

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<sup>9-11</sup>. However, when hemolysate samples from the parents were electrophoresed, only 4 alleles were found with *Es-1<sup>87</sup>* absent. From the offspring data, 1 parent in each of 5 of the 16 pairs should have had the *Es-1<sup>87</sup>* 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<sup>87</sup>* allele. Parents were then sacrificed, and analysis of their liver samples revealed the *Es-1<sup>87</sup>* 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<sup>9,10</sup>. 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)<sup>1</sup>

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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<sup>3</sup> on the basis of specimens from Chiloé Island (Southern Chile). During many years some authors<sup>4-6</sup> 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.

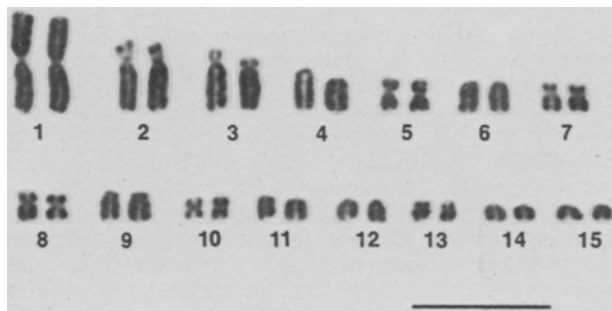
In this paper, the chromosomes of *E. calcaratus* are described for the first time, and its chromosomal set is compared with the karyotypes of *E. vittatus*<sup>7</sup>, *E. migueli*<sup>8</sup>, and *E. roseus*<sup>9</sup>, in order to establish the karyological evolutionary trends of the genus *Eupsophus*. 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<sup>10</sup>.

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*<sup>7</sup>, which has 28 biarmed chromosomes and a fundamental number of 56. In the 2nd group (B) are the following species; *E. roseus*<sup>8</sup> and *E. calcaratus* (2n=30, 8 biarmed pairs and 7 monoarmed, and NF 46), and *E. migueli*<sup>8</sup> (2n=30, 7 biarmed pairs and 8 monoarmed pairs and NF 44). The 2 karyological groups are completely separate and Bogart<sup>7</sup> 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  $\mu$ m.

Although the B group species (*roseus*, *migueli* and *calcaratus*) have the same chromosomal number ( $2n=30$ ), their fundamental numbers and telocentric pairs are different. For this reason the group B has been split into 2 sections. Section 1 only contains *E. migueli* (NF 44, 8 telocentric pairs) and in section 2 are *E. roseus* and *E. calcaratus* (NF 46, 7 telocentric pairs). If the number of telocentric chro-

Summary of primary and secondary constrictions and length as a percentage of the largest chromosome of *Eupsophus calcaratus*

Chromosomes	r*	Type	%	C
1	1.1	m	100	
2	2.6	sm	73.6	sm
3	3.2	st	62.1	
4	∞	t	40.2	
5	1.3	m	39.3	
6	∞	t	34.3	
7	1.6	m	33.3	
8	1.3	m	32.8	
9	∞	t	31.3	
10	1.1	m	27.8	
11	∞	t	25.3	
12	∞	t	24.8	
13	1.0	m	21.8	
14	∞	t	19.9	
15	∞	t	19.4	

\*r is the ratio of the short arm divided into the long arm. For a ratio of 1.0–1.7 the chromosome type is metacentric (m); 1.7–3.0 is submetacentric (sm); 3.0–7.0 and above is subtelocentric (st); 7.0 and above is telocentric (t). The positions of the secondary constrictions (C) are based on similar ratios. The chromosome lengths are expressed as a percentage of the longest chromosome in the karyotype.

mosomes is considered to be a characteristic of karyological primitiveness<sup>11</sup> *E. migueli* should show the most primitive karyological form (8 telocentric pairs) and *E. vittatus* the most derived (no telocentric chromosomes are present). Bogart<sup>7</sup> considered that the karyotype of *E. roseus* (here *E. migueli*) is very reminiscent of those encountered in some species of *Eleutherodactylus* and *Syrrophus marnocki* and that the genus *Eupsophus* is chromosomally more similar to the *Leptodactylus marmoratus* group than to *Pleurodema*<sup>12</sup>. On the other hand, Duellman and Veloso<sup>13</sup> consider that it is easiest to derive the chromosome complement of *Pleurodema* from a stock resembling *E. roseus*. If all the telocentric chromosomes of *E. migueli* are fused among themselves, the *Pleurodema*<sup>14</sup> karyotype could be reconstructed.

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## Esterase polymorphism in a population of *Zaprionus paravittiger*

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**Summary.** Esterase isozyme variation in *Zaprionus paravittiger* is controlled by multiple alleles at 2 autosomal loci (Est-1 and Est-3). Est-1 codes for dimeric esterases while Est-3 codes for monomeric esterases. The degree and pattern of esterase polymorphism have been described.

Hubby and Lewontin<sup>1</sup> pioneered the use of gel electrophoresis to reveal genetic variability at the level of proteins. Since proteins are primary gene products, electrophoretic variations (mobility differences between proteins) can be interpreted in terms of genetic variation. Such analysis has not been attempted to reveal the genetic architecture of fruit-fly populations in this region. The present studies have been undertaken to analyze the genetic control of esterase polymorphism in the most abundantly available species i.e. *Zaprionus paravittiger*.

Esterase polymorphism was studied by starch gel electrophoresis<sup>2</sup> using a discontinuous system of buffers<sup>3</sup>. The adult flies were individually homogenized, and the homogenates run electrophoretically in 12% starch gel for 3 h at 200 V and 25 mA. The gels were stained for esterases following Brewer<sup>4</sup>. Genetic control of esterase variation was studied from the zymograms of parents and progeny of single pair matings. The nomenclature of banding patterns proposed by Ayala et al.<sup>5</sup> has been followed in this study. Electrophoretic variants at any esterase zone have been

indicated by letters A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub> etc. in an anodal to cathodal sequence. The phenotypes of homozygous and heterozygous banding patterns have been represented as A<sub>1</sub>A<sub>1</sub> and A<sub>1</sub>A<sub>2</sub> respectively.

Individuals of *Z. paravittiger* exhibit consistently 3 zones of esterase activity. The Est-1 zone is represented by 3 single bands and 3 triple-banded patterns (figure). The end bands of each triple-banded pattern have the same mobility value as that of 2 single variant bands; the middle band is of intermediate mobility. However there is no electrophoretic variation at the Est-2 zone. The Est-3 zone is represented by a single band in any of 2 different positions or by a 2-band pattern. In the latter case, the 2 bands occupy the same positions as those of 2 variant single bands (figure). The esterase genotypes of parents and progeny of single pair matings have been analyzed to reveal bands which are under the control of separate loci and those coded by allelic variants at a locus (table).

The segregating esterase genotypes/phenotypes at any Est zone appear in the expected 1:2:1 proportions. Thus, the