Inheritance and expression of *Esterase-1* allozymes in *Peromyscus leucopus*[1]

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Summary. We analyzed inheritance of 5 *Es-1* alleles in *P. leucopus* and found them to be co-dominant and segregating from a single autosomal locus, thereby verifying assumptions of Mendelian inheritance implicit in field data. We also described an allele that is 'silent' in hemolysate, but is active in liver extract.

Electrophoresis and histochemical staining techniques have been widely used to measure genic heterozygosity, proportion of loci polymorphic in populations and systematic relationships for various organisms[4-8]. Presently, the analysis of genic variability is becoming increasingly important for studies of genetic population structure. These studies tacitly assume that the electrophoretic phenotypes are inherited in a Mendelian fashion, although experimental examination has frequently not been undertaken. Inheritance of allozymes encoded by the *Esterase-1* locus has been investigated from erythrocytes for several species of *Peromyscus: P. maniculatus*[9], *P. leucopus*[10] and *P. truei*[11]. Because *Es-1* has considerable potential as a genetic marker for *P. leucopus*[12], we examined the inheritance of 5 codominant alleles of *Es-1* scored from the hemolysate fraction of blood and liver extracts.

Materials and methods. P. leucopus were obtained from the laboratory colony of Dr J.J. Christian at the State University of New York at Binghamton. The colony was established from mice captured in the vicinity of West Windsor, Broome County, New York. Matings were made for the production of mice for other studies prior to any knowledge of *Es-1* genetic variability in the colony. We, therefore, made use of a series of blind, genetic crosses when the parent and offspring mice were made available to us. *Es-1* in the parents was initially analyzed from blood samples taken from the suborbital canthal sinus using a heparinized capillary tube. After centrifugation, the hemolysate fraction was diluted with 5 drops of cold distilled water and electrophoresed immediately. Ultimately all parental mice and offspring were sacrificed and *Es-1* genotypes determined from electrophoresis of liver extracts. Livers were prepared by removing and freezing after mice had been sacrificed by chloroforming. After thawing, each liver was chopped with scissors and placed in a centrifuge tube with an equal volume of cold distilled water. Tubes were cooled with ice in a refrigerator for 1-2 h before centrifugation at 20,000 × g for 10 min at 0-4 °C. Supernatant was poured into a culture tube and stored at −70 °C.

Samples were analyzed with horizontal starch gel electrophoresis using a lithium hydroxide buffer system for liver extracts and a trishydrochloric acid buffer system for hemolysate[4]. Liver extracts were electrophoresed at 350 V for 3.5 h and hemolysate at 250 V for 1.5 h. The esterase substrate used for both liver extracts and hemolysate was naphthol-AS-D-acetate[12]. The staining solution was 1 ml (1% in acetone) naphthol-AS-D-acetate (1% acetone solution), 25 mg Fast Blue RR salt and 49 ml phosphate buffer pH 6.1[4].

All *Esterase-1* bands migrated anodally on the buffer systems utilized. Two types of banding patterns were observed. Single-banded phenotypes were interpreted as being homozygous (with the exception discussed below), whereas double-banded phenotypes were considered to be heterozygous. Alleles were designated according to their relative mobilities and do not necessarily correspond to designations of other investigators.

Results and discussion. Genetic data for 236 offspring from 16 pairs of mice (13 different crosses) are given in the table. Sexual differences were not present in inheritance patterns; sexes were combined for further analysis. Observed numbers of different genotypes in 11 of 13 crosses were consistent with expected numbers (table) and revealed 5 codominant alleles segregating at a single autosomal locus. For the other 2 crosses, highly significant deviations occurred. The deficient genotypes, however, were observed in other crosses and it is likely that deviations were the result of small sample size. It is interesting to speculate that some effect other than chance was important since *Es-1*$^{110/96}$ heterozygotes were numerically deficient in both crosses. Unfortunately each cross is represented by only 1 pair of mice and considerable numbers of matings would be needed to document a consistent distortion in genotypic ratios. Overall, our results for inheritance of these 5 alleles are in agreement with earlier studies on the inheritance of *Es-1* in other species of *Peromyscus* and other alleles in *P. leucopus.*

In contrast to the patterns of inheritance, which fit the expected, our electrophoretic analysis demonstrated an unexpected difference in the phenotypic expression of *Es-1* between hemolysate and liver tissues. The liver extracts from offspring were electrophoresed first and indicated the

Esterase-1 patterns and genotypes of parents and offspring in *Peromyscus leucopus*

Number of offspring	*Esterase-1* pattern of parents	Genotypes of offspring				χ^2
54*	110/110 × 110/ 96	28:110/110	26:110/ 96			0.07
19*	110/110 × 89/ 87	9:110/ 89	10:110/ 87			0.05
13	110/110 × 110/ 89	7:110/110	6:110/ 89			0.08
12	110/110 × 100/ 96	6:110/100	6:110/ 96			0.00
6	110/110 × 110/100	3:110/110	3:110/100			0.00
10	110/ 96 × 110/100	8:110/110	0:110/100	1:110/96	1:100/96	16.40**
10	110/ 96 × 89/ 87	4:110/ 89	1:110/ 87	2: 96/89	3: 96/87	2.00
18	110/ 96 × 110/ 89	3:110/110	6:110/ 89	2:110/96	7: 96/89	3.78
19	110/ 96 × 110/ 87	2:110/110	11:110/ 87	0:110/96	6: 96/87	14.90**
13	110/ 96 × 89/ 89	6:110/ 89	7: 96/ 89			0.08
12	110/ 89 × 89/ 89	6:110/ 89	6: 89/ 89			0.00
31*	110/ 89 × 110/ 89	8:110/110	19:110/ 89	4: 89/89		2.61
19	100/ 96 × 89/ 87	6:100/ 89	4:100/ 87	4: 96/89	5: 96/87	0.58

