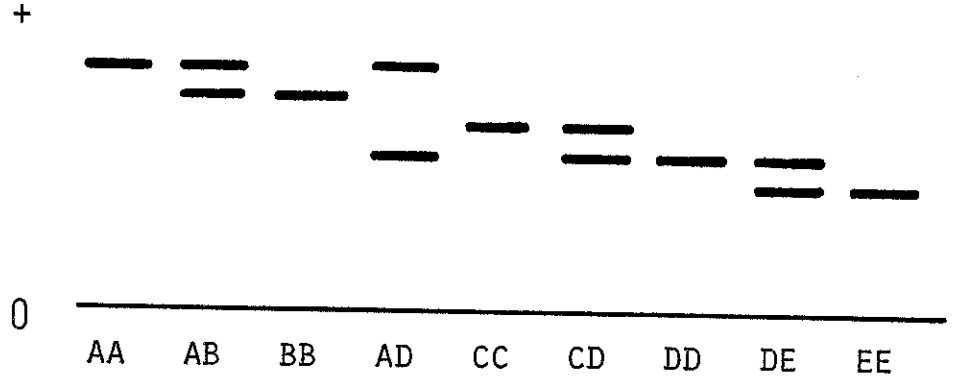

Fig. 7 -- Variation in tissue esterase (Es-3).

Table 5. Allelic frequency and genetic variability of the esterase-2 loci of seven species of the family Icteridae.

	Loci			
	a	b	c	d
Sturnella magna	0.16	0.31	0.44	0.09
Sturnella neglecta	0.12	0.19	0.46	0.23
Agelaius phoeniceus	0.88	0.08	0.04	. .
Molothrus ater	1.00	. .	. .	. .
Euphagus cyanocephalus	1.00	. .	. .	. .
Cassidix mexicanus	1.00	. .	. .	. .
Quiscalus quiscula	1.00	. .	. .	. .

Table 6. Allelic frequency and genetic variability of the esterase-3 loci of seven species of the family Icteridae.

	Loci				
	a	b	c	d	e
Sturnella magna	0.04	0.11	0.31	0.44	0.10
Sturnella neglecta	. .	0.08	0.44	0.40	0.08
Agelaius phoeniceus	0.67	0.20	0.07	0.04	0.02
Molothrus ater	0.88	0.12	. .	. .	. .
Euphagus cyanocephalus	0.89	0.11	. .	. .	. .
Cassidix mexicanus	0.81	0.19	. .	. .	. .
Quiscalus quiscula	0.92	. .	0.08	. .	. .

S. magna showed five alleles at frequencies of 0.04 for a, 0.11 for b, 0.31 for c, 0.44 for d, and 0.10 for e. S. neglecta, showing no a alleles, had alleles with the following frequencies: 0.08 for b, 0.44 for c, 0.40 for d, and 0.08 for e. Five alleles were evident in A. phoeniceus at frequencies of 0.67 for a, 0.20 for b, 0.07 for c, 0.04 for d, and 0.02 for e.

Esterase-4 was the slower migrating esterase system in liver extract showing six alleles (Fig. 8). Alleles a and b were absent in the genus Sturnella as was f in M. ater and the Quiscaline group (Table 7). The prominant allele in S. magna was the e allele occurring at a frequency of 0.43. The other three alleles were the c allele occurring at a frequency of 0.03, the d allele occurring at a frequency of 0.20, and the f allele occurring at a frequency of 0.34. S. neglecta also had four alleles occurring at frequencies of 0.06 for c, 0.17 for d, 0.19 for e, and 0.58 for f.

Five alleles occurred in A. phoeniceus at frequencies of 0.09 for b, 0.26 for c, 0.31 for d, 0.17 each for e and f. Alleles e and f were absent in M. ater. Alleles a, b, c, and d occurred at frequencies of 0.21, 0.50, 0.22, and 0.07, respectively.

E. cyanocephalus showed 3 alleles, a at a frequency of 0.11, b at a frequency of 0.83, and c at a frequency of 0.06. The alleles and their frequencies in C. mexicanus were 0.38 for b, 0.50 for c, and 0.06 each for d and e. Q. quiscula showed frequencies of 0.08 for the a allele, 0.42 for the b

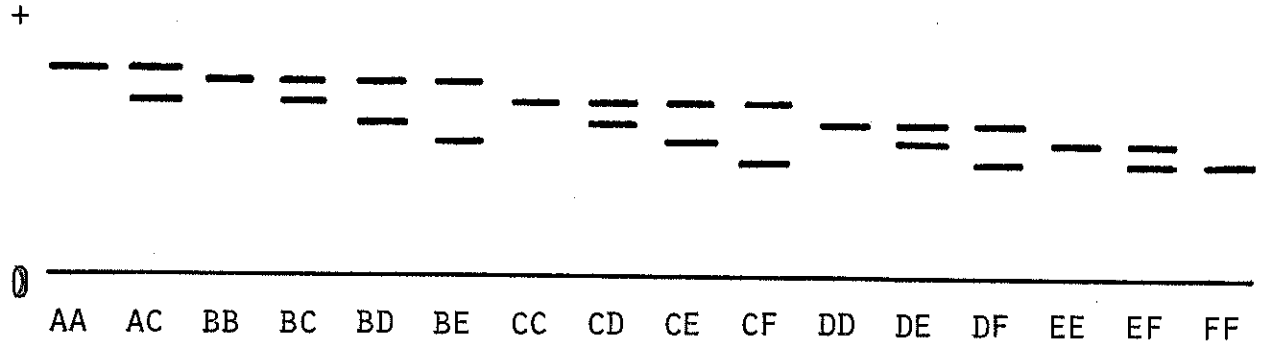

Fig. 8--Variation in tissue esterase (Es-4).

