of 1975, stratified in December, and planted in a 16°-24°C growth chamber in late February. These plants developed tight rosettes, were transplanted to 15 cm pots in May 1976, and were grown in a lathhouse (30 percent of full sunlight) where rosettes expanded to a diameter of 4 to 5 cm by September. Shoot elongation and flowering began in May 1977. Thus, it appears that flowering follows vernalization after an initial season of vegetative growth.

Though very little is yet known about the relationship of growth to light and temperature, the sum of these preliminary observations indicates that the Roan Mountain bluet can be grown easily as container stock. Populations could be expanded by transplanting this container stock to suitable high elevation sites. Some of these planted sites could subsequently be used as cultivated seed production areas.

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ANALYSIS OF THE ELECTROPHORETIC PATTERN OF NONSPECIFIC ESTERASES OF BLACK BEAR SERUM

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ABSTRACT

The serum of 43 black bears (*Ursus americanus*) from the Great Smoky Mountains National Park was analyzed for nonspecific esterases by use of concave gradient polyacrylamide gel electrophoresis. The sera were resolved into approximately 11 bands of enzyme activity. Certain esterase bands are quantitatively different in males and females, and alterations in the electrophoretic profile may be correlated with the physiological state and previous history of the animals.

INTRODUCTION

The black bear population of the Great Smoky Mountains National Park is being studied in detail by Pelton and co-workers to obtain information which may establish base-line data for the species. Preliminary investigations have been concerned with the relationships between body measurements and weight (Cherry & Pelton, 1976) and with the chemical analysis of serum (Eubanks et al., 1976).

The various molecular forms of tissue, plasma, and serum esterases are sensitive to changes in the physiological state of living systems. Specific esterase patterns have been observed during differentiation in humans (Blanco & Zinkham, 1966) and mice (Sherman, 1972). The serum esterase activity of female mice is increased during pregnancy (Oki et al., 1966), and specific alterations in esterase activity have been described in uterine tissue and in washings of the uterus of rabbits (Tyndall & Daniel, 1975). Differences in several mouse plasma esterases are sex-associated (Augustinsson & Olsson, 1960; Allen & Moore, 1966a). Alterations in the electrophoretic profile of esterases are associated with neoplasia in hamsters (Kreusser, 1966) and mice (Tyndall et al., 1971; Daugherty et al., 1976).

Since electrophoretic esterase profiles may serve as sensitive indicators of the physiological state of numerous mammals, we have analyzed the electrophoretic pattern of nonspecific esterases in black bear serum. The present study provides an additional biochemical parameter in the characterization of black bears.

MATERIALS AND METHODS

ANIMALS

Serum was obtained from 43 black bears (25 males, 18 females) between June 13, 1975, and September 13, 1975. The animals were captured in the Great Smoky Mountains National Park by use of spring-activated snares. After the animal's weight was estimated, each animal was immobilized with Sernylan (phencyclidine hydrochloride; 2.2 mg/100 kg body weight). Panhandlers (animals which beg or steal food along the roads or at campgrounds) were shot with the tranquilizer while free-ranging. After an animal was immobilized, its sex, weight, and general condition were recorded, and one premolar was extracted for use in estimating the animal's age. A 20-ml sample of blood was withdrawn from the femoral vein with a 20 gauge 1-1/2-in. needle and a 20-ml Vacutainer tube containing no additives. Blood samples were returned from the field within 2-5 hr after collection and refrigerated overnight to allow maximum clot retraction. The serum was then decanted and centrifuged at 3300 rpm in a clinical centrifuge (International Equipment Company, head 211) for 20 min to remove any remaining cells. This process yielded 4-5 ml of serum, which was refrigerated and later taken to the Biology Division of Oak Ridge National Laboratory and stored at —70°C until analyzed.

