

Molecular Genetics of Avian Proteins

XIII.* Protein Polymorphism in Three Species of Australian Passerines

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Abstract

An introduced species, the house sparrow (*Passer domesticus*), and two Australian native species, the welcome swallow (*Hirundo tahitica neoxena*) and the fairy martin (*Petrochelidon ariel*), have moderately low levels of protein polymorphism compared with domesticated or semi-wild 'managed' species of birds.

Genetically variant proteins in these birds include transferrin, esterase, phosphoglucomutase, NADP-dependent isocitrate dehydrogenases, phosphogluconate dehydrogenase (decarboxylating) and glucose-6-phosphate dehydrogenase.

Egg-white protein polymorphism confirms heterogeneity of egg colour, markings and shape, and suggests that approximately 10% of the 'clutches' in house sparrow nests represent infidelity (intraspecific nest parasitism).

For the four enzymes capable of supplying reduced NADP for reductive biosyntheses in growth and detoxification, the house sparrow has more heterozygosity (29%) than either the welcome swallow (9.4%) or the fairy martin (2.3%) and the difference is highly significant statistically. The results are discussed in relation to possible biochemical correlates of MacArthur and Wilson's (1967) evolutionary strategies of *r* or *K* selection.

Introduction

In an attempt to understand the evolutionary significance of protein polymorphism, a number of researchers have used electrophoresis to survey a variety of proteins in plant and animal species. Correlations have been drawn between the amount of genetically based protein variation and various factors: phylogenetic position, for example invertebrate versus vertebrate (Selander and Kaufman 1973); type of environment (Manwell and Baker 1970); and breeding system (Brown and Allard 1970; Marshall and Allard 1970).

As Corbin *et al.* (1974) point out, data for different species of birds are still quite meagre. Such data are desirable in view of differences in the natural history of various avian species.

In the present study we compared two species of native Australian passerines, the welcome swallow, *Hirundo tahitica neoxena* Gould, and the fairy martin, *Petrochelidon ariel* Gould (taxonomy based on Peters 1960), with another species of passerine, the house sparrow, *Passer domesticus* L., which is not native to Australia but, following introductions in the middle of the 19th century, has spread to many parts of Australia (Hill 1967).

Questions asked in this study include the following. (i) Do wild species of birds have less protein polymorphism than domesticated species, or species 'managed' in

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a semi-wild state by repeated introductions (see discussion in Corbin *et al.* 1974)? (ii) Does a species that has been introduced relatively recently have as much variability as native species? (iii) Do egg-white protein polymorphisms, which reflect the genotype of the mother bird, confirm the suggestion based on discontinuous differences in the appearances of some clutches of eggs that there is intraspecific nest infidelity?

Materials and Methods

Birds

Welcome swallows, fairy martins and house sparrows were sampled in rural areas along roads from Williamstown to Strathalbyn, South Australia. Although there are some places where the nesting areas of the three species do not overlap, in the region where specimens were collected all three species commonly nest under bridges or in large drains associated with roads.

Birds were sampled as adults (collected by mist-netting) or as nestlings or as embryos. The two native species are protected by law and the collections were carried out with a permit from the Fisheries and Fauna Department.

No developmental changes which could be confused with genetic variation were noted except for differences observed in the haemoglobin patterns of early embryos, late embryos, nestlings and adults, especially in the appearance of the major anodal haemoglobin zone. However, these differences largely disappeared upon lysis of erythrocytes in dilute phosphate buffer, or upon electrophoresis of haemolysates in buffers containing phosphate, as observed in other studies on avian haemoglobins (Manwell *et al.* 1963; Baker *et al.* 1966). It is known that inorganic phosphate binds strongly to chicken haemoglobin and alters the electrophoretic mobility of certain fractions (Huisman *et al.* 1964), and it is likely that these changes are related to the binding of organic phosphates which alter in amount during development (Benesch and Benesch 1969). Because most of the developmental differences between embryos and later stages disappear when the samples are treated with inorganic phosphate, it is likely that the major haemoglobin chains are similar throughout most if not all of the development of these passerines. There are two major haemoglobins in each of these passerines and, by analogy with studies on the polypeptide chain composition of the two major late embryo and adult chicken haemoglobins (D'Amelio 1966; Hashimoto and Wilt 1966; Manwell *et al.* 1966), there are three major haemoglobin loci.

