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Variation of Esterase Isozyme in the Bedford's Red-backed Vole, *Clethrionomys rufocanus bedfordiae* (THOMAS)

By

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Summary : By means of starch-gel electrophoresis, esterases in four organs and plasma of the Bedford's red-backed vole, *Clethrionomys rufocanus bedfordiae* (THOMAS) were separated, and the esterase zymograms were demonstrated by a chemical method with α-naphtyle acetate as a substrate and using eserine and EDTA as inhibitors. All samples used in the present investigation were taken from fourty-two adult voles (21 females and 21 males) captured at three different habitats. The esterase zymograms consisted of twenty-five bands numbered in order of their appearance from the fastest mobility to the latest on the anodal side. No esterase band was observed on the cathodal side. These esterase bands showed different electrophoretic patterns among the organs and plasma. The esterase bands in the liver, kidneys and plasma were clearly more both in total number of isozyme bands and in number per individual than those in the heart and lungs. The liver and kidney esterase bands, however, showed a higher tendency in occurrence frequency than did the plasma ones. For that reason, it is considered that the plasma esterase patterns are more suitable for the purpose of making a comparison among individuals and populations as a genetic marker than the others.

When 10^{-5} M eserine and 10^{-3} M EDTA were used as inhibitors to classify non-specific esterase bands into three types, eserine did not inhibit so clearly ths specific bands in the four organs as those in the plasma. The plasma esterases inhibited by eserine were Es-13 and Es-15 which both were considered to be cholinesterase, wherease EDTA was not effective in inhibiting the esterases in each organ and plasma.

Introduction

Population fluctuation of the Bedford's red-backed vole, *Clethrionomys rufocanus bedfodiae* (THOMAS) is considered to be greatly influenced by the population constitution and reproductive conditions such as litter size and duration between pregnancies, etc. In Hokkaido, the population constitution and reproductive conditions of the vole vary with regions[5)8)16)] having different environmental conditions. Therefore, it is suggested that the vole population fluctuation also varies with regions[4)]. Two factors, i. e., environmental (exogenous) and genetic (endogenous) aspects must be involved in the regional difference of the population fluctuation. As for the former, several reports have been published up to the present[6)10)14)16)]. As to the latter, however, no research has been made except for a paper by KUWAHATA *et al.* (1974) in Japan[9)]. In some foreign countries, KREBS *et al.*[7)] and SEMEONOFF *et al.*[15)] have already engaged in such an investigation.

As the first step of this study, it should be taken into consideration whether the quality of the vole populations which brings about such different fluctuations varies genetically with

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regions or not. Accordingly, the author took up esterase isozymes separated by electrophoresis as a genetic marker, for the purpose of inquiring into possibility of the regional difference in vole population quality[1]. The aim of this study was to make clear the esterase isozyme patterns in four organs and blood plasma of this species, and hereby to find out which parts will be suitable for making a comparison of esterase isozyme patterns among individuals and populations.

Materials and methods

A total of 42 adult voles (21 females and 21 males) of *C. r. bedfordiae* were used in the present study. These were collected at three localities of Hokkaido in 1979. The collection localities, tapping date, sample size and the habitat characterization are summarized in Table 1.

After the voles were brought back to the author's laboratory, each of them was killed by cervical decapitation and drawn blood quickly by means of a heparinized capillary tube with a maximum capacity of 75 μl. Soon after, the blood plasma was spun down at 3,500 r. p. m. for 5 minutes, and the heart, lungs, liver and kidneys were homogenized with three volumes of deionized water, respectively. Each suspension was centrifuged at 10,000 r. p. m. (9,200 × g) for 30 minutes at 0°C and the supernatant was used for electrophoresis. All samples were kept frozen at −20°C until used for electrophoresis.

Electrophoresis was carried out in a horizontal starch-gel system in accordance with a modification of the method by Edwards *et al.*[3], using hydrolysed starch (Connaught Co., Canada) at a concentration of 11%. The electrode buffer contained lithium hydroxide (2.5 g) and boric acid (14.2 g per 1 litre of deionized water) at pH 8.50. The gel buffer was mixed with 5.4 volumes of the solution containing citric acid (1.5 g) and tris aminomethane (9.6 g per 1 litre of deionized water) at pH 8.50, to one volume of the electrode buffer.