ELECTROPHORESIS

The multiple molecular forms of serum esterases were separated by concave gradient polyacrylamide gel electrophoresis (Daugherty, 1977). All chemicals were obtained from Sigma Chemical Company. The concave gradient gel slabs (Pharmacia Fine Chemicals, PAA 4/30) each held 12 samples. The acrylamide concentration ranged from 4% at the top to 30% at the bottom of the gel. The electrophoretic separation was performed in a vertical electrophoresis tank (Pharmacia Electrophoresis Apparatus GE-4), by use of two gradient gel slabs. The current source was a pulsed power supply, model 4100, purchased from Oak Ridge Technical Enterprises Corporation. The electrophoresis buffer was 50 mM Tris (pH 8.4) containing 0.40 M glycine. The separation was conducted at room temperature. The slabs were prerun at 125 V for 25 min to remove the sodium azide in which the gels are stored. Then serum samples (10 pl) in 20% sucrose were applied to each sample well and the slabs were electrophoresed at 80 V for 21 min until the samples had entered the gel. The remainder of the electrophoretic run was conducted with pulsed power: the voltage was increased to 350 V and the samples were electrophoresed at 75 pulses/sec for 16 min, 100 pulses/sec for 28 min, 150 pulses/sec for 19 min, and 350 pulses/sec for 52 min.

After electrophoresis, the gel slabs were stained for nonspecific esterase activity (Allen & Moore, 1966b). The slabs were preincubated in 40 mM Tris buffer (pH 6.6) for 15 min at 37°C, then added to a substrate—dye mixture consisting of 40 mg of alpha-naphthyl acetate and 100 mg of fast blue RR salt per 100 ml of 40 mM Tris buffer (pH 6.6) at 37°C for 18 min. The enzymatic reaction was stopped with a mixture of ethanol:acetic acid:water (10:9:81). Individual sample gels were vertically cut from the slabs and stored in 10% acetic acid. The gels were scanned at 540 nm with a Beckman Acta CIII Spectrophotometer, and the amount of activity was determined with a Gilson planometer and expressed as area under each peak per unit volume of serum.

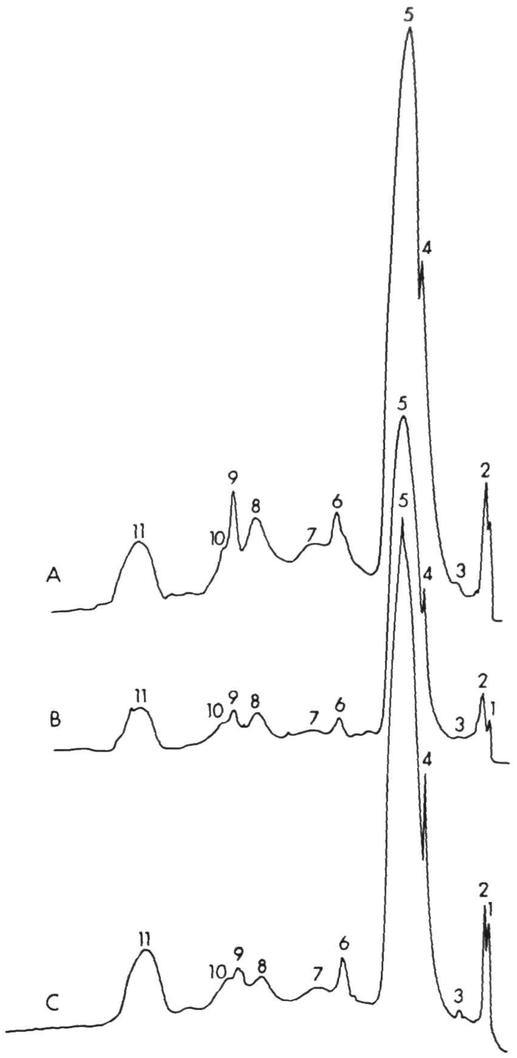

FIG. 1: *Typical spectrophotometric scans of black bear serum esterases. A, Male, age 5.5 yr, 59 kg; B, female, age 4.5 yr, 36 kg; C, same animal shown in A, captured at a later time with a weight of 64 kg. The direction of electrophoresis is from right to left.*

TABLE 1. *Quantitative analysis of typical spectrophotometric scans of male and female black bear serum*

