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Electrophoretic Profiles of Esterases in Starling (*Sturnus vulgaris*) Plasma: An Apparent Simple Genetic Variant

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Using electrophoretic techniques the carboxylic ester hydrolases, EC subgroup 3.1.1, of both mammals and birds have been shown to be a complex of multiple enzyme forms (AUGUSTINSSON 1959 a, b; HOLMES & MASTERS 1968; GRUNDER 1968; BARGIELLO *et al.* 1977). It has been suggested by SELANDER & KAUFMAN (1973) that esterases are the most genetically variable of all vertebrate enzymes. They are, therefore, ideally suited for use as indicators of genetic variability in natural vertebrate populations, a knowledge of which is of value when assessing the potential hazard presented to wildlife by the use of agricultural chemicals. While most toxicological studies are carried out under controlled environmental conditions using experimental subjects of limited genetic variability, introduction into agricultural usage will involve the exposure of a much more genetically heterogeneous population under a wide variety of conditions. An examination of the extent of esterase polymorphism in wildlife species was therefore included as part of a programme intended to evaluate new approaches to assessing the effect of agricultural chemicals on wildlife. Initial findings in respect of Starling (*Sturnus vulgaris*) plasma esterases are reported here. This species was selected for study because it is abundant, is of a convenient size to handle, readily nests in artificial sites and is of agricultural interest.

MATERIALS AND METHODS

The study was carried out for three breeding seasons (1979–1981). Forty nest boxes (thirty-four in 1979 and 1980) suitable for occupation by starlings were sited around a single storey office building in a suburban area of South London. Inspections of the boxes were commenced in the spring and were carried out at intervals in order to determine the dates of laying, clutch size and the date of hatching. At approximately 11 days of age young birds were removed from the nest for ringing and a blood sample (0.2 mL) was taken from the wing vein using a 1-mL disposable syringe which had been rinsed with heparin solution (1000 U/mL) and was fitted with a 25 gauge needle. The birds were then replaced in the nest. Some of the parent birds were also caught in the nest boxes at the beginning and end of the breeding season for ringing and blood sampling. Immediately after collection blood samples were centrifuged at 3,000 rpm for 10 min at 4°C according to the procedure described by HEALING (1978). The plasma thus produced was stored frozen at -18°C until examined electrophoretically.

Iso-electric focusing in agarose gels was carried out with a LKB Multiphor apparatus. Agarose (0.35g I.E.F. grade) was dissolved in water (44 mL) by boiling. Equal quantities (1.4 mL) of Ampholines of the ranges pH 4–6 and pH 5–7 were added and the gel cast by capillary attraction onto a Gelbond film (260 mm x 125 mm FMC Ltd) warmed by circulating water at 50°C below it and held flat by a clean glass plate supported by plastic spacers (1.25 mm thick). The temperature of the

circulating water was rapidly reduced to 10°C to facilitate setting of the gel which was then stored at 4°C in a water saturated atmosphere for up to 3 days before use. Iso-electric focusing was achieved using a constant power source delivery 25 W with current and voltage limits of 50 mA and 1250 V respectively. The gel was cooled by circulating water at 4°C beneath it at all times. Plasma samples (20 µL) were pipetted onto pieces of filter paper (5 mm x 10 mm) which were then placed on the gels. After a 30 min period of focusing, the filter papers were removed and electro-focusing continued for a further 45 min. The gel was then rinsed in 0.1 M tris chloride buffer pH 7.4 for 10 min before staining for esterase activity using N-methylindoxyl acetate and fast blue RR (GALBRAITH & WATTS 1978) in 0.1 M tris chloride buffer pH 7.4. After staining for 15 min, the gels were fixed in 10% acetic acid for 4 hours, washed in water overnight and allowed to dry at room temperature. The pH gradient was determined by removing sections (10 mm x 10 mm) immediately after the completion of electro-focusing and soaking them in 1 mL of distilled water for 2 hours before measuring the pH of the resultant solution. The iso-electric point (pI) of the individual esterase bands was determined by reference to a graphical plot of pH against distance from the cathode (Fig. 1).

RESULTS AND DISCUSSION

Plasma esterase profiles were established in respect of 235 individual starlings, some of which produced sampled young in more than one year, and revealed the existence of three distinct polymorphic types (Fig. 1). The most common type found on 206 occasions, contained only a single major esterase band (designated band A) with an iso-electric point (pI) of 4.75. In the next most frequently occurring type, found in 24 birds, a second band (Band B, pI 4.50) was also present whilst in a further 5 birds band B was found alone.

