Genetical Studies on Liver Esterase Isozymes of the Japanese Quail, *Coturnix coturnix japonica*

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A genetic variation was found in liver esterase isozyme of the Japanese quail. The six phenotypes such as AA, BB, CC, AB, AC and BC were observed. It was clarified that these phenotypes were under control of three co-dominant alleles, *Es-2^A*, *Es-2^B*, and *Es-2^C*, at the same locus located on the autosome. The gene frequency in *Es-2* esterase isozyme of random-bred populations were estimated as very high for *Es-2^B* (0.77~0.88), low for *Es-2^A* (0.10~0.21), and the lowest for *Es-2^C* (0.01~0.02). *Es-2* esterase isozyme showed organ specificity and was found only in liver. *Es-2* esterase isozyme first appeared on the day of hatching but its activity was very weak. The activity was clearly observed in all individuals at about five days of age, thereafter the activity increased gradually with age. At 15 days of age, the activity was approximately the same as that of the matured quail at about 40 days of age. It was also revealed that the liver esterase is an aliesterase in characteristics.

Introduction

Genetical and electrophoretical studies on esterase isozyme in Japanese quail (*Coturnix coturnix japonica*) have been reported with respect to organs, tissues (HASHIGUCHI *et al.* 1966, 1976; KIMURA *et al.* 1971; EMURA *et al.* 1974), erythrocytes (MANWELL and BAKER 1969; SHIIHARA *et al.* 1974), and sera (HASHIGUCHI *et al.* 1966; YOSHIDA *et al.* 1973; KONDO *et al.* 1971).

In the present study, electrophoretic variations in esterase pattern which were detected only in the liver of Japanese quail and the mode of genetic control for liver esterase isozyme were investigated. The phenotype and gene frequencies of some populations were also estimated, using these genes as markers, and the esterase pattern was characterized.

Materials and Methods

The Japanese quails were obtained from a random-bred population maintained at Kagoshima University and at Saga University. Additional three populations (Toyohashi I, II and III), which had been introduced from three different fanciers in Aichi Prefecture to Kagoshima University in 1970, were also analysed. When it was necessary to make sure the phenotypes of birds used for mating, pieces of tissue was collected by liver biopsy technique (MAEDA *et al.* 1973). Otherwise tissues of liver were collected after the birds were decapitated and exsanguinated.

Adult quails over 45 days of age were used in most of the experiments, but embryos on the 9th day of incubation, at 2 days before hatching, and quails at 1, 2, 5, 10, 15, 40 and 60 days after hatching were also subjected to analyse the period of appearance of

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isozyme patterns and their activities. Whole body of each embryo on the 9th day of incubation was homogenated and used for one experiment. The livers collected from three embryos at two days before hatching were used as one specimen.

The agar gel electrophoresis techniques described by OGITA (1964) were applied to detect esterase isozymes with a little modification. The detail of gel composition, methods of electrophoresis, staining and identification of esterase were previously reported (HASHIGUCHI *et al.* 1966, 1976).

Results

1. Genetic control of L-pattern esterases in liver

Of all bands of the liver esterases appeared on the anode side, twelve bands were identified. The bands of liver esterase with the fastest mobility, L-pattern, was found only in the liver as previously reported (HASHIGUCHI *et al.* 1966). Six zymogram variations were observed in the L-pattern.

Mating of various combinations was carried out to clarify the mode of genetic control of L-pattern in liver. Segregation patterns showed a good fit to the Mendelian ratio based on the expected genotype combinations (Table 1). Therefore, activities of L-pattern esterases are controlled by three co-dominant alleles, i.e. A, B and C, at the same locus. When this locus is designated as *Es-2*, six genotypes, i.e. AA, BB, CC, AB, AC and BC are expressed as *Es-2^A/Es-2^A*, *Es-2^B/Es-2^B*, *Es-2^C/Es-2^C*, *Es-2^A/Es-2^B*, *Es-2^A/Es-2^C* and *Es-2^B/Es-2^C*, respectively.