	Age (years)	No. of bears observed	Activity of esterase band 1	2	3	4	5	6	7	8	9	10	11	12	Total activity
Males	1.5	4	3.8±3.4	3.5±3.0	1.3±0.5	23 ±26	414± 42	7.3±1.3	3.0±1.4	7.0±2.0	6.0±3.3	8.5±8.5	77 ±14	0.5 ±1.0	550± 66
	2.5	3	5.7±1.5	6.7±3.1	1.3±0.6	8 ± 7	373± 40	7.7±2.1	4.7±5.5	7.7±6.0	6.3±2.1	1.3±0.6	23 ± 7	0	445± 52
	3.5	4	6.3±4.3	1.5±1.0	1.0±0.0	70 ±52	566± 66	5.0±2.2	5.0±1.6	7.5±4.5	8.3±2.9	4.0±2.2	5.5± 3.7	0	680± 9
	4.5	6	2.5±2.0	8.2±4.7	1.0±0.6	69 ±42	438± 60	6.7±1.0	4.2±3.4	11.5±6.4	8.0±3.5	1.5±0.8	83 ±51	0	634±160
	5.5	7	2.6±2.4	8.7±6.0	1.1±0.4	26 ±33	343±104	6.7±2.6	7.0±6.3	7.1±5.2	4.0±3.1	2.4±1.4	53 ±28	0	462±127
	6.5	1	0	0	1	0	340	1	3	21	18	1	14	0	399
		25	3.8±3.0	5.8±5.0	1.1±0.4	38 ±41	415±100	6.2±2.4	4.8±4.2	8.5±5.7	6.6±4.1	3.2±4.0	49 ±41	0.08±0.40	
Females	1.5	1	1	1	1	1	377	9	4	8	10	2	18		432
	4.5	6	3.7±2.6	1.0±0.0	1.0±0.0	9.5±11	199± 80	3.7±1.4	3.8±2.0	6.3±4.2	5.0±2.7	6.8±7.7	38 ±33	0	278± 71
	5.5	5	1.0±0.0	2.2±1.3	2.2±1.3	12 ±21	273± 87	4.8±2.7	5.4±4.0	4.4±2.7	7.0±4.8	2.8±1.3	7.8± 4.8	0	323±120
	6.5	4	1.0±0.0	1.0±0.0	1.0±0.0	4.0± 5.4	175± 34	1.8±1.0	3.0±1.4	7.5±3.1	6.3±3.0	4.0±2.4	5.0± 2.4	0	270±150
	8.5	1	0	0	0	0	47	1	2	0	1	0	1	0	52
	9.5	1	0	0	0	0	85	2	0	6	8	0	0	0	101
		18	1.8±2.0	1.2±1.0	1.2±0.9	7.6±13	209± 98	3.6±2.4	3.8±2.7	5.8±3.5	6.1±3.5	4.2±4.9	17 ±24	0	
Total		43	3.0±2.8	3.9±4.5	1.0±0.7	26 ±36	329±141	5.2±2.7	4.4±3.6	7.4±5.1	6.4±3.8	3.5±4.4	36 ±38	0.05±0.30	

RESULTS AND DISCUSSION

Typical spectrophotometric scans of esterase profiles of male and female black bear serum are shown in Fig. 1. Table 1 summarizes the quantitative analyses of the esterase bands as a function of age of male and female animals. The serum of the majority of animals was resolved into 11 electrophoretic bands of esterase activity. To aid in discussion, the bands of enzyme activity are designated numerically, beginning with the esterase of least electrophoretic mobilty.

The total esterase activity, obtained by summation of the individual band activities, was greater in the males than in the females of all age groups. The total esterase activity of both sexes generally decreased with increasing age of the animal.

Quantitative differences between the sexes were also evident in several serum esterase bands. Comparison of the total sample of animals by the Student's *t*-test (Remington & Schork, 1970) revealed that five esterase bands were significantly different between males and females at the 0.01 level of significance (bands 2, 4-6, and 11), and two bands (bands 4 and 5) were different at the 0.001 level of significance. The esterase bands not showing sex dependence were 1, 3, and 7-10. All of the esterase bands exhibiting a significant difference had higher activities in the males. Significant differences between males and females were also noted when the esterase activities of different age groups were examined. However, the bands showing a difference varied with the age group. Males had greater esterase activity in bands 2, 4-6, and 8 in the 4.5-year age group, and in band 11 in the 5.5-year age group. Quantitative differences between males and females have also been reported for mice (Allen & Moore, 1966a).

Alterations in individual bands of esterase activity were also associated with the age of the animal. However, these alterations were not consistent. The activity of band 11 in males decreased as the age increased from 1.5 to 3.5 years. However, the activity at 4.5 years of age was higher than the activity observed at 1.5 years of age. The activity again decreased from 4.5 to 8.5 years of age. Furthermore, the alterations of activity observed for one esterase band did not parallel the alterations observed in other bands (compare bands 4 and 11 in males and bands 5 and 11 in females).