The distribution of the variants among the nestlings from the 55 boxes from three seasons that produced viable young was found to be consistent with their being under simple genetic control. Thus in 43 nests all 168 nestlings possessed the common variant, band A, alone, as did all 34 of the parent birds associated with them from which plasma samples were obtained. Of the profiles obtained with the 48 young from the other 12 nests, 24 contained band A alone. In the remaining 24 birds band B was found to be present, in 19 cases in combination with band A and in the remaining 5 on its own. Esterase profiles were established for 12 of the parent birds from these nests and band B was found to be present together with band A in 8 of them, the remaining 4 adults were found to have band A alone (Table 1).

TABLE 1

Distribution of major bands in the esterase profiles of parent and nestling starlings.

	Nest box No.	Parent esterase complement ♂	♀	Nestling esterase complement (second brood) Band A only	Band A + Band B	Band B only
1979	2	-	-	2	0	0
	4	-	-	3	0	0
	5	-	-	4	0	0
	6	-	-	4	0	0
	11	-	-	2	1	0
	16	-	A	4	0	0
	17	A	-	2	0	0

TABLE 1 (cont'd)

	Nest box No.	Parent esterase complement ♂	♀	Nestling esterase complement (second brood) Band A only	Band A + Band B	Band B only
	20	–	A	2	0	0
	23	–	–	3	0	0
	27	–	A	5	0	0
	28	–	AB	1	1	0
	32	–	–	4	0	0
1980	3	–	A	(1)	(2)	(0)
	4	–	AB	0	1	3
	5	–	A	4(1)	0(0)	0(0)
	6	–	A	3	0	0
	8	–	–	3	0	0
	10	–	–	3(1)	0(0)	0(0)
	11	AB	–	2	3	0
	12	–	–	4	0	0
	15	A	A	5	0	0
	16	–	A	(3)	(0)	(0)
	19	A	–	4(1)	0(0)	0(0)
	20	–	A	2(2)	0(0)	0(0)
	21	–	A	3	0	0
	23	A	–	5	0	0
	24	–	A	3	0	0
	26	–	–	2	0	0
	27	–	AB	1	3	0
	32	–	–	3	0	0
1981	1	–	–	(3)	(0)	(0)
	3	–	A	2	2	0
	4	–	–	(3)	(0)	(0)
	5	–	A	5	0	0
	6	–	A	4	0	0
	7	A	–	3	0	0
	8	A	AB	3(2)	1(0)	0(0)
	9	AB	–	3	1	0
	10	–	A	3(4)	0(0)	0(0)
	11	AB	–	2	3	0
	12	A	A	5	0	0
	15	A	A	5	0	0
	19	–	A	(3)	(0)	(0)
	20	–	A	4	0	0
	21	–	A	3(1)	0(0)	0(0)
	22	A	A	3	0	0
	23	–	A	4	0	0
	26	A	A	2	0	0
	27	–	AB	2	0	2
	28	–	AB	2	1	0
	30	–	–	(3)	(0)	(0)
	32	–	A	2	0	0
	33	–	A	4(2)	0(0)	0(0)
	34	A	–	(4)	(0)	(0)
	36	–	A	(3)	(0)	(0)

