# GENETIC POLYMORPHISM IN NEW GUINEA STARLINGS OF THE GENUS *APLONIS*

KENDALL W. CORBIN,[1] CHARLES G. SIBLEY, ANDREW FERGUSON[2]
Peabody Museum of Natural History and
Department of Biology
Yale University
New Haven, Connecticut 06520

ALLAN C. WILSON
Department of Biochemistry
University of California
Berkeley, California 94720

ALAN H. BRUSH
Biological Sciences Group
The University of Connecticut
Storrs, Connecticut 06268

AND

JON E. AHLQUIST
Peabody Museum of Natural History and
Department of Biology
Yale University
New Haven, Connecticut 06520

Protein polymorphisms presumed to have a genetic basis are known to exist in natural populations of most species examined to date. Among protein morphs, the observed differences usually are detected as electrophoretic mobility changes that are assumed to be due to amino acid substitutions rather than to post-translational modification. Since the studies of Hubby and Lewontin (1966) and Lewontin and Hubby (1966), there has been considerable interest in determining the percentage of the genome that may be either heterozygous in individuals or polymorphic in populations. Comparable data for natural populations of many species, both vertebrates and invertebrates being represented, are now available. However, the extent of protein polymorphism and individual heterozygosity in natural avian populations is known for relatively few species (Bush 1967; Sibley and Corbin 1970; Nottebohm and Selander 1972). Several other studies of protein polymorphism in bird populations have dealt with only a few loci (Stratil and Valenta 1966; Brush 1968, 1970; Bush et al. 1970; Ferguson 1971; Brush and Scott 1972).

Most of the work on the specific variation of bird proteins involved domestic species. In this category, proteins found to be polymorphic include the serum esterases of the Japanese Quail (*Coturnix coturnix*) (Kaminski 1964; Manwell and Baker 1969), several species of pigeons (species of *Columba* and *Streptopelia*) (Boehm and Irwin 1970; Ferguson 1971), and the Domestic Chicken (*Gallus gallus*) (Kimura 1969a, b). Lactate dehydrogenase polymorphisms were found in the Rock Dove (*Columba livia*) (Zinkham et al. 1963, 1964, 1966).

One aim of the present study is to determine the extent of protein polymorphism in natural populations of two bird species found in the lowlands of Papua-New Guinea. These species are the Metallic Starling (*Aplonis metallica*) and its congener the Singing Starling (*Aplonis cantoroides*). The Singing Starling is a solitary nester, using holes or crevices in trees or coconut palms; the Metallic Starling nests in colonies of one hundred or more pairs in isolated rain-forest trees. The colonies are normally several miles apart and the breeding season is more or less synchronized throughout the distribution of this species. For these reasons we assume that each nesting colony constituted a breeding population of the Metallic Starling. The four localities where Singing Starlings were collected are distant from one another and probably represent distinct breeding populations.

The topography of Papua-New Guinea is extremely varied. Mountain ranges, flooded lowlands around some of the major rivers, and long stretches of open water between islands probably constitute an extensive system of natural barriers to gene flow among starling populations. Given this potential system of natural barriers to gene flow, we wanted to determine if variation in allelic frequencies among populations was correlated with mor-

[1] Present address: Bell Museum of Natural History and Department of Ecology and Behavioral Biology, University of Minnesota, Minneapolis, Minnesota 55455.
[2] Present address: Department of Zoology, The Queen's University of Belfast, Northern Ireland.


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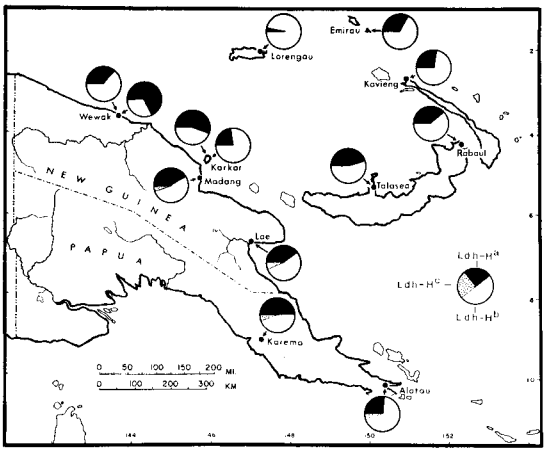


FIGURE 1. The geographic variation in the allelic frequencies at the lactate dehydrogenase-H locus in populations of *Aplonis metallica*. The frequency of an allele in a population is proportional to the area of the circle occupied by its symbol. Allelic frequencies at Rabaul localities 1 and 2 are essentially identical for the purpose of illustration. The same is true of Karkar localities 2 and 3 (see table 1). The Lorengau locality is on Manus Island.

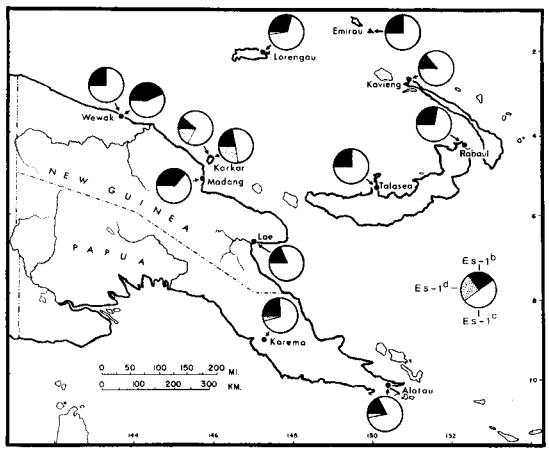


FIGURE 2. The geographic variation in the allelic frequencies at the esterase-1 locus in populations of *Aplonis metallica*. The frequency of an allele in a population is proportional to the area of the circle occupied by its symbol. Allelic frequencies at Karkar Island localities 2 and 3 are essentially identical for the purpose of illustration (see table 2).

phological variation in the respective species. *A. cantoroides* is a monotypic species, whereas five subspecies of *A. metallica* have been recognized (Mayr and Greenway 1962). Three of the subspecies of *A. metallica* inhabit the geographical areas sampled. *A. m. metallica* is found on New Guinea, *A. m. nitida* inhabits islands of the Bismarck Archipelago and Rambutyo Island of the Admiralty group, and *A. m. purpureiceps* is found on some of the islands of the Admiralty group including Manus Island.