* 2 sibships; ** significant at $p < 0.01$.

presence of 5 alleles as discussed above. These were expressed as single and double-banded individuals and were consistent with gel patterns described by earlier investigators[9-11]. However, when hemolysate samples from the parents were electrophoresed, only 4 alleles were found with *Es-1*^{87} absent. From the offspring data, 1 parent in each of 5 of the 16 pairs should have had the *Es-1*^{87} allele present in a heterozygous state (table). These parents had been scored as homozygotes from the hemolysate samples on our original gels, based on appearance of single-banded phenotypes for the non-*Es-1*^{87} allele. Parents were then sacrificed, and analysis of their liver samples revealed the *Es-1*^{87} band as predicted. The other 4 alleles were examined and found to be expressed in both liver and hemolysate. Other investigators have used hemolysate for analysis of *Es-1* and reported 'silent' alleles, which produced no electrophoretic bands[9,10]. Use of erythrocytic esterase patterns alone may lead to misinterpretation of banding patterns on gels and may also result in underestimation of the number of alleles segregating at the *Es-1* locus.

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The chromosomes of *E. calcaratus* and the karyological evolution of the genus *Eupsophus* (Anura: Leptodactylidae)[1]

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Summary. The karyotype of the Chilean frog *Eupsophus calcaratus* is described for the first time. The evolutionary karyological trends of this genus are presented.

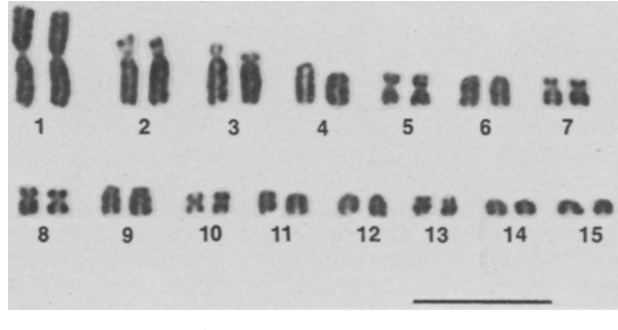
Eupsophus calcaratus was described by Günther[3] on the basis of specimens from Chiloé Island (Southern Chile). During many years some authors[4-6] have considered this frog as identical with *E. roseus;* however some of them had no personal experience with adult live animals. During our herpetological researches in the Nothofagus forests of Southern Chile (Valdivia and Osorno) we collected adult frogs, which are consistent with Günther's description and show the external morphology of the holotype. In this frog the upper part of the iris is yellow there are 2 dorsal fringes convergent behind, and the belly is gray with minute irregular spots. 2 dark-brown rounded spots are present on the lumbar area which stand out on the light-brown background.

In this paper, the chromosomes of *E. calcaratus* are described for the first time, and its chromosomal set is compared with the karyotypes of *E. vittatus*[7], *E. migueli*[8], and *E. roseus*[9], in order to establish the karyological evolutionary trends of the genus *Eupsophus.* In addition, the karyotypes of *Eupsophus* species are compared with the chromosomes of other leptodactylid frogs.

The frogs used in this study included: 3 males and 7 females from La Picada (Osorno Province, 480 m, Los Andes Range), 11 males and 1 female from Cordillera Pelada (Valdivia Province, 1080 m, Coastal Range) and 2 females from Parque Nacional Puyehue (Osorno Province, 960 m, Los Andes Range). The methodology and nomenclature used are described in a previous paper[10].

The analysis of 42 c-metaphasic plates shows that *E. calcaratus* has 30 chromosomes, 8 biarmed pairs and 7 monoarmed pairs. The fundamental number is 46. Pairs 1-3 are large, pair 4 is median and pairs 5-15 are small. Pairs 1,5,7,8,11 and 13 are metacentric (m), pair 2 is submetacentric (sm) and pair 3 is subtelocentric (st). Pairs 4,8,9,11,12,14, and 15 are telocentric (t). Pair 2 shows a remarkable secondary constriction. No sexual chromosomes were observed. The karyotype is presented in the figure and the chromosome measurements are shown in the table.

When the karyotypes of *Eupsophus* species are compared, 2 karyological groups can be established. The 1st group (A) contains *E. vittatus*[7], which has 28 biarmed chromosomes and a fundamental number of 56. In the 2nd group (B) are the following species; *E. roseus*[8] and *E. calcaratus* (2n=30, 8 biarmed pairs and 7 monoarmed, and NF 46), and *E. migueli*[8] (2n=30, 7 biarmed pairs and 8 monoarmed pairs and NF 44). The 2 karyological groups are completely separate and Bogart[7] considered that any attempt at combination of t chromosomes present in the karyotype of *E. roseus* (here named *E. migueli*) would not produce a karyotype similar to *E. vertebralis* (here *vittatus*).



Karyotype of *Eupsophus calcaratus*. The bar equals 10 µm.