Hybrid enzyme formation was observed in genotypes of AC and BC. Genotype AC demonstrated three bands, i.e. band A, band C and a new hybrid band. The activity of band A was relatively strong, whereas that of band C was weak, and the hybrid band was intermediate between the two. Genotype BC also demonstrated three bands including

Fig.1. Six phenotypic patterns of liver esterase detected on the anode side.

Table 1. Distribution of phenotype of the esterase isozyme in the progenies from various matings

Matings Male×Female	Number of progenies	Phenotypes of progeny						χ^2	P
		AA	AB	BB	BC	AC	CC		
AA×AA	6	6/6 ¹⁾						—	—
AA×AC	17	10/ 8.5				7/ 8.5		0.529	0.25—0.50
AA×BC	11		4/ 5.5			7/ 5.5			
AB×AA	31	14/15.5	17/15.5					0.290	0.50—0.75
AB×AB	116	25/29	61/58	30/29				0.741	0.50—0.75
AB×AC	10	2/ 2.5	2/ 2.5		4/ 2.5	2/ 2.5			
AB×BB	50		24/25	26/25				0.080	0.75—0.90
AB×BC	38		10/ 9.5	9/ 9.5	14/ 9.5	5/ 9.5		4.316	0.10—0.25
AC×AA	19	10/ 9.5				9/ 9.5		0.053	0.75—0.90
AC×AB	17	3/ 4.25	3/ 4.25		4/ 4.25	7/ 4.25			
AC×AC	15	5/ 3.75				8/ 7.5	2/ 3.75		
AC×BB	13		9/ 6.5		4/ 6.5				
AC×BC	16		5/ 4		3/ 4	5/ 4	3/ 4		
BB×AA	5		5/ 5					—	—
BB×AB	19		11/ 9.5	8/ 9.5				0.474	0.25—0.50
BB×BB	23			23/23				—	—
BB×BC	36			20/18	16/18			0.444	0.50—0.75
BC×AA	20		11/10			9/10		0.200	0.50—0.75
BC×AB	75		21/18.75	16/18.75	17/18.75	21/18.75		1.107	0.75—0.90
BC×AC	7		3/ 1.75		1/ 1.75	2/ 1.75	1/ 1.75		
BC×BB	23			11/11.5	12/11.5			0.043	0.75—0.90
BC×BC	102			29/25.5	52/51.0		21/25.5	1.294	0.50—0.75
CC×AB	8				4/ 4	4/ 4			
CC×AC	4					1/ 2	3/ 2		
CC×BB	14				14/14			—	—
CC×CC	28						28/28	—	—

1) Observed/expected numbers.

a clear hybrid band. In genotype AB, since band A and B were overlapped each other, impossible to determine whether hybrid enzymes were formed or not (Fig.1).

The zymograms of the *lobus dexter* and *lobus sinister* of liver were compared to determine the exact localization of esterase isozymes in liver. However, any interlobular difference was not identified.

2. Phenotypic distribution and gene frequency in the *Es-2* esterase locus

The phenotypic distribution and gene frequencies in *Es-2* esterase isozyme of five different populations were estimated (Table 2).

In all populations, the observed numbers of each phenotype were in good agreement with the number expected from Hardy-Weinberg's equilibrium for gene frequencies.

The frequency of BB type was higher than that of other phenotypes in all populations and the gene frequency of *Es-2^B* showed 0.77~0.88. The frequency of phenotype AB was also higher in comparison with those of AA or BC types. Phenotype AC was not found in Kagoshima, Saga, and two Toyohashi (I and III) populations. The gene frequency of *Es-2^A* was estimated as 0.10~0.21. The CC type did not appear in the ran-

Table 2. Phenotype and gene frequencies of the liver esterase isozyme (*Es-2*) in five different populations

Name of population	Number of birds	Phenotypes						Gene frequencies			P of χ^2
		AA	AB	BB	BC	AC	CC	<i>Es-2</i> ^A	<i>Es-2</i> ^B	<i>Es-2</i> ^C	
Kagoshima	205	5 ¹⁾ 2.1 ²⁾	31 36.1	160 158.8	9 7.2			0.10	0.88	0.02	>0.10
Saga	140	9 4.5	32 40.3	93 89.6	6 4.5			0.18	0.80	0.02	>0.05
Toyohashi I	57	2 2.5	20 18.4	33 33.8	2 1.8			0.21	0.77	0.02	>0.95
Toyohashi II	127	8 5.1	34 40.1	83 79.3	1 2.0	1 0.5		0.20	0.79	0.01	>0.25
Toyohashi III	75	6 3.3	20 24.6	48 45.6	1 1.2			0.21	0.78	0.01	>0.25

1) Observed number.