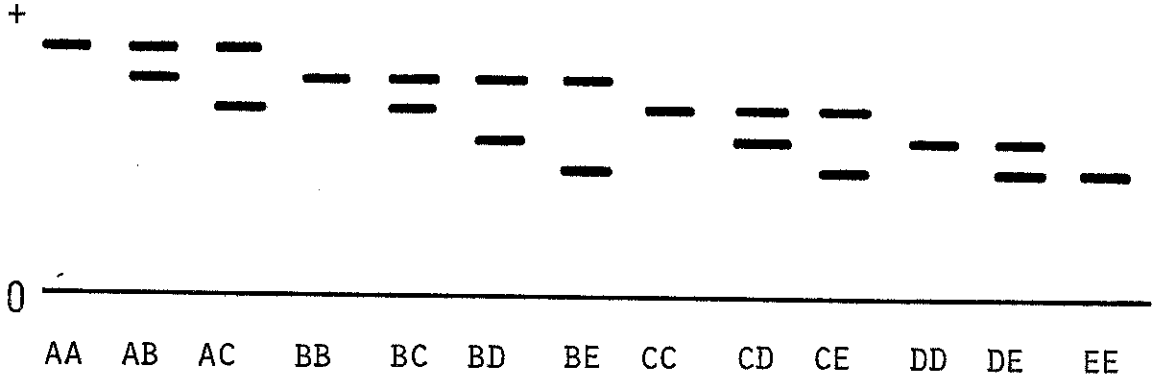

Fig. 9--Variation in hemolysate esterase (Es-6).

Table 7. Allelic frequency and genetic variability of the esterase-4 loci of seven species of the family Icteridae.

	Loci					
	a	b	c	d	e	f
Sturnella magna	. .	. .	0.03	0.20	0.43	0.34
Sturnella neglecta	. .	. .	0.06	0.17	0.19	0.58
Agelaius phoeniceus	. .	0.09	0.26	0.31	0.17	0.17
Molothrus ater	0.21	0.50	0.22	0.07	. .	. .
Euphagus cyanocephalus	0.11	0.83	0.06	. .	. .	. .
Cassidix mexicanus	. .	0.38	0.50	0.06	0.06	. .
Quiscalus quiscula	0.08	0.42	0.25	0.04	0.21	. .

allele, 0.25 for the c allele, 0.04 for the d allele and 0.21 for the e allele.

Esterase-6.--Esterase-6, an erythrocyte esterase, showed five alleles (Fig. 9). The a allele was absent in the Agelaiine group and the e allele was absent in M. ater and the Quiscaline group (Table 8). S. magna showed three alleles, the b allele at a frequency of 0.08, the c allele at a frequency of 0.10, and the e allele at a frequency of 0.82. The predominant allele in S. neglecta was e with a frequency of 0.69. S. neglecta also showed a c allele.

A. phoeniceus had four alleles present, the b allele at a frequency of 0.32, the c allele at a frequency of 0.07, the d allele at a frequency of 0.48, and the e allele at a frequency of 0.13. M. ater had frequencies of 0.37 for the a allele, 0.48 for the b allele, 0.11 for the c allele, and 0.03 for the d allele. E. cyanocephalus only had two alleles present, a with a frequency of 0.38 and b with a frequency of 0.62.

C. mexicanus was polymorphic showing four alleles, a at a frequency of 0.42, b at a frequency of 0.33, c at a frequency of 0.17, and d with a frequency of 0.08. The frequencies of the alleles in Q. quiscula were 0.46 for the b allele, 0.31 for the c allele, and 0.23 for the d allele.

Genetic Relationships

Coefficients of genetic similarity were calculated for all paired combinations of species using Rogers' (S) and

Table 8. Allelic frequency and genetic variation of the esterase-6 loci of seven species of the family Icteridae.

	Loci				
	a	b	c	d	e
Sturnella magna	. .	0.08	0.10	. .	0.82
Sturnella neglecta	. .	. .	0.31	. .	0.69
Agelaius phoeniceus	. .	0.32	0.07	0.48	0.13
Molothrus ater	0.37	0.48	0.11	0.03	. .
Euphagus cyanocephalus	0.38	0.62	. .	. .	. .
Cassidix mexicanus	0.42	0.33	0.17	0.08	. .
Quiscalus quiscula	. .	0.46	0.31	0.23	. .

