

## Serological Relationships in the *Leptodactylus pachypus* Species Group (Amphibia, Salientia)

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Precipitin tests indicate that *Leptodactylus ocellatus* and *L. chaquensis* are more closely related than either is to *L. pentadactylus*. However, the serological differences between *L. ocellatus* and *L. chaquensis* support a separate specific ranking for these forms. Populational variations of the specific antigens occur in all 3 species.

### INTRODUCTION

THE existence of physiological and sibling species of amphibians has been discovered during recent years. In the case of *Leptodactylus*, a very puzzling problem in the *L. ocellatus* complex has been pointed out by one of us (Ceí 1948, 1949, 1950). Among the neotropical forms of the *L. pachypus* stock (or subgenus), we can consider at first 2 groups of frogs: the *ocellatus-bolivianus* group and the *pentadactylus* group, both widely distributed in the cis-Andine continental belt. In addition to many distinctive morphological features, differences in some physiological or metabolic characters of these groups have recently been found, e.g., the presence in the skin of different, probably opposite, enzymatic systems for bioamine synthesis leading to phenylalkylamines (Leptodactyline) in the *ocellatus-bolivianus* group, and to indolalkylamines (5-H-triptamine and derivatives) in the *pentadactylus* group (Erspamer and Ceí 1963). But in the *ocellatus-bolivianus* group, the widespread *ocellatus* complex, formerly a Linnean taxon (*Leptodactylus ocellatus* = *Rana ocellata* Linné), includes 2 closely related, partly sympatric species which can be distinguished by many physiological characters but by few morphological ones. There are, for example, striking differences in the male sex cycle and sex character regulation (Ceí 1948, 1949, 1950, 1962; Cohen 1962), a remarkable difference in the electrophoretic properties of the hemoglobins (Bertini and Rathe 1962), considerable chromatographic difference in the Leptodactyline content of the skin (Ceí and Erspamer 1963) and a strikingly different populational distribution of the seroprotein electrophoretic patterns (Ceí and Bertini 1961, Gallopin 1962). These sibling species, *Leptodactylus chaquensis* Ceí and *L. ocellatus* (Linné), are found in well-defined areas, overlapping in more or less extensive zones from northeastern Brazil to the Parana River

(Santa Fe, Argentina). It appeared worthwhile applying to these sibling species of the *ocellatus* complex the criterion of serological affinity, as indicated by precipitin tests. In the present paper, we report on such tests of serum samples of *Leptodactylus ocellatus* and *L. chaquensis* populations in comparison with those of the more distantly related *L. pentadactylus*.

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### MATERIAL AND METHODS

Hemolysis-free pooled sera (antigens) obtained by cardiac puncture from recently captured adult specimens were used. All stored sera were preserved with merthiolate (1:10,000) as recommended by Boyden and De Falco (1943) and Boyden and Gemeroy (1950). Because of the small quantity of antigens available, the rabbits were injected by a single series of 3 injections, spaced 2 weeks and 1 week apart, successively. In all cases, the initial and each subsequent injection was 2 mg of protein per kg of rabbit body weight, but in every case reinforced by the addition of 1 ml of Twen 80 to each 1 ml of antigen, as adjuvant. Eight days after the last injection, a trial bleeding was taken, and on the following day the animals were bled by cardiac puncture and the blood allowed to clot in the refrigerator. The decanted sera were centrifuged, then Seitz filtered, and the antisera bottled under sterile conditions and stored at 5 C. The precipitin tests followed the techniques of Boyden (1942) and co-workers. A Libby Photroner (AMINCO Universal Model) was used. This unit was specially modified by us to work with direct current (6V). Only thus could we obtain a well-stabilized line adequate to the Photroner's galvanometric sensitivity.

TABLE 1. PRECIPITIN TESTS OF *Leptodactylus* ANTISERA.

Anti-serum	Kind	Homologous Area	Heterologous Area	Kind	Percentage Heterol. Area Homol. Area
CB-11	<i>L. ocellatus</i> -San Luis	253	253	<i>L. ocellatus</i> Mendoza-0197	100.00
"	"	253	168	<i>L. chaquensis</i> Tucuman-0.137	66.4
"	"	253	132	<i>L. chaquensis</i> Resistencia-0179	52.1
"	"	253	149	<i>L. pentadactylus</i> Misiones-0164	58.8
CB-8	<i>L. chaquensis</i> -Tucuman	416	405	<i>L. chaquensis</i> Resistencia-0179	97.3
"	"	416	330	<i>L. ocellatus</i> Mendoza-0197	79.3
"	"	416	298	<i>L. ocellatus</i> San Luis-0136	71.6
"	"	416	346	<i>L. ocellatus</i> Bahia Blanca-0174	83.1
"	"	416	236	<i>L. pentadactylus</i> Misiones-0-164	56.7
CB-10	<i>L. pentadactylus</i> -Misiones	333	160	<i>L. ocellatus</i> San Luis-0136	48.0
"	"	333	208	<i>L. ocellatus</i> Mendoza-0197	62.4
"	"	333	168	<i>L. ocellatus</i> Bahia Blanca-0174	50.4
"	"	333	141	<i>L. chaquensis</i> Tucuman-0137	42.3
"	"	333	203	<i>L. chaquensis</i> Resistencia-0179	60.9

Serum samples of the following species and populations were used, their numbers referring to our register of antigens:

*Leptodactylus ocellatus*:—SAN LUIS (Sierras: 1,000 mosl) Argentina. MENDOZA (near the town: 800 mosl) Argentina. BAHIA BLANCA (Buenos Aires Province: mosl) Argentina.