2) Expected number on the basis of Hardy-Weinberg's equilibrium with gene frequencies of each populations.

Table 3. Substrate specificity and inhibition of the *Es-2* esterase

Substrates	Inhibitors	Staining reaction ¹⁾
α -naphthyl acetate		+
α -naphthyl acetate	EDTA	+
α -naphthyl acetate	Eserine sulphate	+
α -naphthyl acetate	DDVP	—
α -naphthyl acetate	Acetazolamide	+
β -naphthyl acetate		+
α -naphthyl propionate		+
α -naphthyl butyrate		+
β -naphthyl butyrate		+
α -naphthyl caprate		+
β -carbonaphthoxy choline iodide		—
Naphthol AS acetate		—

1) + presence of staining reaction; — absence of staining reaction.

α -naphthyl caprate. By contrast the activities of all esterases, including *Es-2*, were not apparent in the case when naphthol AS acetate or β -carbonaphthoxy choline iodide was used as substrate.

Es-2 esterase activity was inhibited by DDVP, which is an organophosphorus compound, but not by eserine sulphate, EDTA and acetazolamide. These results indicated that *Es-2* esterase had an aliesterase characteristic according to Augustinsson's classification.

4. Appearance period of *Es-2* esterase isozyme and changes in its activities during development

The appearance period of *Es-2* esterase and alteration of its activities during development were investigated by electrophoresis. The band of *Es-2* esterase was not detected in all individuals from about 9th to 16th day of incubation irrespective of sex, but first appeared on the day of hatching though its activity was very weak. The number of bands

dom-bred populations used in this experiment. The frequency of *Es-2*^C was very low (0.01~0.02) in all populations.

3. Characterization of *Es-2* esterase

The substrate specificity and susceptibility to various inhibitors of *Es-2* esterase were examined in order to determine the esterase characteristics. The results are shown in Table 3. *Es-2* esterase activity was specific to the substrate as such as α - or β -naphthyl acetate, α -naphthyl propionate, α - or β -naphthyl butyrate, and α -

Fig. 2. Appearance period of *Es*-2 esterase isozymes and changes in their activities during development.

other than *Es*-2 isozyme in individuals before hatching and at the day of hatching was approximately the same as that of adult quails. *Es*-2 isozyme was observed in all individuals only at five days of age but their activities were still weak, thereafter the activities increased gradually with age. At 15 days of age, however, the activities were approximately the same as those at 40 days of age when they were sexually matured.

Discussion

We have observed a genetic variation in liver esterase of the Japanese quail. It was revealed that the expression of liver esterases are controlled by the three co-dominant alleles, A, B and C located at *Es*-2 locus on the autosome. It was also made clear that the liver esterase is an aliesterase in characteristics.

OGITA (1968) classified the isozymes by the mode of genetic control into 1) unigenic, 2) allelic, and 3) non-allelic isozyme. *Es*-2 isozyme of the present study has been reported as genetic variations in esterase isozyme of the Japanese quail and belongs to allelic isozyme. So do all of so far reported such as *Es*-1^{F,S} (MANWELL and BAKER 1969), *Es*-3^{F,S} (MANWELL and BAKER 1969), *Es*-4^{A,B} (HASHIGUCHI *et al.* 1976), *Es*-5^{A,O} (YOSHIDA *et al.* 1973), *Es*-6^{A,O} (YOSHIDA *et al.* 1973), *Es*-7^{A,O} (YOSHIDA *et al.* 1973), *Es*-8^{A,B,C} (SHIHARA *et al.* 1974), *Es*-II^{A,B} (EMURA *et al.* 1974) and *Es*-D^{F,S} (WATANABE *et al.* 1977).

Es-2 esterase isozyme was assumed to be a dimer by the fact that a hybrid enzyme was formed in either the AC or BC, but not in AB type. Bands A and B were overlapped each other in the case of AB type.