Electrophoresis

Two techniques were used: (1) horizontal starch-gel electrophoresis with a range of voltage gradients from 12 to 25 V/cm, the latter allowing electrophoresis to be completed in approximately 2 h; (2) 'Gradipore' electrophoresis in an acrylamide polymer of increasing density at greater distances from the sample insertion slots (Margolis and Kenrick 1968). The main buffers for the starch gels were the discontinuous system (pH 8.0) of Ferguson and Wallace (1961) and the potassium phosphate buffer (pH 6.7, ionic strength 0.02). The most useful buffer in the polyacrylamide electrophoresis was the pH 8.8 buffer of Aronsson and Grönwall (1957). Presence or absence of polymorphism was checked in both electrophoretic systems and, for starch gel, in the buffers of pH 8 and 6.7. Additional buffers were used for certain proteins: haemoglobin was also surveyed in starch gel with potassium phosphate buffer (pH 6.0, ionic strength 0.02). The search for lysozyme meant that egg-white samples were also studied in starch gels made with barbiturate buffer (pH 8.4, ionic strength 0.03) and also the pH 4.7 and 5.7 acetate buffers (Baker and Manwell 1967; Arnheim *et al.* 1969).

Localization of Specific Enzymes and Other Proteins after Electrophoresis

The survey concentrated on 12 different proteins which yielded good electrophoretic resolution. (Some esterases resolved very well and others did not.) Localization of enzymes and other proteins were by methods discussed by Manwell and Baker (1970), the enzyme-staining techniques being similar to those used by Prasad and Shaw (1970).

Some limitations were imposed by the small size and instability of the samples. Haemoglobin was converted to the carbon monoxide derivative to increase stability. Fresh, unfrozen samples gave the sharpest resolution for certain of the enzymes but after 48 h unfrozen, or 2 weeks frozen, resolution deteriorated for glucose-6-phosphate dehydrogenase and some esterases. Corbin *et al.* (1974)

found that, despite care in handling samples, within 6 months avian glucose-6-phosphate dehydrogenase, phosphoglucanate dehydrogenase (decarboxylating) and phosphoglucomutase lost all activity. We have found that high activity and sharp resolution of these enzymes can be obtained with fresh samples used within 48 h.

Resolution of egg white by electrophoresis was good except that lysozyme could not be located using the standard buffers mentioned above. However, weak lysozyme activity was observed by the method of Smolelis and Hartsell (1951). Sibley (1970) noted that many passerine egg-white samples lack the characteristic cathodal zone of lysozyme. Accordingly, this enzyme is not represented with the other common egg-white proteins in Table 1.

We failed to obtain adequate resolution of hexokinase and 6-phosphofructokinase; resolution of NAD-dependent malate dehydrogenases and fructose-bisphosphate aldolase is usually sufficiently diffuse to make resolution an unsuitable method in the search for genetic variation in these avian species. The two NADP-dependent isocitrate dehydrogenases, called here 'locus II' and 'locus III', consistently gave good resolution and high activity after electrophoresis; however, only some extracts yielded one or more sharp zones of a rapidly migrating anodal NADP-dependent isocitrate dehydrogenase; this enzyme ('locus I') showed evidence of genetic variability which paralleled neither of the other two NADP-dependent isocitrate dehydrogenases. Locus I is not included in the present study, however, because it was not present in many samples and it overlapped with zones of a 'nothing dehydrogenase' which was also erratic in its occurrence.

Egg-white Esterases

The fairy martin resembles the domesticated pigeon, the rock dove *Columba livia* (Baker, unpublished data), in having sharp zones of polymorphic esterase in egg white. In other species we have surveyed, egg-white esterases generally resolve poorly.