## MATERIALS AND METHODS

During the 1969, Program B, R/V *Alpha Helix* expedition to Papua-New Guinea (Sibley 1969), 354 Metallic Starlings and 108 Singing Starlings were collected from the localities listed in tables 1–3 and shown in figures 1 and 2. At those localities where two or three colonies of the Metallic Starling were sampled, the colonies were several miles apart. Six collecting localities were located along the Bismarck Archipelago. Lorengau is at the east end of Manus Island of the Admiralty group, Emirau Island is in the Saint Matthias group, Kavieng is at the northwestern tip of New Ireland, Rabaul localities 1 and 2 are at the northeastern end of New Britain, and Talasea is on the Willaumez Peninsula on the northern coast of New Britain. The remaining nine localities are on or near the coast of New Guinea. Specimens were taken during the period beginning 15 July and ending 9 September 1969. The number of individuals taken at each locality can be obtained from tables 1–3 by summing the number of individuals distributed among the respective genotypes.

Individuals of *A. metallica* were collected by shooting. Mist nets and shot guns were used to collect *A. cantoroides*. Blood samples from all birds were taken by cardiac puncture, using either heparin or 10% EDTA-$Na_2$ as ar anticoagulant. Those birds shot were bled immediately, while those birds caught in mist nets were bled after returning to a field laboratory. Blood samples were centrifuged at ambient temperature to separate plasma and red cells. The latter were washed once with 0.9% NaCl. Both plasma and red cell samples were stored at –10°C in the field and then transferred to a Revco Freezer held at –100°C. During transit to the United States, samples were kept frozen with dry ice. The majority of the heart and breast muscle samples were taken at the time the birds were collected. To extract enzymes, the tissues were placed in approximately four times their weight of a solution containing phenoxyethanol, sucrose, and phosphate buffer (Nakanishi et al. 1969; Karig and Wilson 1971). Tissues were extracted overnight at room temperature or at 4°C and then frozen. A few heart and breast muscle samples were not taken immediately after the collection of a bird. In these cases the whole carcass was frozen and the tissue samples taken at a later date.

Aliquots (0.01 ml) of tissue extract or plasma were analyzed by horizontal starch-gel electrophoresis at either pH 6.0 or 8.6. The pH 6.0 buffer composition was that described by Karig and Wilson (1971). The pH 8.6 system was composed of 0.076 M Tris(hydroxymethyl) aminomethane, 0.005 M citric acid, 0.015 M boric acid, and 0.005 M lithium hydroxide. The electrode buffer of the pH 8.6 system was composed of 0.3 M boric acid and 0.1 M lithium hydroxide. In each electrophoretic separation, a control sample of a recognizable phenotype was run on each side of the starch-gel slab to facilitate the scoring of phenotypes. Many of the samples were analyzed on board the R/V *Alpha Helix* and the accuracy of the scoring was verified by independently analyzing samples in laboratories at Yale University, the University of California, Berkeley, and the University of Connecticut, Storrs. Both buffer systems satisfactorily resolved the lactate dehydrogenase (LDH) and the major anodal esterase variants but only the pH 6.0 system resulted in the sharp resolution of malate dehydrogenase (MDH) bands. After electrophoresis,

the gels were sliced into three slabs and each was assayed for a different protein.

Esterases were detected using 20 mg α-naphthyl acetate and 50 mg of either Fast Garnet GBC salt or Fast Blue RR salt in 100 ml of 0.1 M phosphate buffer, pH 6.0. The lactate dehydrogenase reagent contained 10 ml of 1.0 M Na-DL-lactate, 30 mg NAD, 20 mg nitro blue tetrazolium, and 2 mg phenazine methosulphate in 90 ml of 0.1 M Tris-HCl buffer, pH 7.1. NAD-dependent malate dehydrogenase was detected with the same reagent as LDH except that 1.0 M Na-L-malate was substituted for the lactate. Total protein assays used 1% Amido Black 10B in methanol, acetic acid, and water, 5:1:5. Other enzymes, all of which were monomorphic, were detected with reagents described by Shaw and Prasad (1970). LDH was also assayed spectrophotometrically in the pyruvate to lactate direction (Rose and Wilson 1966).

Characterization of the major (i.e., highest concentration) heart LDH band of one *A. cantoroides* specimen involved tests of thermal stability, sensitivity to pyruvate inhibition, and reactivity to an antiserum prepared against pure chicken $H_4$ LDH using standard methods (Rose and Wilson 1966).

Allelic frequencies and their standard deviations, Hardy-Weinberg equilibrium distributions, and $\chi^2$ values for observed deviations from the expected Hardy-Weinberg distributions were calculated by the computer program MAXIM (Kurczynski and Steinberg 1967).

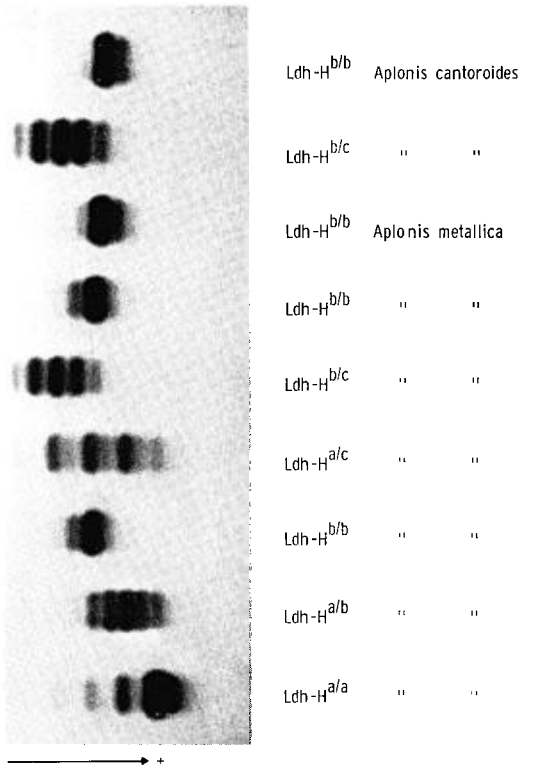


FIGURE 3. Starch-gel electrophoretic patterns of the lactate dehydrogenases-H of *Aplonis metallica* and *Aplonis cantoroides*. The patterns are designated according to the genotypes inferred to code for the respective enzymes.