Electrophoresis was performed with a constant current of 2.2 mA/cm of gel width for 5 hours at about 5°C. After electrophoresis, esterases of plasma and organs were detected chemically by incubation of the gel (37°C) for 1 hour and 25 minutes, respectively, in a mixture containing α-naphtyl acetate, as a substrate, with Fast Blue RR salt in a phosphate buffer at pH 6.80. Inhibition experiments were carried out only in the male for 60 minutes in a phosphate buffer (pH 6.80) containing 10^{-5} M eserine and 10^{-3} M EDTA (ethylenediamine-tetraacetic acid) separately at a room temperature, before staining.

Table 1. Collection localities of the voles and their habitat characterization

Localities	Date	No. of samples (♀, ♂)	Habitat characterization
Toyohoro (Ebetsu City)	Nov. 7～10 '79	29 (16, 13)	Snow break forest for railway and its vicinity
Islet in Lake Dōya	Aug. 8～ 9 '79	6 (3, 3)	Todo-fir planted forest of the second age class
Nakashibetsu (Konsen Districts)	Oct. 15～19 '79	7 (2, 5)	Thicket of bamboo grass along a mountain stream

Results

Esterase activity in the four organs and blood plasma of all materials from three different regional populations was examined, and twenty-five esterase bands were revealed on the starch gel (Fig. 1). These bands were numbered consecutively in the decreasing order of anodal mobility. But, the esterase bands of organs placed between Es-5 and 6, and between Es-9 and 10 of plasma esterase bands were expressed as Es-5(6) and Es-9(10), respectively in the following. No band was detected toward the cathode.

The total numbers of esterase bands observed in the heart, lungs, liver, kidneys and plasma were 14, 13, 19, 18 and 21, respectively. Among these organs and plasma, the bands in the liver, kidneys and plasma were relatively more in number than those in the heart and lungs. The bands of Es-20 to 23 were highly concentrated in all samples examined, except for the plasma. The staining intensity of the bands in the plasma was strong in five parts, that is, Es-1 and 2, Es-8 to 10, Es-12 and 13, Es-14 and 15, and Es-20 to 22. The rest varied with

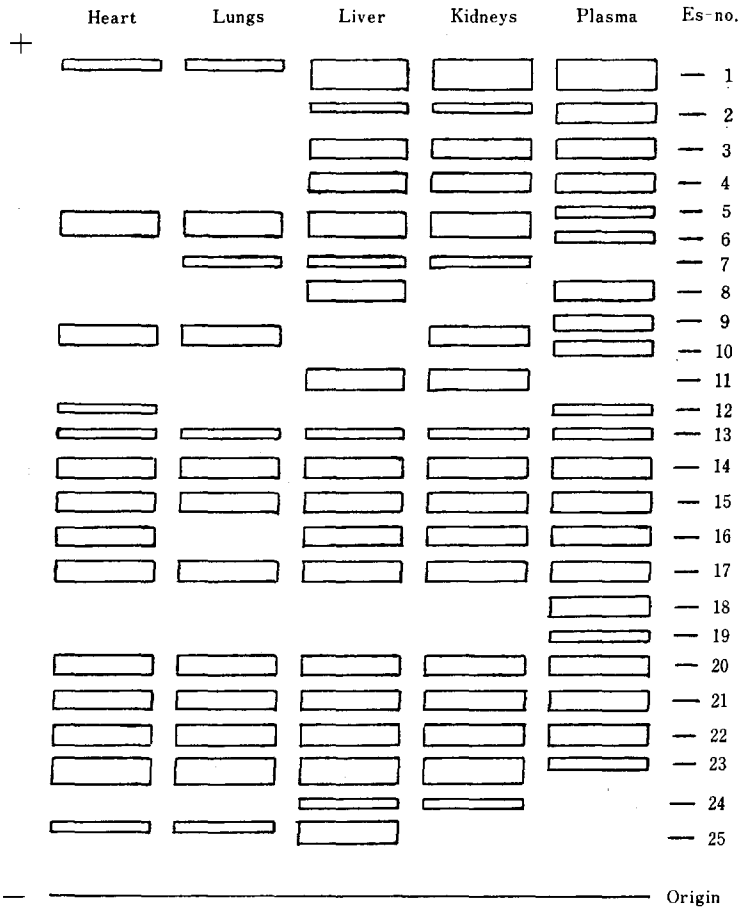

Fig. 1 Schematic representation of the esterase zymograms separated by starch-gel electrophoresis on the four organs and blood plasma of the vole, *Clethrionomys rufocanus bedfordiae*.