The fairy martin also has another esterase in egg white, a diffuse zone which migrates less rapidly than the polymorphic set of zones. The sharp and the diffuse fairy martin egg-white esterases are the product of distinct genetic loci. Not only is there no parallel variation, but the sharp and diffuse esterases can be differentiated by inhibitors: the sharp esterases are inhibited only slightly by 10^{-3} M eserine, which completely inactivates the diffuse esterases. Pre-incubation with 10^{-3} M Malathion [*O,O*-dimethyl *S*-(1,2-dicarbethoxyethyl)phosphorodithioate] did not inhibit the diffuse esterases although it did inhibit the sharp esterases completely; the inhibition was reversible by overnight rinsing of the gel.

The polymorphic sharp egg-white esterase does not occur in the welcome swallow or the house sparrow. Because of its non-homology in the species comparison, this esterase is not included in the estimate of enzyme heterozygosity; however, its extensive polymorphism makes it useful in checking the fidelity of nesting habits in the fairy martin.

Measures of Variability

With respect to differences between individuals for a given protein, three categories are used:

- (1) *Monomorphic*—the electrophoretic zones are identical in position.
- (2) *Variable*—slight differences in position and appearance of the zones exist but there are no clear genetically interpretable patterns of polymorphism. (Whether the variation is a pre-parative artefact, physiological, or genetic can not be determined without further characterization.)
- (3) *Polymorphic*—discrete differences among individuals exist which involve a set of electrophoretic patterns that have been established as genetic in other species (Manwell and Baker 1970).

Definitions of polymorphism emphasize that the frequency of the condition must be greater than can be accounted for by mutation alone. Different lower limits are used by different research workers. For the sake of consistency with other studies on protein polymorphism in populations we adopt the same numerical value as Avise and Selander (1972): a protein is monomorphic if the most common allele has a frequency of 0.95 or greater. Recognizing the uncertainties about the role of selection in the maintenance of less common genetic variants, we suggest that the word 'polymorphic' be placed in quotation marks when referring to such genetic variation where the estimate of the allele frequencies of the less common variants is below Avise and Selander's (1972) threshold of 0.05.

Definition of a protein locus is fairly straightforward. As mentioned previously, it appears that for the two major avian haemoglobins, there are three polypeptide chain types, one common chain and two unique chains; thus, there are three haemoglobin loci. In the present study lactate dehydrogenase is counted as only one locus, for only one major zone occurred in erythrocyte samples.

Recent research on protein polymorphism in populations has often employed three measures of the amount of genetic variability (see, for example, Avise and Selander 1972; Nottebohm and Selander 1972; Selander and Kaufman 1973):

- (1) the percentage of all protein loci which are polymorphic (recognizing a 'cut-off' frequency, as discussed above),
- (2) the average number of alleles per polymorphic locus,
- (3) the average proportion of loci (H) for which an individual is heterozygous.

It is becoming clear that certain proteins are more frequently found with electrophoretic polymorphism than others; accordingly, we also express separately the heterozygosity for the seven homologous enzymes and for the five different proteins from various body fluids (blood or egg white).

Results

Protein Polymorphism

Classification of the 12 different proteins in the three species into monomorphic, variable, polymorphic and 'polymorphic' is shown in Table 1, together with the number of individuals surveyed for each protein.

The patterns of electrophoretic protein polymorphism are shown in Fig. 1 which is a copy of the electrophoretic results. The position of the haemoglobin zones, which are very similar if not identical electrophoretically in the three species, are shown on the left-hand side of the figure for reference. The small rapidly migrating cathodal zone of haemoglobin was found only in some late embryos and nestlings. The relative positions of the enzymes and variants for the three species are consistent, although in some experiments the NADP-dependent isocitrate dehydrogenase II zones migrated slightly faster than the anodal haemoglobin. The satellite zones of glucose-6-phosphate dehydrogenase often had different appearances in repeated experiments, probably as a consequence of aging of the samples; however, this did not interfere with the classification of variants providing the samples were analyzed within 48 h of preparation.

There are no unusual findings with regard to the patterns of protein polymorphism and the results agree with those of other research workers who have examined enzymes in other passerine birds—for example enzymes in the Chingolo sparrow, *Zonotrichia capensis* (Nottebohm and Selander 1972); enzymes in the metallic starling, *Aplonis metallica*, and in the singing starling, *Aplonis cantoroides* (Corbin *et al.* 1974); and ovotransferrin in the willow sparrow, *Passer montanus* (Kimura and Makita 1966). The egg-white electrophoretic patterns resemble those obtained by Sibley (1970) for the same or closely related species of passerines and the pattern of protein polymorphism is similar to that observed in other birds and some other groups of organisms (Manwell and Baker 1970).