## RESULTS AND DISCUSSION

*Protein polymorphism within species.* It is well established that birds and mammals normally have five major forms of L-lactate dehydrogenase (Appella and Markert 1961; Cahn et al. 1962; Chilson et al. 1964; Markert 1968). These are each tetramers that result from the random combination of two types of polypeptide subunits known respectively as M (muscle) and H (heart) or A and B, or 2 and 1. The subunit compositions of the five tetramers are given by the formulae $H_4$, $H_3M_1$, $H_2M_2$, $H_1M_3$, and $M_4$. Although similar in size, the polypeptide subunits are the products of different genes. The gene controlling the synthesis of the H subunit is normally most active in heart tissue, while the gene for M subunits is most active in skeletal muscles.

After electrophoresis, heart extracts prepared from *A. cantoroides* exhibited a major anodal band having LDH activity. Other faint anodal bands that migrated less far were observed; these were presumably the $M_4$, $M_3H_1$, $M_2H_2$, and $M_1H_3$ tetramers. On the basis of each of the tests listed in the methods section, the major heart LDH band of one *A. cantoroides* specimen was identified as being homologous to the $H_4$ isoenzyme of the chicken.

An extensive polymorphism was found at the LDH-H locus of *A. metallica*. Five electrophoretic pattern types (phenotypes) were observed among the 354 specimens sampled (fig. 3). The subunit composition of these five phenotypes is assumed to be identical to the model postulated for other similar data (Vesell 1965). Thus the five phenotypes are accounted for by postulating that three allelic forms of the LDH-H gene control the synthesis of the H subunits of *A. metallica*. These we designate *Ldh-H*$^a$, *Ldh-H*$^b$, and *Ldh-H*$^c$. Assuming random combination of the allelic products in heterozygous individuals, each such individual would produce five tetramer types in the proportions 1:4:6:4:1. Three heterozygous phenotypes each having the five tetramer types present in the expected proportions were found (fig. 3). Only two of the predicted phenotypes produced by homozygous individuals, those of *Ldh-H*$^{a/a}$ and *Ldh-H*$^{b/b}$ genotypes, were found due to the rarity of the *Ldh-H*$^c$ alleles in all populations.

In contrast to the predominantly polymorphic character of the LDH-H locus of *A. metallica* that of *A. cantoroides* was essentially monomorphic. All but 2 of the 108 individuals sampled were homozygous for the same allele of heart muscle lactate dehydrogenase. This

TABLE 1. Distributions of LDH-H genotypes of *Aplonis metallica*, allelic frequencies, and Chi-squared values for deviations from expected Hardy-Weinberg equilibrium distributions.

| | Distribution of genotypes | | | | | | Allelic frequencies | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Locality | AA | AB | AC | BB | BC | CC | A | B | C | $\chi^2$ | *P* |
| Lorengau | 0 | 2 | – | 26 | – | – | 0.04 ± 0.03[a] | 0.96 ± 0.03 | – | 0.038[b] | 0.85 |
| Emirau | 3 | 13 | – | 13 | – | – | 0.33 ± 0.06 | 0.67 ± 0.06 | – | 0.009 | 0.93 |
| Kavieng | 1 | 9 | – | 11 | – | – | 0.26 ± 0.07 | 0.74 ± 0.07 | – | 0.247 | 0.64 |
| Rabaul 1 | 5 | 13 | – | 12 | – | – | 0.38 ± 0.06 | 0.62 ± 0.06 | – | 0.209 | 0.66 |
| Rabaul 2 | 3 | 14 | – | 10 | – | – | 0.37 ± 0.07 | 0.63 ± 0.07 | – | 0.337 | 0.58 |
| Talasea | 5 | 17 | – | 10 | – | – | 0.42 ± 0.06 | 0.58 ± 0.06 | – | 0.254 | 0.63 |
| Lae | 2 | 8 | 0 | 4 | 2 | 0 | 0.38 ± 0.09 | 0.56 ± 0.09 | 0.06 ± 0.04 | 1.975 | 0.58 |
| Madang | 8 | 14 | 1 | 13 | 2 | 0 | 0.41 ± 0.06 | 0.55 ± 0.06 | 0.04 ± 0.02 | 1.356 | 0.72 |
| Wewak 1 | 9 | 3 | – | 4 | – | – | 0.66 ± 0.08 | 0.34 ± 0.08 | – | 5.465 | 0.02 |
| Wewak 2 | 2 | 10 | – | 8 | – | – | 0.35 ± 0.08 | 0.65 ± 0.08 | – | 0.196 | 0.67 |
| Karkar 1 | 0 | 6 | – | 7 | – | – | 0.23 ± 0.08 | 0.77 ± 0.08 | – | 1.170 | 0.28 |
| Karkar 2 | 5 | 17 | – | 2 | – | – | 0.56 ± 0.07 | 0.44 ± 0.07 | – | 4.629 | 0.04 |
| Karkar 3 | 5 | 10 | – | 2 | – | – | 0.59 ± 0.08 | 0.41 ± 0.08 | – | 0.781 | 0.39 |
| Alotau | 0 | 9 | 0 | 6 | 2 | 0 | 0.26 ± 0.08 | 0.68 ± 0.08 | 0.06 ± 0.04 | 3.888 | 0.28 |
| Karema | 5 | 13 | 0 | 3 | 2 | 0 | 0.50 ± 0.07 | 0.46 ± 0.07 | 0.04 ± 0.03 | 3.702 | 0.30 |

[a] Allelic frequency ± one standard deviation.
[b] $\chi^2$ tests involving 3 genotypes have 1 degree of freedom. Those involving 6 genotypes have 3 d.f.

we designate as *Ldh-H*$^b$ since its enzyme is identical in electrophoretic mobility to the *Ldh-H*$^b$ enzyme of *A. metallica*. The other two individuals were heterozygous, possessing a second allele that is presumably identical to *Ldh-H*$^c$ of *A. metallica* on the basis of the electrophoretic mobility of their respective enzymes.