Nei's coefficients (Table 9). From a matrix of coefficients for paired combinations of the seven species, cluster analysis was performed by the weighted pair group method (Sokal and Sneath 1963). The resulting dendrograms are shown in Figs. 10 and 11.

Values of S are used for most of the discussion of genetic similarity, since these values have been obtained by several investigators and comparable data are available (Zimmerman et al. 1974). Coefficients of genetic similarity between species (S) ranged from a low of 0.620 between S. neglecta and E. cyanocephalus to a high of 0.939 between S. neglecta and S. magna. The S values between all species of the Quiscaline group ranged above 0.900 with a mean value of 0.907. The S values for the Agelaiine group ranged from 0.789 to 0.939 with a mean value of 0.834. The mean S values between M. ater and the other two groups were 0.673 for the Agelaiine group and 0.811 for the Quiscaline group. The mean S value between all species in this study was 0.754.

Although the values calculated by Nei's coefficient appear to be slightly higher, they correlate very closely to those values calculated by Rogers' coefficient between the same species. The I values ranged from 0.637 to 0.989 with a mean of 0.797. All members of the Quiscaline group had I values above 0.964, with a mean of 0.970. The mean I values between members of the Agelaiine group was 0.895 with a range of 0.841 to 0.989. The mean I values between the ancestral

Table 9. Mean coefficients of genetic similarity (Nei's above, Roger's below) among seven species of the Family Icteridae.

	Sturnella magna	*Sturnella neglecta*	*Agelaius phoeniceus*	*Molothrus ater*	*Euphagus cyanocephalus*	*Cassidix mexicanus*	*Quiscalus quiscula*
Sturnella magna	. .	.989	.857	.666	.658	.698	.702
Sturnella neglecta	.939	. .	.841	.651	.637	.681	.683
Agelaius phoeniceus	.789	.773	. .	.804	.789	.811	.843
Molothrus ater	.638	.625	.757	. .	.848	.832	.843
Euphagus cyanocephalus	.629	.620	.731	.814	. .	.964	.972
Cassicix mexicanus	.661	.645	.771	.810	.900	. .	.975
Quiscalus quiscula	.660	.646	.789	.808	.908	.914	. .

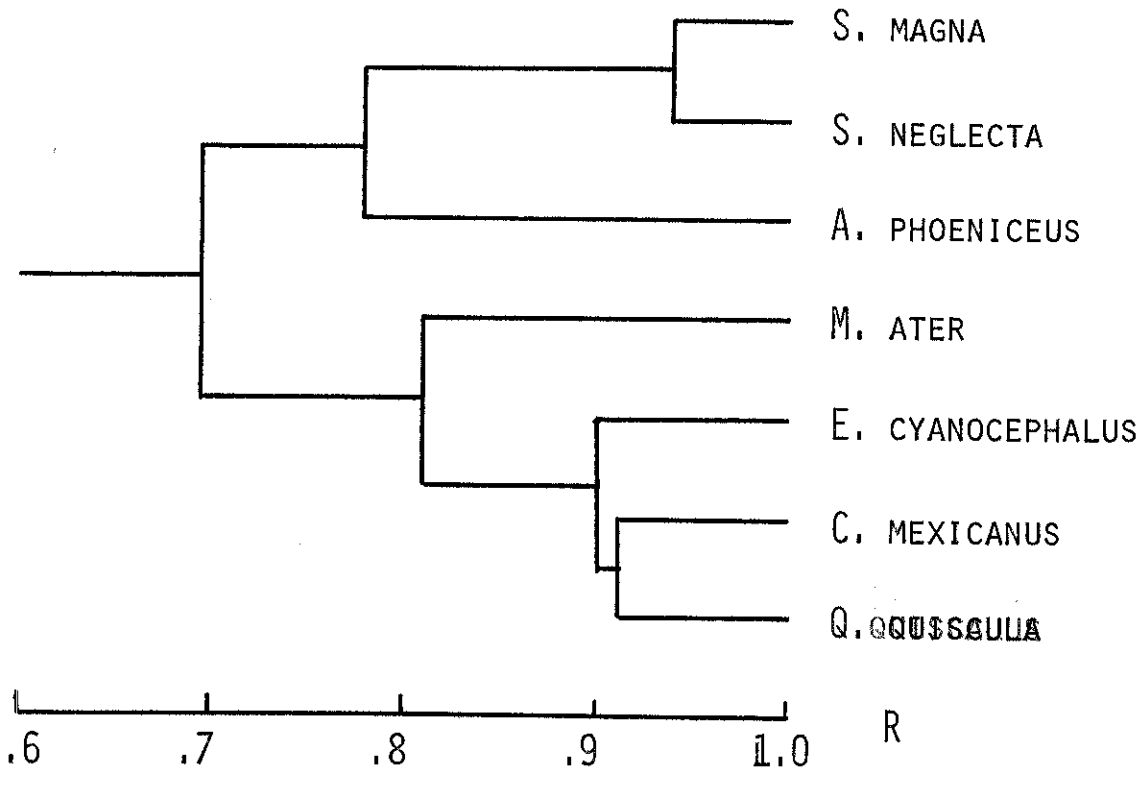

Fig. 10--Dendrogram as determined by Rogers' coefficient of genetic similarity.