In contrast to polymorphisms of mammalian erythrocyte glucose-6-phosphate dehydrogenase, hybrid zones in heterozygotes are characteristic of the polymorphism of this enzyme and its autosomal inheritance in several avian species (Bhatnagar 1969; Cooper *et al.* 1969; Manwell and Baker 1969; Baker and Manwell, unpublished data on hybrids between *Cairina moschata* and *Anas platyrhynchos*).

Table 1. Variability of proteins in three species of passerines

Protein	Source	Welcome swallow No. Remarks	Fairy martin No. Remarks	House sparrow No. Remarks
Lactate dehydrogenase (1.1.1.27)	Erythrocytes	17 Monomorphic	11 Monomorphic	19 Monomorphic
Isocitrate dehydrogenase (NADP ⁺) (1.1.1.42)	Erythrocytes	23 Polymorphic	33 Polymorphic	25 Polymorphic
Locus II	Erythrocytes	23 Monomorphic	33 'Polymorphic'	25 Polymorphic
Locus III	Erythrocytes	25 Monomorphic	33 Monomorphic	25 Polymorphic
Phosphogluconate dehydrogenase (decarboxylating) (1.1.1.44)	Erythrocytes	25 Variable	32 Variable	25 Polymorphic
Glucose-6-phosphate dehydrogenase (1.1.1.49)	Erythrocytes	28 Polymorphic	31 'Polymorphic'	25 'Polymorphic'
Phosphoglucomutase (2.7.5.1)	Egg white	7 ^A Variable	40 ^A Polymorphic	6 ^A Variable
Esterase (3.1.--)	Erythrocytes	12 Monomorphic	26 Monomorphic	30 Monomorphic
Glucosephosphate isomerase (5.3.1.9)				
Haemoglobin, major anodal and major cathodal				
(3 polypeptide chains)	Egg white	31 Monomorphic	30 Monomorphic	30 Monomorphic
Transferrin (conalbumin)	Egg white	18 ^A Polymorphic	49 ^A Polymorphic	32 ^A Polymorphic
Ovomacroglobulin	Egg white	18 ^A Variable	49 ^A Monomorphic	32 ^A Monomorphic
Ovalbumin	Egg white	18 ^A Monomorphic	49 ^A Monomorphic	32 ^A Monomorphic
Pre-ovalbumin	Egg white	18 ^A Monomorphic	49 ^A Monomorphic	32 ^A Monomorphic

^A As theoretically all eggs in a given nest reflect the one genotype of the mother in terms of egg-white proteins, this number is the number of nests sampled. The actual number of eggs surveyed individually is greater, e.g. 21 welcome swallow eggs, 60 fairy martin eggs and 81 house sparrow eggs.

Phenotypes and estimates of gene frequencies for the genetically variant proteins are shown in Table 2.