Table 2. The vole numbers used for starch-gel electrophoresis and individual variation in number of esterase bands

Numbers of esterase bands	Heart	Lungs	Liver	Kidneys	Plasma
2	1	2			
3	0	0			
4	5	4			
5	2	8			
6	8	6			
7	9	8		1	
8	7	3		0	
9	3	4		2	5
10	5	3		3	6
11	2	1	1	4	8
12		1	2	11	9
13			2	11	7
14			12	5	7
15			11	2	
16			9	1	
17			4	1	
18			1		
Total	42	40	42	41	42
Average*	7.1 ± 2.1	6.6 ± 2.3	14.9 ± 1.4	12.4 ± 1.9	11.7 ± 1.4

* Mean ± S. D.

Table 3. Uncommon esterase bands in the four organs and blood plasma of the vole

Band no.	Heart	Lungs	Liver	Kidneys	Plasma
2	−	−	+	+	+
3	−	−	+	+	+
4	−	−	+	+	+
7	−	+	+	+	−
8	−	−	+	−	+
9	+	+	−	+	+
10					+
11	−	−	+	+	−
12	+	−	−	−	+
16	+	−	+	+	+
18	−	−	−	−	+
19	−	−	−	−	+
24	−	−	+	+	−
25	+	+	+	−	−

\+ : present, − : absent

Table 4. Occurrence frequencies of the 25 esterase bands in the four organs and blood plasma of the vole.

Band no.	Heart (%)	Lungs (%)	Liver (%)	Kidneys (%)	Plasma (%)
1	31.0	27.5	100.0	97.6	100.0
2			95.2	73.2	100.0
3			11.9	14.6	7.1
4			78.6	46.3	26.2
5	14.3	15.0	100.0	78.0	38.1
6					16.7
7		12.5	97.6	95.1	
8			64.3		59.5
9	16.7	17.5		17.1	78.6
10					73.8
11			52.4	22.0	
12	11.9				100.0
13	16.7	17.5	47.6	58.5	100.0
14	23.8	45.0	76.2	82.9	14.3
15	73.8	75.0	100.0	95.1	95.2
16	81.0		81.0	82.9	38.1
17	61.9	80.0	85.7	78.0	7.1
18					19.0
19					14.3
20	97.6	95.0	100.0	100.0	81.0
21	95.7	97.5	100.0	100.0	85.7
22	97.6	95.0	100.0	100.0	97.6
23	85.7	82.5	100.0	92.7	14.3
24			4.8	2.4	
25	2.4	2.5	90.5		

individuals in concentration. The esterase bands in the liver and kidneys, on the whole, were concentrated a little more than those of the heart, lungs and plasma.

Individual variation in occurrence frequency of esterase bands in the zymograms of the four organs and plasma is shown in Table 2. The average numbers of esterase bands per each individual were 7.1 ± 2.1 in the heart, 6.6 ± 2.3 in the lungs, 14.9 ± 1.4 in the liver, 12.4 ± 1.9 in the kidneys and 11.7 ± 1.4 in the plasma.

Table 3 shows the esterase bands not observed commonly in the four organs and plasma. As shown in this table, the esterase patterns of the heart and lungs are similar to each other, and those of the liver and kidneys are also alike. The plasma esterase pattern resembles the latter, especially on the anodal side of the starch gel. The band of Es-25 in the liver was apparently broader and more concentrated than in the others (Fig. 1). The occurrence frequency of this band in the liver was 90.5 percent in all individuals (cf. Table 4).