Table 1 presents the number of individuals of *A. metallica* of each inferred LDH-H genotype, the frequency of alleles in each population, $\chi^2$ values testing the goodness of fit of genotype distributions to expected Hardy-Weinberg equilibrium distributions, and the *P* values associated with each $\chi^2$ value. Table 3 presents comparable data for the LDH-H locus of *A. cantoroides*.

Only one region of esterase activity in the zymograms could be scored accurately. This was true for samples of plasma and extracts of heart muscle, breast muscle, and liver of both species of starling. This esterase migrated rapidly toward the anode at pH 6.0 and 8.6, and is probably homologous to the carboxyl esterase of the Muscovy Duck (*Cairina moschata*) described by Holmes and Masters (1968) and to the Es-1 region of zymograms containing samples of chicken plasma (Kimura 1969a, b).

This major anodal esterase was polymorphic in both *A. cantoroides* and *A. metallica*. Three phenotypes of the former and six phenotypes of the latter species were observed. The genetic model postulated to explain the obser-

TABLE 2. Distributions of Esterase-1 genotypes of *Aplonis metallica*, allelic frequencies, and Chi-squared values for deviations from expected Hardy-Weinberg equilibrium distributions.

| | Distribution of genotypes | | | | | | Allelic frequencies | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Locality | BB | BC | BD | CC | CD | DD | B | C | D | $\chi^2$ | *P* |
| Lorengau | 4 | 8 | 0 | 16 | 1 | 0 | 0.28 ± 0.06[a] | 0.71 ± 0.06 | 0.02 ± 0.02 | 2.989[b] | 0.40 |
| Emirau | 2 | 11 | – | 17 | – | – | 0.25 ± 0.06 | 0.75 ± 0.06 | – | 0.015 | 0.90 |
| Kavieng | 1 | 4 | – | 15 | – | – | 0.15 ± 0.06 | 0.85 ± 0.06 | – | 0.930 | 0.35 |
| Rabaul 1 | 4 | 11 | – | 15 | – | – | 0.32 ± 0.06 | 0.68 ± 0.06 | – | 0.700 | 0.66 |
| Rabaul 2 | 0 | 13 | – | 14 | – | – | 0.24 ± 0.06 | 0.76 ± 0.06 | – | 2.714 | 0.10 |
| Talasea | 2 | 12 | – | 18 | – | – | 0.25 ± 0.05 | 0.75 ± 0.05 | – | 0.000 | 1.00 |
| Lae | 1 | 3 | – | 12 | – | – | 0.16 ± 0.06 | 0.84 ± 0.06 | – | 1.335 | 0.25 |
| Madang | 6 | 17 | – | 19 | – | – | 0.35 ± 0.05 | 0.65 ± 0.05 | – | 0.460 | 0.50 |
| Wewak 1 | 0 | 8 | – | 8 | – | – | 0.25 ± 0.08 | 0.75 ± 0.08 | – | 1.778 | 0.19 |
| Wewak 2 | 6 | 5 | – | 9 | – | – | 0.43 ± 0.08 | 0.57 ± 0.08 | – | 4.772 | 0.03 |
| Karkar 1 | 0 | 4 | 1 | 5 | 0 | 3 | 0.19 ± 0.08 | 0.54 ± 0.10 | 0.27 ± 0.09 | 9.869 | 0.02 |
| Karkar 2 | 0 | 5 | 0 | 13 | 1 | 3 | 0.11 ± 0.05 | 0.73 ± 0.07 | 0.16 ± 0.06 | 15.758 | <0.01 |
| Karkar 3 | 0 | 2 | 0 | 11 | 2 | 2 | 0.06 ± 0.04 | 0.77 ± 0.07 | 0.17 ± 0.07 | 6.214 | 0.10 |
| Alotau | 0 | 5 | 0 | 11 | 1 | 0 | 0.15 ± 0.06 | 0.82 ± 0.07 | 0.03 ± 0.03 | 0.781 | 0.85 |
| Karema | 1 | 9 | 0 | 11 | 2 | 0 | 0.24 ± 0.06 | 0.72 ± 0.07 | 0.04 ± 0.03 | 1.035 | 0.79 |

[a] Allelic frequency ± one standard deviation.
[b] $\chi^2$ tests involving 3 genotypes have 1 degree of freedom. Those involving 6 genotypes have 3 d.f.