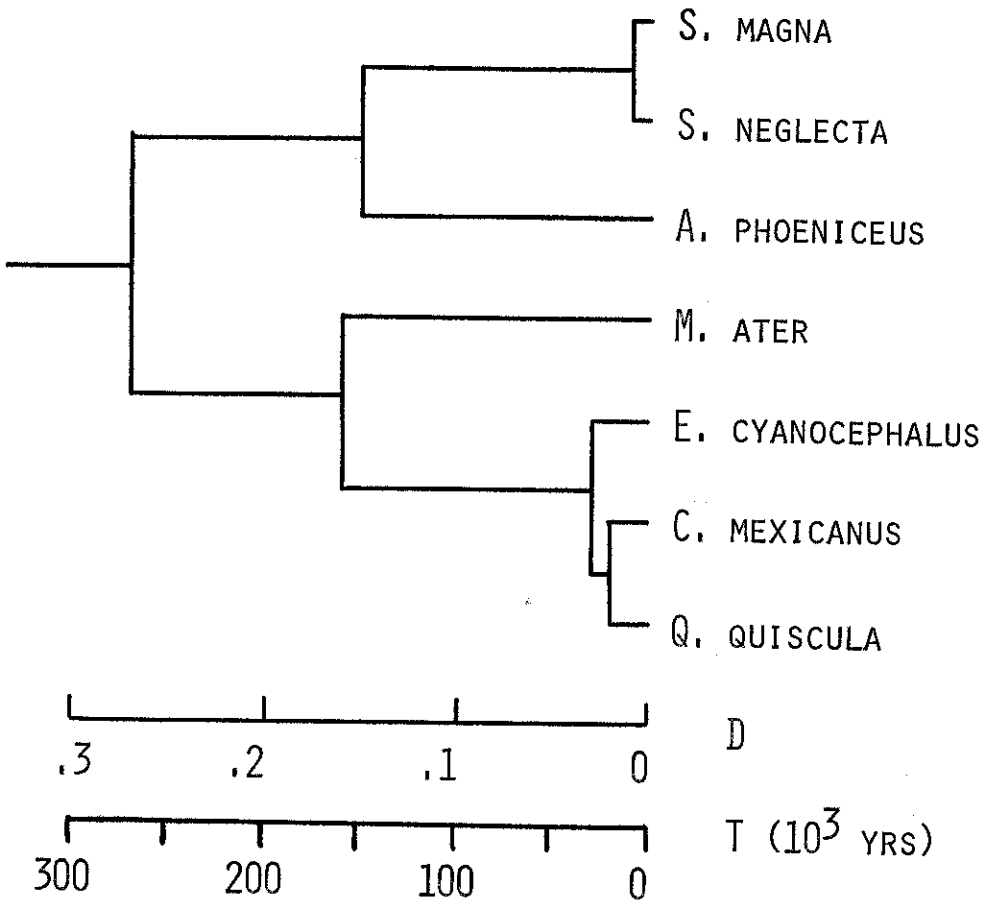

Fig. 11--Dendrogram as determined by Nei's coefficient (I) of genetic similarity showing genetic distance and divergence time (t).

M. ater and the Agelaiine group and Quiscaline groups were 0.707 and 0.841, respectively.

Genetic variability in a population can be expressed in several ways, including number of alleles per population that are polymorphic and the affective number of alleles. Three breeding populations of S. magna and four breeding populations of S. neglecta were compared for polymorphism (Tables 10 and 11). S. neglecta was monomorphic for all loci except the five esterases studied. The total number of alleles per locus in esterases ranged from two to four alleles per locus with the mean number of alleles ranging from 1.25 to 3.00. The affective number of alleles for the esterases ranged from 1.197 to 2.485, indicating a low level of heterozygosity.

S. magna was polymorphic for the esterases as well as albumin, transferrin, GOT-1, and 6-PGD. All proteins polymorphic in S. magna except the esterases demonstrated two alleles per locus. The mean number of alleles ranged from 1.33 to 4.33, whereas the affective number of alleles ranged from 1.020 to 3.303.

The proportions of loci polymorphic per population and polymorphic per individual for each of the seven populations were also determined (Table 12). The proportion of polymorphic loci per population in S. neglecta were low, ranging from 0.13 to 0.33. The proportion of loci polymorphic per individual ranged from 0.030 for the western population of S. neglecta from Hutchinson Co. to 0.17 on the eastern edge of their range.

Table 10. Genetic variation in proteins of Sturnella magna.

Protein	Phenotypic Variation	Number of Alleles		Affective No. Alleles
		Total	Mean Per Population	
Hemoglobin	Monomorphic	1	1.00	1.00
Albumin	Polymorphic	2	2.00	1.193
Transferrin	Polymorphic	2	1.33	1.131
GOT-1	Polymorphic	2	1.33	1.020
GOT-2	Monomorphic	1	1.00	1.000
6-PGD	Polymorphic	2	1.33	1.073
MDH-1	Monomorphic	1	1.00	1.000
MDH-2	Monomorphic	1	1.00	1.000
LDH-1	Monomorphic	1	1.00	1.000
LDH-2	Monomorphic	1	1.00	1.000
Esterase-1	Polymorphic	3	2.00	1.101
Esterase-2	Polymorphic	4	3.67	2.758
Esterase-3	Polymorphic	5	4.33	3.303
Esterase-4	Polymorphic	4	3.67	2.570
Esterase-6	Polymorphic	2	1.33	1.035

Table 11. Genetic variation in proteins of Sturnella neglecta.