Several examples of isozyme forming hybrid enzyme in organisms have been reported;

i. e. esterase of the butterfly, *Colias eurytheme* (JOHNSON and BURNS 1966), phosphohexose isomerase of the horse (SANDBERG 1973), acid phosphatase of the chicken (OKADA and HACHINOHE 1968), 6-PGD of the dove (COOPER *et al.* 1969), 6-PGD of the Japanese quail (OHNO *et al.* 1968), esterase-D of the Japanese quail (WATANABE *et al.* 1977) and LDH of the deer mouse (SHAW and BARTO 1963).

The gene frequency in *Es*-2 esterase isozyme of random-bred populations were estimated as a very high frequency for *Es*-2^B (0.77~0.88), a low value for *Es*-2^A (0.10~0.21) and the lowest frequency for *Es*-2^C (0.01~0.02). The phenotype CC was not observed in random-bred populations used probably because *Es*-2^C gene was held at a lower frequency in a random-bred population.

OHNO (1969) classified the expression of structural genes in the genome into two groups; 1) genes which are expressed in almost all of the tissues in the body, and 2) genes which are expressed only in specific tissues. The acid phosphatase isozyme (*Acp*-2 locus) of the Japanese quail has been observed in all tissues and organs such as liver, pancreas, kidney, ovary and testis (MAEDA *et al.* 1976), and it belongs to the first group. On the other hand, *Es*-2 can be assumed to belong to the second group as it indicates organ specificity similar to pancreatic esterase isozyme (*Es*-4 locus; HASHIGUCHI *et al.* 1976). *Es*-2 isozyme was not observed in the embryonic stage, similar to *Es*-4 isozyme, and it first appeared after hatching. In contrast to this, the activity of *Acp*-2 isozyme is detected approximately on the 5th day of incubation and the activity increases thereafter with age. Thus, the appearance periods of isozyme patterns of *Es*-2, *Es*-4, and *Acp*-2 isozymes differ, indicating that genes controlling the isozyme with organ specificity is expressed at a later stage of development. Whereas genes controlling the isozyme without organ specificity is expressed at a relatively early stage of development.

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日本ウズラの肝臓エステラーゼアイソザイムに関する遺伝学的研究

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日本ウズラの肝臓エステラーゼについて、薄層寒天ゲル電気泳動法を用いて分析を行った。その結果、陽極側の移動性の最もはやい域に個体変異、すなわち AA, BB, CC, AB, AC および BC 型の6つのタイプが検出された。交配実験の結果から、これらは常染色体上の同一座位に存在する3つの共優性対立遺伝子群によって支配されていることが判明した。そこでこの座位を *Es-2* と命名すると、表現型 AA, BB, CC, AB, AC および BC 型に対する遺伝子型は、それぞれ $Es-2^A/Es-2^A$, $Es-2^B/Es-2^B$, $Es-2^C/Es-2^C$, $Es-2^A/Es-2^B$, $Es-2^A/Es-2^C$ および $Es-2^B/Es-2^C$ となる。

ウズラの集団における遺伝子構成をしらべる目的で、鹿児島大学および佐賀大学で飼養中のもの、ならびに豊橋市の別々の3つの業者から導入した集団の合計5つの無作為交配集団について、*Es-2* 座位の表現型出現分布と遺伝子頻度を推定した。その結果、各集団ともに $Es-2^B$ の頻度が最も高く (0.77~0.88), $Es-2^A$ がこれにつぎ (0.10~0.21), $Es-2^C$ の頻度は極めて低かった (0.01~0.02)。そして表現型 CC のホモ接合体は、いずれの集団においても検出されなかった。

Es-2 アイソザイムは、臓器特異性を示し肝臓においてのみ検出される。またエステラーゼの同定実験の結果から、アリエステラーゼであることも判明した。

Es-2 アイソザイムは、胚の時期では検出されず孵化当日になってはじめて一部の個体で検出されるが、その活性は極めて弱い。5日齢になるとすべての個体で検出されるが、その活性はまだ弱く以後日齢の増加と共に活性も高まり、15日齢に達すると40日齢以後の成熟ウズラとほぼ同様の活性を示した。