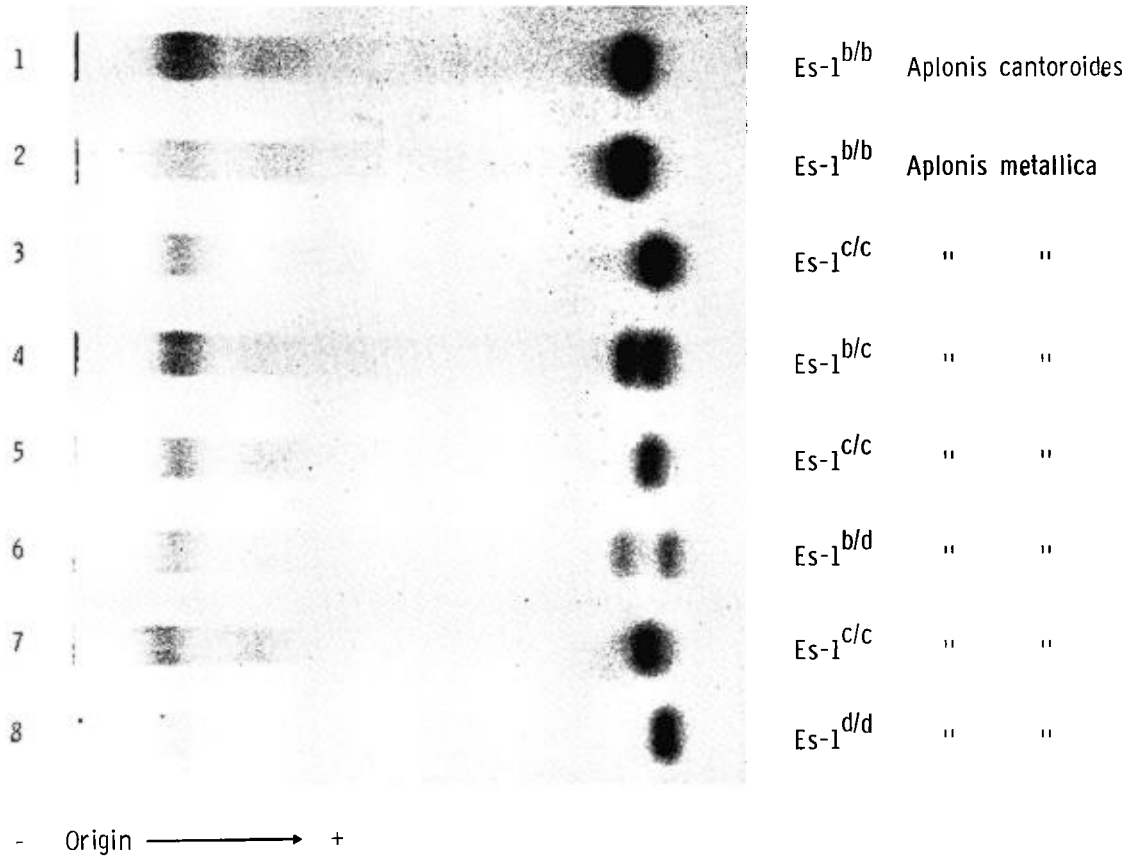


FIGURE 4. Starch-gel electrophoretic patterns of the esterases-1 of *Aplonis metallica* and *Aplonis cantoroides*. The patterns are designated according to the genotypes inferred to code for the respective enzymes.

vations was that of two codominant alleles at the esterase locus of *A. cantoroides* and three codominant alleles at the esterase locus of *A. metallica*. The esterase phenotype of homozygous individuals was a single band of esterase activity, while the phenotype of heterozygous individuals consisted of two equally dense bands of esterase activity. Thus three esterase phenotypes produced respectively by the genotypes $Es\text{-}1^{a/a}$, $Es\text{-}1^{a/b}$, and $Es\text{-}1^{b/b}$ were observed in the case of *A. cantoroides*; six esterase phenotypes produced respectively by genotypes $Es\text{-}1^{b/b}$, $Es\text{-}1^{b/c}$, $Es\text{-}1^{b/d}$, $Es\text{-}1^{c/c}$, $Es\text{-}1^{c/d}$, and $Es\text{-}1^{d/d}$ were observed for *A. metallica*. Some of these patterns are shown in figure 4. Tables 2 and 3 give the number of individuals of each esterase genotype, the frequencies of the alleles at each locality sampled, the $\chi^2$ values for the deviation of the observed distribution of genotypes from the expected Hardy-Weinberg equilibrium distribution for each population, and the $P$ values associated with each $\chi^2$ value.

Two forms of malate dehydrogenase (MDH) occur in birds and mammals, one in the mitochondrial fraction and the other in the supernatant fraction of cell cytoplasm preparations (Kitto and Wilson 1966; Karig and Wilson 1971). Both forms are dimers composed of identical subunits. However, the subunits of mitochondrial MDH are structurally and chemically distinct from those of supernatant MDH and each form is synthesized under the control of separate genes (Davidson and Cortner 1967). Our analyses of the heart extracts revealed no electrophoretic variants of either mitochondrial or supernatant MDH in either species of starling. The supernatant MDH of both species was identical in electrophoretic mobility to that of chicken supernatant MDH; mitochondrial MDH of both starling species was identical in mobility to that of other species of passerine birds (Kitto and Wilson 1966; Karig and Wilson 1971).

The hemoglobins of individuals of *A. metallica* collected at Madang, Wewak, and Karkar Island were identical electrophoretically with the exception of one individual. In addition to the assays for LDH, MDH, and esterase, extracts of heart muscle, breast muscle, liver, and plasma were also assayed electrophoretically for the following enzymes: alkaline phos-

TABLE 3. Distributions of LDH-H and Esterase-1 genotypes of *Aplonis cantoroides*, allelic frequencies, and Chi-squared values for deviations from expected Hardy-Weinberg equilibrium distributions.

| Locality | Distribution of LDH genotypes | | | Allelic frequencies | | | |
|---|---|---|---|---|---|---|---|
| | BB | BC | CC | B | C | $\chi^2$ | *P* |
| Lorengau | 26 | 2 | 0 | 0.96 ± 0.03[a] | 0.04 ± 0.03 | 0.038[b] | 0.85 |
| Rabaul | 23 | 0 | 0 | 1.0 | 0.0 | 0 | 1.0 |
| Karkar | 11 | 0 | 0 | 1.0 | 0.0 | 0 | 1.0 |
| Madang | 32 | 0 | 0 | 1.0 | 0.0 | 0 | 1.0 |
| | Esterase Genotypes | | | | | | |
| | AA | AB | BB | A | B | | |
| Lorengau | 0 | 0 | 28 | 0.0 | 1.0 | 0 | 1.0 |
| Rabaul | 0 | 2 | 21 | 0.04 ± 0.03 | 0.96 ± 0.03 | 0.048 | 0.83 |
| Karkar | 0 | 5 | 6 | 0.23 ± 0.09 | 0.77 ± 0.09 | 0.952 | 0.34 |
| Madang | 5 | 11 | 13 | 0.36 ± 0.06 | 0.64 ± 0.06 | 0.928 | 0.35 |

[a] Allelic frequency ± one standard deviation.
[b] $\chi^2$ tests for these distributions have 1 degree of freedom.

phatase, leucine aminopeptidase, NADP-dependent isocitrate dehydrogenase, xanthine dehydrogenase, and transferrin. Each of these was monomorphic in both species of starlings. In samples examined 6 months after collection, no glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, or phosphoglucomutase activity was found.