Occurrence frequencies of twenty-five esterase bands in every organ and the plasma are shown in Table 4. Among eleven esterase bands (Es-1, 5, 6, 13 to 15, 17 and 20 to 23) which appeared commonly in the respective organs and plasma, the occurrence frequencies of five anodal bands (Es-1, 5(6), 13 and 14) in the zymograms of the heart and lungs were lower than those of six other bands (Es-15, 17 and 20 to 23), but those of the liver and kidney

esterases were all alike. The plasma esterases varied individually in occurrence frequency. The occurrence frequencies of Es-20 to 22 bands were very high in each organ and the plasma. On the other hand, occurrence frequencies of fourteen esterase bands (Es-2 to 4, 7 to 12, 16, 18, 19, 24 and 25) not observed commonly were different from each other among the four organs and plasma. Es-25 in the heart and lungs was remarkably lower in occurrence frequency than that in the liver.

The bands of Es-13 and 15 were almost completely inhibited (92.3%) by 10^{-5} M eserine only in the plasma and so these were assumed to be cholinesterases. All the esterase bands observed in the present study were not vividly inhibited by 10^{-3} M EDTA, showing resistibility to it.

Discussion

All the adult specimens used here were autoptically normal; among them the females were all non-pregnant. Thus, uniformity of physiological conditions seemed to be kept in all the specimens. Noticeable sexual difference was not observed about twenty-five esterase bands in the four organs and plasma using α-naphtyl acetate substrate. The variation in esterase isozyme pattern and in occurance frequency of the esterase bands among the organs and plasma was remarkable, and the number of esterase bands in the liver, kidneys and plasma was larger than in the heart and lungs. The esterase bands with a high occurence frequency were abundant in the liver and kidneys as compared with those in the plasma (Table 4). Therefore, it is suggested that the esterase bands of the liver and kidneys are unsuitable, compared with those of the plasma, as a marker gene for the purpose of making a comparison among individuals or populations of this kind of vole.

Accordingly, as an example, occurrence frequencies of the plasma esterase bands in the specimens obtained from three parts are shown in Fig. 2. Although the result was obtained from a small number of populations, it is assumed that occurrence frequencies of Es-10, 14 to 17, 19 and 21 of plasma esterase bands vary regionally. Further, there were some parts where the voles lacked such esterase bands in the plasma as Es-3 to 6 and 23 in Dōya populations

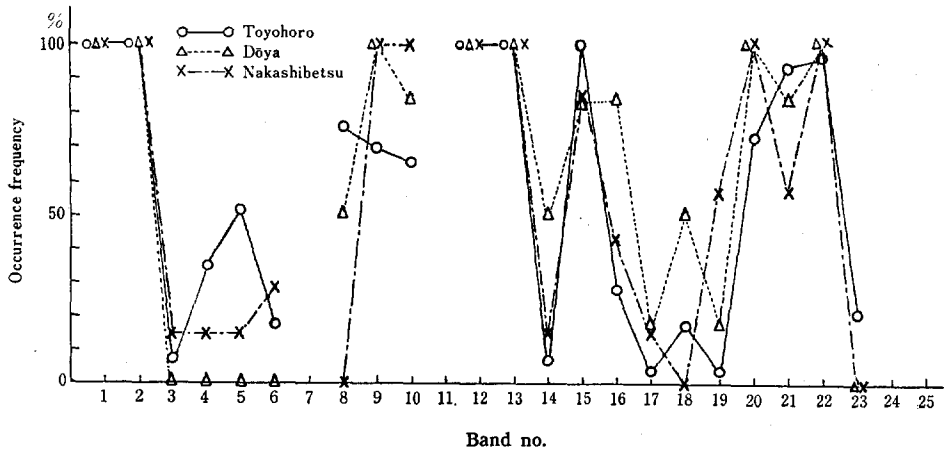

Fig. 2 Occurence frequencies of the blood plasma esterase bands in the voles obtained from three regions.

Table 5. Substrate specificity and inhibition of the esterase bands in starch-gels

Substrates and inhibitors	Esterase types		
	A-esterase	B-esterase	C-esterase
α-Naphtyl acetate	+	+	+
β-Carbonaphthoxy-choline iodide*	−	−	+
Eserine 10^{-5} M	+	+	−
EDTA 10^{-3} M	−	+	+
Tetraethyl pyrophosphate (TEPP) 10^{-5} M*	+	−	−

+ : presence of staining reaction, − : absence of staining reaction.
* : after MANDA *et al.* (1969) (in mouse and rat).

and Es-8, 18 and 23 in Nakashibetsu ones.