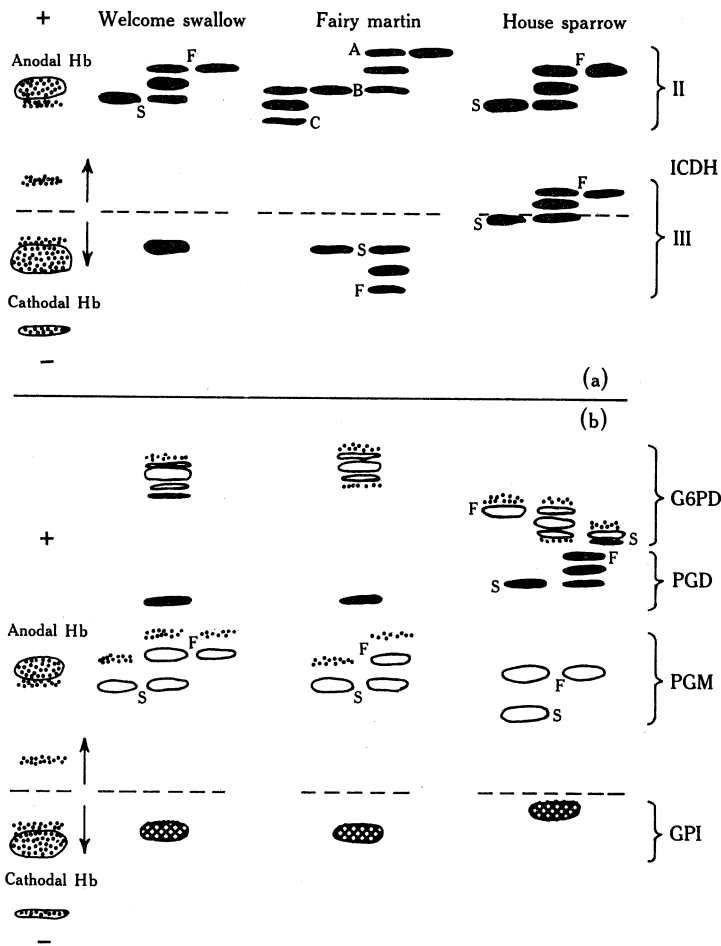


Fig. 1. Patterns of electrophoretic protein polymorphism. The diagram is based on copies of the actual electrophoretic separations achieved in the pH 8 buffer of Ferguson and Wallace (1961). (a) Diagram for the two NADP-dependent isocitrate dehydrogenases (ICDH II and III). (b) Diagram for four other enzymes: glucose-6-phosphate dehydrogenase (G6PD), phosphogluconate dehydrogenase (decarboxylating) (PGD), phosphoglucomutase (PGM) and glucosephosphate isomerase (GPI). Variants are labelled by letters, F = fast, S = slow. For the tri-allelic 'polymorphism' of isocitrate dehydrogenase II in the fairy martin the variants are labelled A, B and C. The dashed line represents the place where the samples are inserted into the starch gel. There is no significance in the use of solid, open or shaded zones other than to make the diagram easier to interpret visually in comparing the location of different zones of the same protein.

The results for the penta-allelic polymorphism of egg-white esterase in the fairy martin are shown in Table 3. All but two of the 15 possible combinations of phenotype for a penta-allelic polymorphism were observed in this sample of 49 eggs. Studies

on pedigree animals would be desirable to check the genetic interpretation of the phenotypes, for although patterns, 2 3 and 4 represent presumably the commonest homozygotes, the various combination of these for possible heterozygotes are less frequently observed than would be expected. The zones are sharply resolved in electrophoresis and the results are reproducible; however, zones vary in activity and this may be a source of complication. One egg sampled from a fairy martin nest yielded no esterase I zone.

Table 2. Genetically variable proteins in three species of passerines

Protein	No. of birds ^A	Phenotypes	Percentage heterozygotes	Gene frequencies of less common alleles
Welcome swallow				
Isocitrate dehydrogenase II	23	10S, 4F; 9FS	39.1	$p_F = 0.37$
Phosphoglucomutase	28	2S, 18F; 8FS	28.6	$p_S = 0.21$
Transferrin	18 (21)	9S, 1F; 8FS	44.5	$p_F = 0.28$
Fairy martin				
Isocitrate dehydrogenase II	33	1A, 30B; 1AB, 1BC	6.1	$p_A = 0.045, p_C = 0.015$
Isocitrate dehydrogenase III	33	32S; 1FS	3.0	$p_F = 0.015$
Phosphoglucomutase	31	30S; 1FS	3.2	$p_F = 0.016$
Esterase (egg white)	39 (49)	See Table 3	51.3	$p_1 = 0.21; p_3 = 0.15$ $p_4 = 0.23; p_5 = 0.09$
Transferrin	47 (60)	37S; 10FS	21.3	$p_F = 0.11$
House sparrow				
Isocitrate dehydrogenase II	25	10S, 1F; 14FS	56.0	$p_F = 0.32$
Isocitrate dehydrogenase III	25	15S, 5F; 5FS	20.0	$p_F = 0.30$
Phosphogluconate dehydrogenase (decarboxylating)	25	22S; 3FS	12.0	$p_F = 0.06$
Glucose-6-phosphate dehydrogenase	25	7S, 11F; 7FS	28.0	$p_S = 0.42$
Phosphoglucomutase	25	23F; 2FS	8.0	$p_F = 0.04$
Transferrin	36 (81)	29S; 7FS	19.4	$p_F = 0.097$

^A The numbers in parentheses are the total number of eggs surveyed for egg-white proteins; estimates of phenotype frequencies are based on the assumption that a given female lays all the eggs in a clutch (see discussion in text).