*Genic heterozygosity and protein polymorphism within populations.* Several previous studies of electrophoretic variation of enzymes and nonenzymatic proteins have estimated the proportion of loci that were either polymorphic within a species or per population or heterozygous per individual. Estimates are available for many species (cf. Selander and Kaufman 1973). The proportion of loci polymorphic per population ranges from 0.42 to 0.08, while the proportion of loci heterozygous per individual ranges from 0.25 to 0.01.

Until 1970, only commercially kept or semi-managed populations of birds were sampled to study protein polymorphisms on a non-selective basis (see review by Sibley et al. 1974); that is, the selection of proteins assayed was random in so far as an attempt was made to detect and score all assayable enzymes and nonenzymatic proteins present in the tissue sampled. In two European stocks of the Japanese Quail, 57–58% of 24 loci were polymorphic per "population" (Baker and Manwell 1967). Of 44 loci of the Ring-necked Pheasant (*Phasianus colchicus*), 19 were polymorphic among all the populations examined (Baker et al. 1966).

In contrast to this, the amount of polymorphism in natural populations of passerine birds appears to be much less. Sixteen loci of the Rose-breasted Grosbeak (*Pheucticus ludovicianus*) were examined and all were monomorphic (Sibley and Corbin 1970). In the Baltimore-Bullock's Oriole complex (*Icterus*) and the Rufous-sided Towhee (*Pipilo erythrophthalmus*), 3 of 18 loci (17%) were polymorphic (Sibley and Corbin 1970 and unpubl. data). An average of 3.53% of 24 loci of the Andean Sparrow (*Zonotrichia capensis*) was polymorphic (Nottebohm and Selander 1972).

In our study of *A. cantoroides* and *A. metallica*, 16 proteins were scored. These include 11 enzymes and 5 nonenzymatic proteins; they are assumed to be coded for by 18 loci. Expressing the polymorphism as the percentage of protein types found to be variant among all individuals sampled, 18.8% (3 out of 16 including the single case of a hemoglobin variant) of the proteins of *A. metallica* and 12.5% (2 out of 16) of the proteins of *A. cantoroides* were polymorphic. The proportion of loci polymorphic per species was 0.166 and 0.111, respectively. For the Karkar Island populations only, the proportion of loci polymorphic per population of each species was 0.166 for *A. metallica* and 0.111 for *A. cantoroides*. For all other populations of these two species the proportion of polymorphic loci was 0.111. In these populations (excluding Karkar Island) the average proportion of loci that was heterozygous per individual was 0.047 in *A. metallica* and 0.008 in *A. cantoroides*. While the latter is very low, comparable values (0.02) were reported for several species of *Dipodomys* (Johnson and Selander 1971).

A lactate dehydrogenase polymorphism previously was reported for one avian species, the Rock Dove or common pigeon (Zinkham et al. 1963, 1964, 1966). No LDH electrophoretic variant was found in the comparison of 44 Peafowl (*Pavo cristatus*) heart and breast muscle extracts (Rose and Wilson

1966). LDH variants are rare in man (Vesell 1965) and *Mus musculus* (Selander and Yang 1969; Selander et al. 1969a,b), but common in some poikilotherms (Markert and Faulhaber 1965; Odense et al. 1966, 1969; Ohno et al. 1967; Bailey and Wilson 1968; Neaves and Gerald 1969; Salthe 1969; Salthe and Nevo 1969; Wright and Atherton 1970; Levy et al. 1971). Thus the extensive polymorphism at the LDH-H locus of the starling species is somewhat unique only with respect to other avian species studied to date. Unpublished data of C. G. Sibley, K. W. Corbin, A. Ferguson, and P. Bottjer show that LDH is monomorphic in populations of the Baltimore and Bullock's Orioles (*Icterus*), Red-eyed and Spotted Towhees (*Pipilo*), Rose-breasted and Black-headed Grosbeaks (*Pheucticus*), Yellow-shafted and Red-shafted Flickers (*Colaptes*), Eastern and Western Meadowlarks (*Sturnella*), the Slate-colored Junco (*Junco*), and the House Finch (*Carpodacus*). Brush and Scott (1972) found that LDH was monomorphic in the Red-winged Blackbird (*Agelaius*).

Polymorphism at the esterase loci is a common feature of vertebrate populations (Harris 1969; Shaw 1965; Selander and Yang 1969; Selander et al. 1969a,b; Dessauer and Nevo 1969; deLigny 1968). In addition to the esterase polymorphisms reported here, geographic variation in allelic frequencies of esterase alleles has been found in several other species of passerine birds. These include the Baltimore and Bullock's Orioles (*Icterus*), the Yellow-shafted and Red-shafted Flickers (*Colaptes*), the Red-eyed and Spotted Towhees (*Pipilo*), the Slate-colored Junco (*Junco*) (Sibley, Corbin, Ferguson and Bottjer, unpublished data), and the Red-winged Blackbird (*Agelaius*) (Brush and Scott 1972). Not all bird esterases are polymorphic, however. Those of the Rose-breasted and Black-headed Grosbeaks (*Pheucticus*) and the House Finch (*Carpodacus*) are monomorphic (Sibley and Corbin 1970; Sibley, Corbin, Ferguson, and Bottjer, unpubl. data), and the esterases of the lesser Bird of Paradise (*Paradisaea minor*), Count Raggi's Bird of Paradise (*P. raggiana*), and their hybrids are monomorphic (Sibley, Corbin, and Ferguson, unpubl. data).