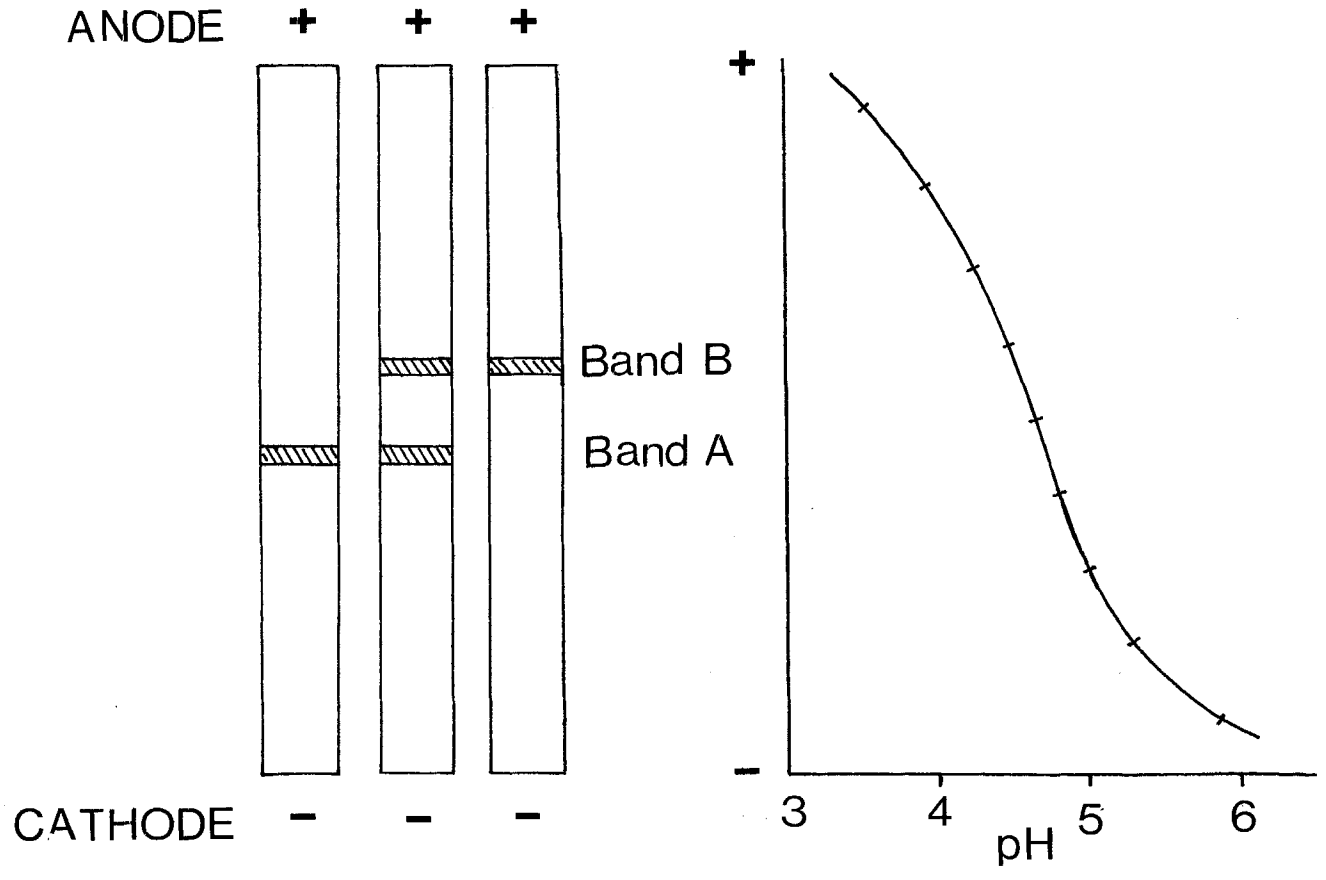

Fig. 1 Esterase profiles, showing major bands, and pH gradient produced by the iso-electric focusing of starling plasma.

It was also observed during the course of this study that while the major bands A and B were found to be present in the esterase profiles of nestlings in older birds a number of additional bands were also revealed. However, although these bands were located in the same area as the major bands after electro-focusing (their iso-electric points ranging from pH 4.4 to 4.8) they were all of a minor nature and did not interfere in any way with the identification of the 2 major bands reported.

It would appear from these results that that the electrophoresis technique employed involving the use of the N-methylindoxyl acetate and fast blue RR has identified a simple genetic variant in starlings. This finding will be of considerable interest to workers studying avian genetic morphology.

This study has revealed that the great majority of a given wildlife species, in this case approximately 80% of a starling population, can show a high degree of genetic homogeneity. However, the presence of a small number of individuals with a more heterogeneous genetic complement may be of considerable importance when assessing the likely environmental impact of an agricultural chemical. With particular reference to esterases, ECOBICHON & COMEAU (1974) have correlated the pharmacologic and toxicological effects of certain drugs with polymorphism of plasma esterases in the rabbit (*Oryctolagus cuniculus*). In addition BUNYAN *et al* (1968) demonstrated that the components of the liver esterase complex in both the pigeon (*Columba livia*) and the pheasant (*Phasianus colchicus*) were differentially inhibited by a number of organophosphorus pesticides, while, in more general terms, the genetic heterogeneity of the rat (*Rattus norvegicus*) has enabled this species to exhibit resistance to the anticoagulant rodenticides (GREAVES & AYRES 1967).

The advantages of using agarose gels for iso-electric focusing has also been demonstrated. The method described was simple, rapid, gave excellent resolution and produced, as a final product, an electropherogram which was permanent and easily stored. It is, therefore, to be recommended to other workers interested in the genetic morphology of avian and other species.

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