Fidelity of Nesting Habits

As egg-white proteins are a representation of the genotype of the maternal parent, it was possible in those cases where protein polymorphism of egg-white proteins existed to type several eggs from one nest in order to check nesting faithfulness. As with medico-legal tests for human paternity, the protein character data can not prove parentage but can exclude individuals with certain genotypes.

We found no mismatched egg-white patterns among the samples of two or more eggs from one clutch where the eggs of the clutch were similar in shape, colour and pattern. Of some 32 clutches of house sparrow eggs which did not have incubated embryos in them, and thus were suitable for egg-white protein examination, four clutches contained eggs which clearly did not match in shape, colour or pattern of markings on the egg shell. That the mismatched eggs came from different female birds was confirmed in two cases by a consistent difference in transferrin genotype.

Similarly, for the fairy martin, one nest contained a clutch where two eggs differed markedly in shape from the other two eggs. The eggs also differed consistently in esterase I type and thus must have come from two different birds.

Redfield (1973) has used incomplete family data in studies on an unidentified (serum peptidase?) protein polymorphism in the blue grouse, *Dendragapus obscurus*. Such a procedure should be accompanied by ‘maternity testing’. There is evidence that nest infidelity, a type of intraspecific nest parasitism, occurs in a number of species of birds and may well be an early stage in the evolution of interspecific nest parasitism, which has occurred in at least six separate phylogenetic lines in the class Aves (see, for example, Yom-Tov *et al.* 1974). Ornithological literature also includes a number of examples of egg stealing and chick stealing (fostering). In Table 2 of Redfield’s (1973) paper he reports three examples where, for the blue grouse, “The single offspring which belong to each of these cells [of incomplete family data] was not compatible with genetic theory”; the problem was that on three occasions the female adult blue grouse was homozygous for one allele and her associated chick was homozygous for another allele.

Table 3. Frequency of egg-white esterase I phenotypes in the fairy martin, *Petrochelidon ariel*
Esterase zones are labelled 1 (fastest) to 5 (slowest) for electrophoretic mobility. Phenotypes with two zones, presumed to be heterozygotes, are identified with the corresponding numbers. One additional nest, containing one egg, was sampled and yielded no sharp esterase zone

Phenotype	No. of eggs surveyed	No. of nests surveyed	Phenotype	No. of eggs surveyed	No. of nests surveyed
1	1	1	2, 3	—	—
2	10	7	2, 4	5	2
3	5	4	2, 5	1	1
4	7	6	3, 4	2	1
5	1	1	3, 5	2	2
1, 2	9	8	4, 5	—	—
1, 3	1	1	Homozygotes	24	19
1, 4	3	3	Heterozygotes	25	20
1, 5	2	2	Total	49	39

Discussion

Protein Polymorphism in Native versus Introduced or Domesticated Species of Birds

Although the three species of passerines have approximately similar amounts of overall protein polymorphism (summarized in Table 4), the introduced species, the house sparrow, has greater genetic variability by criteria (1) and (2) below.

(1) The house sparrow has a higher average heterozygosity, *H*, over the seven enzyme loci homologous in each of the three species. For the house sparrow there were 31 heterozygotes out of 174 opportunities for these seven enzyme loci, compared with 17 heterozygotes out of 149 opportunities for the welcome swallow and with 4 heterozygotes out of 190 for the fairy martin. Using the χ^2 test for 2×2 contingency tables and correcting for continuity (Bailey 1959), we find that the difference between the house sparrow and the welcome swallow is not statistically significant ($\chi^2_{1\text{d.f.}} = 2.12, P > 0.05$) but the difference between the house sparrow and the fairy martin is very highly statistically significant ($\chi^2_{1\text{d.f.}} = 24.02, P < 0.001$).