Since supernatant malate dehydrogenase is normally an invariant protein not only in birds (Kitto and Wilson 1966; Karig and Wilson 1971) but also in mammals (Davidson and Cortner 1967), it was anticipated that NAD-MDH would be monomorphic in the starling species.

Although the blood plasma transferrins of *A. metallica* and *A. cantoroides* are monomorphic, serum transferrin and ovotransferrin polymorphisms are present in other avian species (Mueller et al. 1962; Baker and Hanson 1966; Desborough and Irwin 1966; Baker 1967; Baker and Manwell 1967; Brush 1968, 1970; Stratil 1968; Sibley and Corbin 1970; Ferguson 1971; Brush and Scott 1972).

*Genetic differentiation among populations.* Within a breeding population with an effective size of 200 or more individuals, which is of the order of magnitude of a Metallic Starling breeding colony, a number of factors may operate simultaneously, resulting in variation in allelic frequencies. These factors are gene flow, drift, mutation pressure, and selection pressure (Prakash et al. 1969).

With the exception of the Wewak-1 and Karkar-2 populations of *A. metallica*, the distributions of LDH-H genotypes within populations are in agreement with the numbers expected on the basis of Hardy-Weinberg equilibria (table 1). Likewise, excepting the Wewak-2, Karkar-1, and Karkar-2 populations, the distributions of esterase-1 genotypes within populations are in agreement with those expected (table 2). Excepting the populations mentioned above, the observed allelic frequencies might be accounted for by the simplest genetic model, that of random mating in the apparent absence of disrupting gene flow, mutation pressure, and selection pressure. If genotype frequencies are being modified by selection for or against homozygotes or heterozygotes, the number of individuals sampled from each population is too small for this to be demonstrated statistically.

The interpopulation homogeneity of the observed genotype frequencies of *A. metallica* was determined by Chi-squared tests. Since expected values are less than 5 for several esterase and LDH genotype categories in each population, not all genotype classes were included in the Chi-squared calculations. An analysis of the esterase-1 genotypes $Es\text{-}1^{b/b}$, $Es\text{-}1^{b/c}$, and $Es\text{-}1^{b/d}$ indicated that there were no significant differences in the interpopulation variation of genotype frequencies. If all populations are included in the calculation of a Chi-squared value for LDH genotypes $Ldh\text{-}H^{a/a}$, $Ldh\text{-}H^{a/b}$, and $Ldh\text{-}H^{b/b}$, the observed values differ very significantly from those expected. This indicates, in contrast to the situation observed for the esterase genotypes, that the lactate dehydrogenase-H genotype frequencies are not homogeneous among populations. The populations sampled have been partitioned in various ways to identify the source of this heterogeneity. It was found that the LDH-H genotype frequencies at

Lorengau contributed significantly to the overall Chi-squared value. It was also found that the genotype frequency data for Wewak-1 and 2 and Karkar Island-1, 2, and 3 contributed to the heterogeneity. The fact that *Ldh-H*$^a$ is low in frequency on Manus Island and relatively high at Wewak-1 may indicate that selection favors one LDH allele in one locality while another allele is selectively superior elsewhere. The results for the LDH-H genotype data suggest that the Lorengau population on Manus Island, representing the subspecies *purpureiceps*, not only is set off geographically from the other populations but also does not exchange genes with other populations. An alternative hypothesis is that genetic drift in the relatively isolated Lorengau population has resulted in the marked shift in allelic frequencies. By contrast, Emirau Island apparently does belong to part of the larger gene pool involving the other populations of the Bismarck Archipelago. Barriers to gene flow may exist on mainland New Guinea as indicated by the fact that significant Chi-squared values are obtained for various combinations of the mainland populations. However, this situation is not necessarily due to barriers to gene flow since differences in selective regimes could establish similar allelic frequency differences. Indeed, a grouping of the three Karkar Island populations results in a significant Chi-squared value. Since Karkar Island has a diameter of about 12 miles, it is highly probable that the various colonies on this island exchange individuals. Thus, it is logical to infer either that selection pressures vary significantly on different parts of Karkar Island or that these results are an artifact of sampling. The same conclusion also applies to the Wewak localities.

In contrast to the results obtained for the LDH genotype frequency data, the homogeneity of the esterase genotype frequency data indicates that either there is rather extensive gene flow among the localities sampled for *A. metallica*, or that selection pressures on the three esterase alleles are more or less uniform for all populations.

Patently, there are several possible explanations for the observations made here. If there is extensive gene flow among populations, then selection tends to oppose changes in allelic frequencies at the LDH locus whereas allelic frequencies at the esterase-1 locus are essentially uniform due to panmixia and weak and/or uniform selection pressures. Alternatively, if gene flow between populations is restricted or nonexistent, then selection pressures for the esterase alleles must be more or less uniform throughout the distribution of *A. metallica*, while selection pressures acting on the LDH alleles vary among the populations sampled.

The above two alternatives primarily give consideration to those factors that maintain allelic variation rather than to the mechanism that gave rise to the observed polymorphisms. It is possible that the LDH-H and Es-1 alleles were and perhaps still are selectively equivalent. In the absence of migration, it would take approximately $4N_e$ generations to fix a selectively equivalent allele in a population (Kimura and Ohta 1969), where $N_e$ is the effective population size. For *A. metallica* this time might be as few as 200 years, given a colony size of 50 individuals and one generation per year. Migration would subsequently alter this situation and could result in various distributions of allelic frequencies depending on the rate of exchange of individuals between populations and on the population size (Kimura and Ohta 1971; Maruyama 1971). Given a situation where different alleles became fixed in isolated populations, subsequent exchange of alleles between these populations could result in clines even though the alleles were selectively equivalent (Maruyama 1971).