Protein	Phenotypic Variation	Number of Alleles		
		Total	Mean Per Population	Affective No. Alleles
Hemoglobin	Monomorphic	1	1.00	1.000
Albumin	Monomorphic	1	1.00	1.000
Transferrin	Monomorphic	1	1.00	1.000
GOT-1	Monomorphic	1	1.00	1.000
GOT-2	Monomorphic	1	1.00	1.000
6-PGD	Monomorphic	1	1.00	1.000
MDH-1	Monomorphic	1	1.00	1.000
MDH-2	Monomorphic	1	1.00	1.000
LDH-1	Monomorphic	1	1.00	1.000
LDH-2	Monomorphic	1	1.00	1.000
Esterase-1	Polymorphic	2	1.50	1.197
Esterase-2	Polymorphic	3	2.00	1.827
Esterase-3	Polymorphic	4	2.75	2.482
Esterase-4	Polymorphic	4	3.00	2.485
Esterase-6	Polymorphic	2	1.25	1.279

Table 12. Genetic variation in populations of Sturnella magna and Sturnella neglecta.

Populations	Proportions of Loci Polymorphic Per Population	Proportion of Loci Polymorphic Per Individual
Sturnella magna		
Denton Co., Tex. (6)	0.27	0.03
Alvord, Wise Co., Tex. (21)	0.40	0.029
Slidell, Wise Co., Tex. (38)	0.53	0.043
Sturnella neglecta		
Alvord, Wise Co., Tex. (3)	0.13	0.17
Slidell, Wise Co., Tex. (4)	0.20	0.05
E. Panhandle Tex. and W. Okla. (5)	0.33	0.118
Hutchinson Co., Tex. (7)	0.33	0.03

The number of loci polymorphic per population in S. magna was higher, ranging from 0.29 in the easternmost population to 0.53 for the Slidell, Wise Co., population in north-central Texas. The proportion of polymorphic loci per individual was 0.03 for the Denton Co. population, 0.029 for the Alvord, Wise Co., population, and 0.043 for the Slidell, Wise Co., population. These values are lower than those of S. neglecta.

CHAPTER IV

DISCUSSION

The coefficients of genetic similarity, S and I, for the species of the family Icteridae observed in this study were extremely high considering that six different genera were compared. The mean S and I values were 0.754 and 0.797, respectively. The mean S value was compared to known values for several species of organisms as listed in Table 13. Most S values that are higher than the mean for the Icteridae are for conspecifics or closely related species complexes namely: Spalax ehrenbergi (Nevo and Cleve 1974), Drosophila paulsitorum (Richmond 1972), Thomomys talpoides (Nevo et al. 1974), Thomomys bottae and T. umbrinus (Patton et al. 1972), Geomys bursarius and G. personatus (Kim 1972), Drosophila (Zouros 1972), D. bipectinata (Yang et al. 1972), Mus musculus (Selander et al. 1969) and Sigmodon (Johnson et al. 1972). Several species comparisons showed S values considerably lower than the mean S value obtained for the Icteridae. These include Peromyscus boylei and truei complexes (Zimmerman et al. 1974), Dipodomys (Johnson and Selander 1971), Drosophila (Nair et al. 1971), Peromyscus polionotus, P. floridanus (Selander et al. 1971, Smith et al. 1973) and Anolis (Webster et al. 1972).

Table 13. Comparison of genic similarity (S) between populations and between species in a variety of taxa in ascending order.

Taxon	Number of Loci	Genic Similarity Mean & Range	Reference
Spalax ehrenbergi	25	.94 (.90-.98)	Nevo and Cleve 1974
Drosophila paulistorum	12	.92 (.90-.94)	Richmond 1972
Thomomys talpoides complex	29	.87 (.80-.97)	Nevo et al. 1974
Thomomys bottae and *T. umbrinus*	27	.84 (.93-.86)	Patton et al. 1972
Geomys bursarius and *G. personatus*	19	.81 (.76-.86)	Kim 1972
Drosophila (closely related species	12-26	.81 (.69-.93)	Zouros 1972
Drosophila bipectinata	23	.77 (.65-.91)	Yang et al. 1972
Mus musculus	41	.77 (.76-.77)	Selander et al. 1969, Rogers 1972
Sigmodon	23	.76 (.76-.77)	Johnson et al. 1972
Family Icteridae	15	.75 (.62-.94)	Present study
Peromyscus boylei and *truei*	15	.70 (.52-.93)	Zimmerman et al 1974
Dipodomys	18	.61 (.31-.89)	Johnson and Selander 1971
Drosophila	24	.50 (.30-.77)	Nair et al. 1971
Peromyscus polionotus and *P. floridanus*	32	.32 (.31-.33)	Selander et al. 1971, Smith et al 1973
Anolis	25	.21 (.16-.29)	Webster et al. 1972

The high similarity coefficients among the birds in this study reflects the close relationship between the seven species.