Atypical spectrophotometric scans were recorded on the sera of a few animals (Fig. 2); the quantitative analysis is presented in Table 2. The esterase profile shown in Fig. 2A was obtained from a male panhandler with a gunshot wound. Two features unique to this animal are: 1) the increased amount of activity associated with band 10, and 2) the appearance of a new band (designated band 12) which migrated faster than those bands observed in the remainder of the bears. It is not known if the increased activity in band 10 and the appearance of band 12 is due to the gunshot wound or to some other abnormality. Additional male panhandlers were captured, and the first four esterase bands were greatly diminished as is shown for the animal in Fig. 2A.

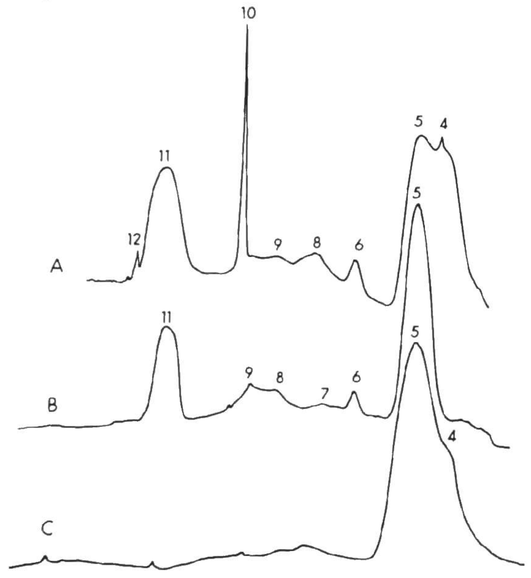

FIG. 2: *Atypical spectrophotometric scans of black bear serum esterases. A, Male, age 1.5 yr, 34 kg; B, female, 4.5 yr, 43 kg; C, male, 4.5 yr, 66 kg. The direction of electrophoresis is from right to left.*

TABLE 2. *Quantitative analysis of atypical spectrophotometric scans of male and female black bear serum*

Animal description	Age (years)	Activity of esterase band 1	2	3	4	5	6	7	8	9	10	11	12
Panhandler, gunshot, male	1.5	1	1	1	1	380	7	4	4	2	20	95	2
Panhandler, male	1.5	1	1	1	1	377	9	4	8	10	2	68	0
Panhandler, male	5.5	1	1	1	1	370	10	9	2	4	3	62	0
Convulsion, male	4.5	1	0	0	0	340	6	1	0	1	0	1	0
Lactating, female	3.5	1	1	1	1	180	5	7	2	3	1	70	0
Lactating, female	6.5	1	1	1	1	200	4	5	3	2	1	83	0
Panhandler, female	4.5	1	1	1	1	377	9	4	8	10	2	18	0
Lactating, panhandler, female	3.5	1	1	1	1	180	5	7	2	3	1	70	0

The esterase activity shown in Fig. 2B was obtained from a lactating female. The activities of bands 1-4, 8, and 10 are greatly decreased, while that of band 11 is increased. One additional female, a lactating panhandler, showed a similar increase in band 11; band 5 was also increased.

One animal convulsed prior to the blood sample being withdrawn. The highly unusual electrophoretic profile is shown in Fig. 2C. The majority of esterase bands are absent. Because the tissue origin of these serum esterases is unknown, it is not possible to discuss the physiological nature of the alterations.

The results of these preliminary studies are subject to considerable variation because: 1) the use of snares may produce tissue trauma resulting in the release of tissue-specific esterases (Tietz, 1970), 2) the immobilizing drug could influence the activity of the esterases, and 3) the varying time between collection of samples and centrifugation to obtain serum results in varying degrees of lysis which could influence enzyme activity. In addition, new esterase species can be formed by hybridization of esterases (Choudhury, 1972), and the dissociation-reassociation phenomenon could be influenced by various factors to produce enzymes with different electrophoretic mobilities and/or different activities. However, the two electrophoretic esterase profiles from the animal that was recaptured are in close agreement. Fig. 1A shows the profile for July 8, 1975, and Fig. 1C shows the profile for September 13, 1975.

Although the studies reported here are subject to the variables just discussed, they nonetheless provide baseline data that will be useful for additional studies of the biochemistry of black bears. The studies demonstrate that alterations in the serum esterases do occur and that these are reflective of the sex of the animal and possibly of environmental determinants.

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