Because the Chi-squared test for homogeneity cannot deal adequately with expected values less than 5, the biologically interesting aspects of our data, i.e., the rarity of the *Es-1*$^d$ and *Ldh-H*$^c$ alleles in most populations and the prevalence of the *Es-1*$^d$ allele on Karkar Island (tables 1 and 2), are necessarily ignored when the contributions of some genotypes are excluded from the Chi-squared calculations. Furthermore, the LDH allelic frequencies appear to vary clinally along the Bismarck Archipelago (table 1 and fig. 1). This suggests that either selection acting on LDH function varies in some linear fashion over this part of the range of *A. metallica* and is independent of any selection pressure acting on esterase function, or that migration of individuals from areas characterized by a high frequency of the *Ldh-H*$^b$ allele (Lorengau or Kavieng) to areas with a lower proportion of this allele accounts for the observed cline.

Unfortunately, we know nothing about the selection pressures acting on Metallic Starling LDH and esterase loci nor can we estimate the extent of immigration into and emigration from the various breeding populations since virtually nothing is known about the long-term movements of this species. If the enzymes synthesized under the control of different alleles are functionally different, they

may be selected differentially at the various geographic locations (Koehn 1969). Hypothetically, if selection favors one allele in one part of the distribution of a species and a second allele is favored in another part of the range, then the allelic frequencies may vary clinally from one locality to another. The frequencies of the two alleles will vary proportionately to one another; as one increases the other decreases. If populations exchange individuals each generation, this situation should result in gradients of allelic frequencies regardless of whether selection pressures vary gradually across the distribution of the species. Gene flow between populations may act to negate the effects of differential selection and will tend to equalize the frequency of an allele in the various populations if they actually contribute to a larger, panmictic population. The larger the effect of migration relative to the force of selection, the more nearly will the allelic frequency at each locality equal an average value (Prakash et al. 1969). Given the limitations of the data presented herein, one might conclude that gene flow rather than differential selection is the more important in reducing the allelic variation at the Es-1 locus. On the other hand, interpopulation variation at the LDH-H locus suggests that differential selection, acting in opposition to gene flow, plays a greater role in maintaining the allelic frequencies at this locus.

With regard to genetic differentiation among populations of *A. cantoroides*, several points can be made. Genetic theory predicts that in small, isolated populations genetic drift will play a predominant role in determining allelic frequencies and one expects to find relatively little polymorphism in such populations. Those loci that are polymorphic near the center of distribution of a species may be monomorphic in isolated or peripheral areas. This appears to be the situation for the Es-1 locus of *A. cantoroides*. *Es-1*$^b$, which is highest in frequency in the Rabaul, Karkar Island, and Madang populations, is fixed in the Lorengau, Manus Island population (table 3). However. the opposite condition is found at the LDH-H locus. It is polymorphic in the Lorengau population and *Ldh-H*$^b$ is fixed in the other three populations (table 3).

We can only speculate on the origin of *Ldh-H*$^c$ in the Lorengau population of *A. cantoroides*. As mentioned above, it appears to be identical to *Ldh-H*$^c$ of *A. metallica* on the basis of its electrophoretic behavior. It seems unlikely that this allele would have arisen independently in the two species subsequent to their divergence from a common ancestor. Rather, it is probable that *Ldh-H*$^c$ was present in ancestral populations prior to speciation and has subsequently been maintained in some populations of both species. In those populations in which it has not been found, it may exist but at frequencies too low to be detected given the sample sizes of this study.

In contrast to the interpopulation similarity in the allelic frequencies of esterase-1 of *A. metallica*, the variation at this locus of *A. cantoroides* is significant. The genotype frequencies in the Rabaul and Lorengau populations are significantly different from those of Madang and Karkar Island. Using the Chi-squared test to partition homogeneous genotype distributions, various combinations of the Rabual and/or Lorengau genotype distributions compared with the Karkar Island and/or Madang distributions all yield $\chi^2$ values with values of $P < 0.01$.

The arguments set forth above to explain the observed differences in allelic variation in populations of *A. metallica* apply equally to the data for *A. cantoroides*. However, the breeding behavior of the Singing Starling tends to space out individuals to a much greater degree than is true for the Metallic Starling. Therefore, it seems that gene exchange between adjacent localities may occur more frequently for *A. cantoroides*. If this is true, then the population differences in allelic frequencies at the Es-1 locus are probably due to differential selection acting in opposition to gene flow. Assuming our inferences to be correct, we may deduce that selection pressures on the Es-1 and LDH-H loci of the Singing Starling are substantially different from those acting on the homologous loci of the Metallic Starling.

## SUMMARY

During the 1969 *Alpha Helix* expedition to Papua-New Guinea, 354 Metallic Starlings were collected from 15 localities and 108 Singing Starlings were collected from 4 localities. Starch-gel electrophoresis was used to detect protein variants in muscle tissue extracts and plasma samples. Of 16 protein types assayed, 13 were monomorphic and 3 were polymorphic. The variable loci of *A. metallica* included three alleles at the lactate dehydrogenase-H locus and three alleles at the esterase-1 locus. Two alleles for esterase-1 and two alleles for lactate dehydrogenase-H were found in populations of *A. cantoroides*. The 16 proteins assayed are thought to be coded for by 18 loci. Thus the proportion of

loci ploymorphic per species is 0.166 for *A. metallica* and 0.111 for *A. cantoroides*. The average proportion of loci heterozygous per individual is 0.047 for *A. metallica* and 0.008 for *A. cantoroides*. The latter value is lower than those values calculated for most other animal species examined.

Allelic frequencies at the lactate dehydrogenase-H and esterase-1 loci vary among populations and in most of these populations the distributions of genotypes are in agreement with those expected for Hardy-Weinberg equilibrium distributions. The frequencies of lactate dehydrogenase-H alleles of *A. metallica* vary clinally among populations on the islands of the Bismarck Archipelago. *Ldh-H*$^b$ is high in frequency (0.96) at one end of the species' distribution while *Ldh-H*$^a$ is highest (0.65) in populations located elsewhere in the range of *A. metallica*. Interpopulation variation in allelic frequencies at the esterase-1 locus is much less than at the LDH-H locus. The opposite is true of these loci in populations of *A. cantoroides*, where LDH-H is essentially monomorphic and allelic frequencies at the esterase-1 locus vary significantly among populations.

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