M. ater appeared to be the most divergent species of the seven species considered in this study. The mean S and I values between M. ater and the Agelaiine group were 0.673 and 0.707, respectively. The mean S and I values between M. ater and the Quiscaline group were 0.811 and 0.841, respectively, indicating a higher degree of similarity to this group. Beecher (1951) suggested that the genus Molothrus is the most primitive in the family Icteridae, showing in many respects the traits of its finch ancestry. Genetic data appear to correlate well with the morphological evidence.

The similarity coefficients between S. magna and S. neglecta were extremely high with an S value of 0.939 and an I value of 0.989, suggesting a close relationship and probably a very recent divergence for these two species. Reports of hybridization (Lanyon, 1966, Szijj, 1962, Sutton and Dickson 1965) between these two species and morphological similarity substantiate this close genetic similarity. When breeding populations of S. magna and S. neglecta were compared using Rogers' coefficient, it was impossible to determine a significant difference in S values between the two species. The mean S value between conspecific populations of S. magna was 0.876 and between conspecific populations of S. neglecta was 0.906. The mean S value between S. magna populations and S. neglecta populations was 0.885, and the total mean for all

populations was 0.886. Therefore, the genetic similarity between populations of interspecifics is often greater than between populations of conspecifics.

A. phoeniceus had S values above 0.700 and I values above 0.800 when compared with the two species of Sturnella. These values indicate a closer relationship between A. phoeniceus and Sturnella than between M. ater and species of the Quiscaline group. Beecher (1951) proposed that the genus Agelaius was probably the ancestor to Sturnella and placed the genera in the same group.

C. mexicanus and Q. quiscula were genetically similar with an S value of 0.914 and an I value of 0.975. These values indicate a very close relationship between these species as suggested by Beecher (1951). The other species in the Quiscaline group also had S and I values of over 0.900 when paired with the two species of Grackles, indicating all members of this group are closely related.

Dendrograms using Rogers' coefficient (Fig. 10) and Nei's coefficient (Fig. 11) were compared to a dendrogram constructed from Beecher's (1951) study of the family Icteridae (Fig. 12). Distances of divergence in Fig. 12 were chosen arbitrarily, as data from Beecher (1951) were not available to determine similarity coefficients. Beecher (1951) suggested that Molothrus gave rise to two lines that were considered in this study, the Quiscaline line and the Agelaiine line. The dendrograms from this study show that M. ater is genetically more

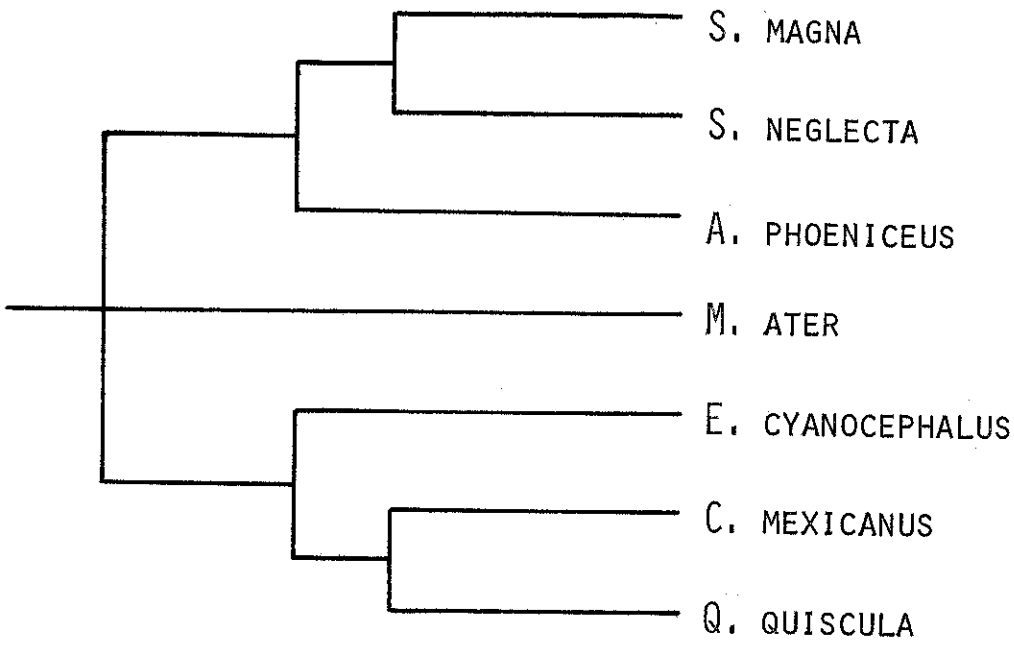

Fig. 12--Dendrogram constructed from Beecher's (1951) data for phylogeny of the Family Icteridae.

divergent from the other six species in this study but has its greatest affinities to the species in the Quiscaline group. This divergence indicates that M. ater could have been closely related to the ancestral form of Icteridae. Biochemical evidence supports the inclusion of Agelaius and Sturnella in a single group. Beecher (1951) indicated that Agelaius probably gave rise to Sturnella which is similar to the results of this study in that A. phoeniceus has a lower genetic similarity to either species of Sturnella.