(2) The house sparrow has a greater number of polymorphic loci, using Avise and Selander's (1972) definition of polymorphic for a cut-off frequency of 0.05: five such loci were present for the house sparrow versus three for each of the two native passerines out of a total of 15 protein loci. If the fairy martin egg-white esterase is removed from such a comparison, then the fairy martin has only two polymorphic loci by Avise and Selander's (1972) criterion.

If the penta-allelic egg-white esterase is considered, however, the fairy martin has more alleles per polymorphic locus: 3.3 versus 2 for each of the house sparrow and the welcome swallow. When the complex variation of the house sparrow esterase is understood, this situation may change: for example, there are at least four alleles specific for α -naphthyl acetate for the major sharply resolved erythrocyte esterase in *Passer domesticus*.

Table 4. Estimates of the amount of genetically interpretable variation in three species of passerines

	Welcome swallow	Fairy martin	House sparrow
No. of loci with clear genetically interpretable variation	3	5	6
No. of loci polymorphic (gene frequency of most common allele < 0.95)	3	3	5
Average No. of alleles per polymorphic locus	2	3.3	2
Average gene frequency of less common alleles	0.29	0.098	0.22
Average heterozygosity for four enzyme loci of NAD-dependent enzymes (%) ^A	9.4	2.3	29.0
Average heterozygosity for seven enzyme loci homologous in the three species (%) ^A	11.4	2.1	17.8
Average heterozygosity for four egg-white and three haemoglobin loci (%)	4.8	3.5	3.4
Average heterozygosity over all 15 loci (includes egg-white esterase)	7.8	6.5	9.8

^A Results of statistical tests for the significance of certain differences between species are given in the text.

Our results agree with those of Corbin *et al.* (1974) in suggesting that wild bird populations have only moderate protein variability. So far the highest protein variability has been found in domesticated or 'managed' species of birds—the chicken, the *Coturnix* quail and the game pheasant (*Phasianus colchicus*) where approximately half of the protein loci are polymorphic.

All such comparisons must include a measure of uncertainty because of two factors. Firstly, the species being compared may be only distantly related; for example, Corbin *et al.* (1974) discuss data on wild passeriforms with data on domesticated or 'managed' galliforms. Secondly, species differ greatly in the range of habitats over which they are distributed (including broad versus narrow niche specificity) and the degree of population stratification (inbreeding versus outbreeding; assortative, random, or disassortative mating; isolation and migration).

For the first of these two factors, we have recently supplied a comparison of wild versus domesticated species which are phylogenetically closer than those in previous comparisons; for example wild quail *Coturnix pectoralis* have significantly less polymorphism and less heterozygosity than domesticated quail *Coturnix coturnix* (Baker and Manwell 1975).

For the second of these factors there are few useful data on bird populations. Comparisons of the amount of protein polymorphism at various egg-white protein loci in different strains and breeds of the domestic fowl serve warning that within a single species a range from monomorphism to extreme polymorphism can be found in different populations (Baker 1968).

Molecular Correlates of r and K Selection?

MacArthur and Wilson (1967) drew attention to two types of evolutionary strategy based on demographic parameters: r and K selection.

(1) r selection is selection for reproductive potential, favouring rapid increases in population. This is often accompanied by selection for rapid growth of the individual, relatively quick growth of the gonads and early sexual maturity. Species which are relatively r -selected occur in changeable environments and include a number of highly successful colonizing or pest species.

(2) K selection is selection for competitive ability through specialization for efficient exploitation of limited resources (rather than competitive ability by producing large numbers of young). K -selected forms are most favoured in stable environments.

It is expected that species where r selection occurs will have greater activity of enzymes involved in biosynthesis so as to maximize growth rate and egg production. This does not imply that these enzymes will have maximum activity under all conditions; rather, what is expected is that regulatory enzymes in anabolic pathways will show less feedback inhibition in response to end products. For enzymes involved in digestion and assimilation of food, high activity will be compromised to some extent by the requirement for broad specificity so as to permit the utilization of a larger variety of food sources. Since many plants produce toxic substances (believed to have evolved as a counter-measure to grazing), it is expected that r -selected herbivores will have evolved both high activity of detoxification enzymes and broad substrate specificity.