Esterase bands separated by α-naphtyle acetate are classified into three types, i. e. A-esterase (Arylesterase), B-esterase (Ali-esterase) and C-esterase (Cholinesterase)[1]. As shown in Table 5, each of those three types is determined by the substrate-specificity and the susceptibility to the selective inhibitors[12]. Since both Es-13 and 15 in the plasma were inhibited by eserine at the rate of 92.3 percent, these bands were considered to be a type of C-esterase, being located at the same middle part of the zymogram as reported by KUWAHATA *et al*[9]. The esterase bands in the four organs, however, were not so sensitive to eserine as those of the plasma. Further, the inhibitive response of esterase to the EDTA was hardly shown in each organ and the plasma. Therefore, it was difficult to divide completely the non-specific esterase bands examined into three types in the present study.

It has been demonstrated that some of the electrophoretically distinguishable esterases of animals, especially in sera, are genetically controlled by autosomal allelic genes[2)11)13)17)]. Thus, it is considered that the esterase bands in the plasma of *C. r. bedfordiae* (THOMAS) also are regulated by genes.

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エゾヤチネズミ個体群におけるエステラーゼ・アイソザイムの変異

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摘　要

北海道の森林におけるエゾヤチネズミ害を防除するために，道内一円において毎年（年3回）予察調査が実施されている。これまでの資料から，道内のエゾヤチネズミ個体群は繁殖様式および個体数の増減傾向などの相違により地域変異が認められる。このような地域変異の特徴を詳細に分析することは，それぞれの地域にそくした有効な発生予察を行ううえに重要である。しかしながら，この地域変異を生ずる要因は大きく二つに類別され，野鼠を取りまく生息条件（環境）によるものと，野鼠自身の本性（遺伝）によるものとが考えられる。したがって，エゾヤチネズミ個体群の地域変異を解明するためには，この両面から追求する必要がある。ところが，前者についてはこれまでに多くの研究がなされてきたが，後者については桑畑ら（1974）の報告を除いては皆無に等しく，まだ研究の緒についたばかりである。

そこで，このような地域変異を惹き起こす母体となるエゾヤチネズミ自身に，遺伝的な地域変異があるかどうかを調べる必要を生じ，その指標として，電気泳動によって得られるエステラーゼのザイモグラムに着目した。本研究では，この研究の手始めとして，先ずエゾヤチネズミの各種臓器および血漿のエステラーゼ・ザイモグラムを作成すること，さらに本種のどの部位（臓器あるいは血漿）のエステラーゼが個体間および個体群間（地域間）の比較をするのに適しているかどうかを判定することにある。

方法としては，水平式澱粉ゲル電気泳動法を用い，エゾヤチネズミの各種臓器（心臓，肺臓，肝臓および腎臓）および血漿中のエステラーゼを生化学的染色法によって，電気的に易動度の異なる活性帯として分離・検出した。供試材料は生息条件の異なる3地域から採集したもので，合計42個体（雌・雄各21頭）を用いた。

エゾヤチネズミの各種臓器および血漿中のエステラーゼには，合計25本の活性帯が陽極側に検出され，易動度の速い順に番号を付した。しかし，陰極側には全く活性帯は検出されなかった。分離・検出されたエステラーゼの電気泳動像は，臓器および血漿によって異なること，また，個体によっても大きな変異を示すことが判明した。すなわち，エステラーゼ活性帯数は肝臓（19本），腎臓（18本）および血漿（21本）において多く，心臓（14本）および肝臓（13本）においてより少なかった。さらに，1個体当りの平均活性帯数についても，前者が後者に比べて多かった。しかしながら，肝臓および腎臓においては，活性帯数が多いにもかかわらず，いずれの個体にも共通してみられる活性帯が多い傾向にあった。したがって，血漿が肝臓および腎臓に比べて個体間または個体群間を比較する目的に適していると思われた。

エステラーゼ活性帯を分類するために，阻害剤としてエゼリン 10^{-5} M および EDTA 10^{-3} M を用いたが，エゼリンの阻害効果は血漿においては認められたが，各種臓器では不明確であった。エゼリンによって阻害された血漿エステラーゼの活性帯は13番目と15番目であり，これらはコリンエステラーゼと考えられた。一方，EDTA によってはいずれの臓器および血漿においても明確な阻害効果は認められなかった。

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