The data in this study show that members of the family Icteridae are probably more closely related than avian taxonomists had predicted. Biochemical data would indicate that there is a very close relationship between C. mexicanus and Q. quiscula, as well as a close relationship of both species with E. cyanocephalus. It is conceivable that C. mexicanus and Q. quiscula should be classified in the same genus. There is the possibility that E. cyanocephalus should also be included in the same genus with these two species, although the superficial morphological features do not bear this out.

Although the I values are somewhat higher than the S values, the dendrogram constructed using Nei's coefficient (Fig. 11) was very similar to the one constructed with Rogers' coefficient. Estimates of divergence time based on Nei's formula are crude, but studies of Peromyscus (Zimmerman et al. 1974) and Thomomys (Nevo et al. 1974) have corresponded

remarkably well to fossil data of these genera. Fossil records of birds are limited, however, and evidence to support estimates of divergence time based on biochemical data is not available.

Using Nei's index (t) for divergence, it appears that the Agelaiine and Quiscaline groups diverged from the ancestral form during the Illinoian glaciation, approximately 270,000 years B.P. The divergence of the Quiscaline group from Molothrus appears to be more recent than divergence of the Agelaiine group, taking place during the Sangamon interglacial, about 160,000 years B.P. The divergence of the Sturnella species from A. phoeniceus appears to have taken place during the Sangamon interglacial, around 150,000 years B.P. The two species of Sturnella diverged within the past 8,000 to 10,000 years B.P., corresponding to the Recent geological period. This prediction would seem reasonable since these two species are very similar morphologically and have been known to hybridize.

C. mexicanus and Q. quiscula appears to have diverged at the end of the Pleistocene, with a divergence time of 18,000 to 20,000 years B.P. The divergence of E. cyanocephalus from these two species appears to have taken place earlier about 30,000 years B.P. during the Plestocene.

Genetic Variation in Sturnella Populations

Estimates of heterozygosity for seven breeding populations of the two species of Sturnella was based on 14 proteins

controlled by 15 loci. The average value of heterozygosity (H) for the two species of Sturnella was 0.067 indicating that 6.7% of the 15 loci are heterozygous in the average individual. Comparable values of H in birds are available from one investigation utilizing several loci for studying genetic structure of populations of birds. Nottebohm and Selander (1972) showed an average value of heterozygosity of 0.0353 for four populations of Zonotrichia capensis. Data from Sturnella indicate a higher mean heterozygosity value when compared to Z. capensis, but H values in Sturnella are generally low. The mean value of H for Sturnella is comparable to estimates of heterozygosity in mammals such as Peromyscus floridanus (Smith et al. 1973), P. polionotus (Selander et al. 1971) and Thomomys (Nevo et al. 1974), all of which are considered to have the lowest levels of heterozygosity among vertebrates. It should be mentioned that two populations contribute most of the variation to the mean value of heterozygosity in these two species, and both were represented by small samples. In addition, the five proteins that were most polymorphic were esterases and have been shown to be highly polymorphic in most vertebrates (Nevo et al. 1974).

Geographic variation in H values is readily apparent. The lowest value of H (0.029) was found in the most marginal population of S. magna. This corresponds with Mayr's (1965) statement that marginal populations are generally less genetically variable than central populations. However, the

S. neglecta population with the highest average value of H (0.17) was the most marginal population of S. neglecta, located on the eastern edge of the breeding range. High levels of heterozygosity in the Slidell, Wise Co., Texas population could result from hybridization and introgression between S. magna and S. neglecta. Frequencies for certain alleles common to both species were more similar in areas of sympatry. In addition, morphological investigations of the specimens collected indicated overlap in characteristics. These morphological characters could be a result of geographic variation, as Rohwer (1972) concluded that hybridization of S. magna and S. neglecta does not occur in north-central Texas. Biochemical evidence from large samples from this area are necessary to substantiate hybridization.

CHAPTER V

SUMMARY

Starch-gel electrophoresis was utilized to study 14 proteins encoded by 15 loci in seven species of the blackbird family, Icteridae, to determine genetic relationships and degree of variation among the species. Beecher (1951) classified the seven species as follows: Molothrus ater which was considered primitive and similar to the ancestral stock; the Agelaiine group, (Blackbirds) which included Agelaius phoeniceus, Sturnella magna, and S. neglecta; and the quiscaline group (Grackles) which included Euphagus cyanocephalus, Cassidix mexicanus, and Quiscalus quiscula. The mean genetic similarity coefficients of Rogers (S) and Nei (I) showed a close relationship of the seven species included in this study. Mean coefficients of genetic similarity among the genera were similar to those previously reported for mammals and higher than the interspecific values of other taxa.

Dendrograms constructed from paired combinations of S and I for the seven species studied suggested that M. ater was the most divergent of the seven species considered in this study, but was shown to be more closely related to the Quiscaline group than the Agelaiine group.