Species where K selection occurs are expected to show different enzymic properties. Regulatory enzymes in biosynthetic pathways are expected to be subject to greater feedback inhibition by end products. Tighter negative feedback loops will mean that greater efficiency of utilization of substrates will occur, though on average with less potential for rapid throughput. On the other hand, K -selected forms, as specialists for narrow niches, will have some digestive enzymes with fast reaction rates but narrow substrate specificities.

In the evolution of enzymes and other proteins there are two major mutational modes: allelic variation (usually the result of single amino acid substitution mutations) and gene duplication.

(1) *Allelic variation* allows the development of polymorphism: on occasions this is transient as one allozyme gradually replaces another or it may be balanced where there is selective advantage to diversity. Estimates are that from one-third to one-half of all proteins have subunit structures, allowing the possibility of interaction between different subunit types in molecules (allomultimers) from heterozygotes. While in a number of cases the heterozygote does not show properties outside the range of those of the homogygotes, there are now several examples known of biochemical 'single gene heterosis' or overdominance where the hybrid protein molecule shows greater

activity or an increase in allosteric effects (e.g. Manwell and Baker 1970; Scandalios *et al.* 1972; Miller *et al.* 1975).

(2) *Gene duplication* allows the potential not only for making more of particular proteins but, given additional mutational divergence, the potential for evolving differently specialized forms of the same enzyme (isozymes), regulatory subunits, or even ultimately different enzymes for different substrates.

Constraints on conformational structure, including subunit organization, force a compromise between enzyme activity, accuracy of feedback regulation and protein stability (reviewed: Manwell and Baker 1970; Hochachka and Somero 1973). Protein stability is expected to be especially critical for *K*-selected forms because of limited resources for the substrate and energy requirements in protein biosynthesis.

Of the three species compared in the present paper, the house sparrow, with its somewhat larger broods and more extended breeding season, its relatively omnivorous feeding habits and its well-known history as a successful colonizing species, is relatively more *r*-selected and less *K*-selected than either the fairy martin or the welcome swallow. We do not know the extent to which the success of the house sparrow as a colonizing species is the result of especially well-adapted enzymes or a moderate level of enzyme polymorphism; nor do we know to what extent the observed polymorphism was present in the populations originally brought to Australia or whether it is the result of subsequent mutation. It would not be unreasonable to expect that *r*-selected forms have higher rates of mutation.

In terms of *r* selection it is of interest that the house sparrow has much more heterozygosity for the four enzymes with hybrid zones in heterozygotes and with the ability to supply reduced NADP needed for reductive biosyntheses in growth, as well as for certain detoxification reactions. These enzymes are NADP-dependent isocitrate dehydrogenases (two major loci), glucose-6-phosphate dehydrogenase and phosphogluconate dehydrogenase (decarboxylating). The house sparrow yielded 29 heterozygotes out of 100 opportunities (29%, data in Table 2), whereas the welcome swallow had 9 heterozygotes out of 96 opportunities (9.4%) and the fairy martin had 3 heterozygotes out of 131 opportunities (2.3%). The differences are highly statistically significant (Bailey 1959, formula 30, p. 60): house sparrow compared with welcome swallow, $\chi^2_{1d.f.} = 10.85$ ($P < 0.01$); house sparrow compared with fairy martin, $\chi^2_{1d.f.} = 31.7$ ($P < 0.001$). Subsequent studies with a larger sample of house sparrows revealed an excess of heterozygotes for NADP-dependent isocitrate dehydrogenase II, significant at the 5% level, and also showed that the fourth enzyme capable of supplying reduced NADP, NADP-dependent malate dehydrogenase ('malic enzyme'), is also polymorphic in this species (Manwell and Baker, unpublished data).

It is not possible to base a general conclusion on a single comparison of three species. However, the possibility is attractive that certain underlying biochemical correlates can be found to explain the type of protein polymorphism in species showing different degrees of *r* and *K* selection. This possibility provides a testable hypothesis in studies on protein variation in populations.

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