The S and I values were extremely high between S. magna and S. neglecta suggesting a close relationship and probably a very recent divergence for these two species. These data are supported by reports of hybridization and close morphological similarity. S and I values for A. phoeniceus when compared with the other six species suggested a closer relationship between A. phoeniceus and the Sturnella species than between M. ater and the Quiscaline group. This similarity supports Beecher's proposal that Agelaius and Sturnella should be included in the same group.

S and I value S between C. mexicanus and Q. quiscula showed a close relationship between the two species. Data also indicated a close relationship of these two species and E. cyanocephalus which Beecher (1951) placed in the same group.

Nei's index (t) for divergence time indicated that the Agelaiine and Quiscaline groups diverged from the ancestral form during the Illinoian glaciation about 270,000 years B.P. The Quiscaline group appeared to diverge from Molothrus during the Sangamon interglacial, approximately 160,000 years B.P., while the divergence of A. phoeniceus from Sturnella appeared to have taken place around 150,000 years B.P. The two species of Sturnella appeared to have diverged within the past 10,000 years B.P. corresponding to the recent geological period. C. mexicanus and Q. quiscula diverged near the end of

the Pleistocene, while E. cyanocephalus diverged around 30,000 years B.P. during the Pleistocene.

Three breeding populations of S. magna and four breeding populations of S. neglecta were compared for genetic variability using number of alleles per population that were polymorphic and affective number of alleles per individual. S. neglecta showed monomorphism in all proteins except the five esterases used in this study. S. magna was polymorphic for the esterases as well as albumin, transferrin, 6-PGD, and GOT-1. Average heterogosity values for all populations were generally low, ranging from 0.17 to 0.029 with a mean value of 0.067. Two small populations of S. neglecta had very high values of heterozygosity. One of these populations, comprised of three individuals, was on the eastern edge of the breeding range.

S. magna showed H (proportion of heterozygous loci per individual) values within the range of most vertebrates, with a distribution of heterozygosity values from 0.029 to 0.043. The low value of 0.029 was found in the most marginal population on the western part of the breeding range. S. neglecta H values showed the opposite distribution with the most variable populations occurring on the margin of the range. Two explanations for this phenomenon are that selection is favoring heterozygosity in this species in the marginal populations or hybridization and introgression are taking place.

APPENDIX

Species and Collecting Localities	Number of Individuals
Sturnella magna	Total 78
1. Lake Kickapoo, Archer County, Tex.	1
2. 1 mi N New York, Clay County, Tex.	1
3. 7 mi E Denton, Denton County, Tex.	12
4. 3 mi NE Coffee Mill Lake, Fannin County, Tex.	3
5. 6 mi E Alvord, Wise County, Tex.	21
6. 1 mi S Slidell, Wise County, Tex.	38
7. 12 mi SE Rankin, Roger Mills County, Okla	2
Sturnella neglecta	Total 26
8. 7 mi E Denton, Denton County, Tex.	2
9. 1 mi S Lake McClellan, Gray County, Tex.	3
10. 4 mi SE San Marcos, Guadalupe County, Tex.	1
11. Lake Merideth, Hutchinson County, Tex.	7
12. 1 mi E Oklaunion, Wilbargher County, Tex.	1
13. 6 mi E Alvord, Wise County, Tex.	3
14. 1 mi S Slidell, Wise County, Tex.	7
15. 12 mi SE Rankin, Roger Mills County, Okla.	2
Agelaius phoeniceus	Total 34
16. 1 mi N Roanoke, Denton County, Tex.	11
17. 3 mi NE Coffee Mill Lake, Fannin County, Tex.	1
18. 5 mi S San Marcos, Guadalupe County, Tex.	1

Species and Collecting Localities	Number of Individuals
19. 12 mi W Borger, Moore County, Tex.	4
20. 3 mi S Keller, Tarrant County, Tex.	12
21. 1 mi E Oklaunion, Wilbargher Co., Tex.	1
22. 1 mi S Slidell, Wise County, Tex.	4
Molothrus ater	Total 45
23. 7 mi E Denton, Denton County, Tex.	9
24. 3 mi NE Coffee Mill Lake, Fannin County, Tex.	1
25. 1 mi S Slidell, Wise County, Tex.	35
Euphagus cyanocephalus	Total 10
26. 7 mi E Denton, Denton County, Tex.	1
27. 4 mi S San Marcos, Guadalupe County, Tex.	1
28. 12 mi W Borger, Moore County, Tex.	1
29. 1 mi E Oklaunion, Wilbargher County, Tex.	1
30. 7 mi NW Decatur, Wise County, Tex.	6
Cassidix mexicanus	Total 8
31. 4 mi SE San Marcos, Guadalupe County, Tex.	4
32. 1 mi N Watauga, Tarrant County, Tex.	4
Quiscalus quiscula	Total 13
33. 7 mi E Denton, Denton County, Tex.	5
34. 3 mi NE Coffee Mill Lake, Fannin County, Tex.	5
35. 3 mi S Keller, Tarrant County, Tex.	3

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