*L. chaquensis*:—TUCUMAN (near the town: 450 mosl) Argentina. RESISTENCIA (Chaco: 60 mosl) Argentina.

*L. pentadactylus*:—OBERÁ (Misiones: 700 mosl) Argentina.

Antisera are conventionally numbered in agreement with the successive operational series of our laboratory. As is customary, we graphed the amounts of turbidity in the reactions between the antisera and the antigens of the various dilutions. The graphed area of a homologous reaction was considered the reference standard for all tests with a particular antiserum, and was rated at 100%. Each

heterologous reaction was then compared to the appropriate homologous one and a percentage relationship determined in accordance with their relative areas of precipitation.

Because of the small quantities of our batrachian sera available, all the progressive antigen dilutions were begun at 1:5 (Evans Buffer) even though the zone of excess antigen often lies to the left of the beginning of the curves of turbidities plotted in our graphs. The zone of excess antigen could be reached with these antisera only by a 1:2.5 or 1:1 dilution. At any rate, the lack of the excess antigen zone at 1:5 dilutions does not substantially change, in the present case, the comparative serological significance of the heterologous/homologous area ratios.

#### RESULTS

Intragenetic and specific variations of serum proteins of *Leptodactylus* and their relationships can be easily appreciated and compared by precipitin tests (Table 1). All

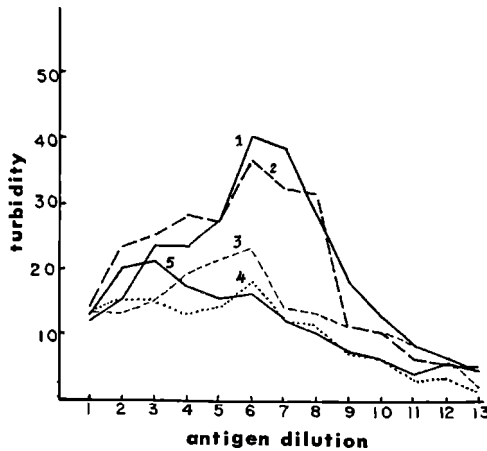


Fig. 1. Results of tests of the antiserum of the *Leptodactylus ocellatus*-San Luis population with its homologous (curve 1) and heterologous antigens (curves 2-5). The amounts of turbidity or precipitate are shown on the ordinate for each of the antigen dilutions indicated along the abscissa. Dilution 1 represents a 1 : 5 dilution of the serum in buffered saline, and each succeeding dilution is double the preceding one. Forms represented in the curves: 2, *L. ocellatus*, Mendoza; 3, *L. chaquensis*, Tucuman; 4, *L. chaquensis*, Resistencia; 5, *L. pentadactylus*, Misiones.

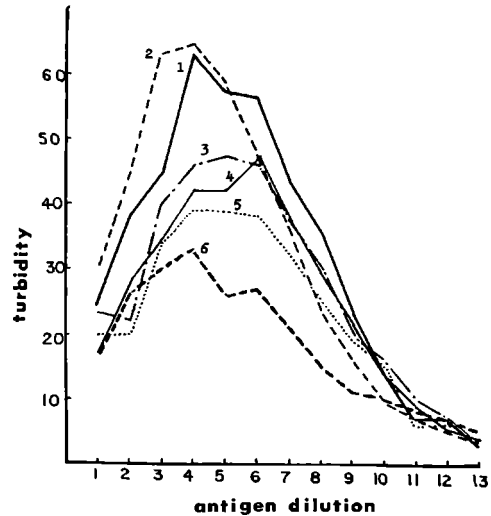


Fig. 2. Results of tests of antiserum of the *Leptodactylus chaquensis*-Tucuman population with homologous (curve 1) and heterologous (curves 2-6) antigens. Forms represented by the latter are: 2, *L. chaquensis*, Resistencia; 3, *L. ocellatus*, Bahia Blanca; 4, *L. ocellatus*, Mendoza; 5, *L. ocellatus*, San Luis; 6, *L. pentadactylus*, Misiones. See legend of Fig. 1 for further explanation.

the antisera here employed show reactions between *Leptodactylus ocellatus*, *chaquensis*, and *pentadactylus*, the progressive weakness of the total turbidity agreeing with the suspected relative systematic and biological distance between these frogs (Figs. 1, 2, 3).

The serological distances which separate *L. pentadactylus* and the sibling species *L. ocellatus* and *chaquensis* are wider than serological distances between these closely related forms. However, the highest reciprocal relationship values of 66.4% and 83.1% found between *ocellatus* of any tested population and *chaquensis* from Tucuman show that these sibling species are really distinct.

Summarizing the results shown by Table 1, the average of percentages of heterologous/homologous areas between *L. ocellatus* populations and *L. chaquensis* from Tucuman is 75.1%, with a range of 66.4 to 83.1%.

Average of percentages between *L. pentadactylus* and *L. ocellatus* populations is 54.9%, range, 48.0 to 62.4%. The average value between *L. pentadactylus* and *L. chaquensis* is 53.3%, range, 42.3 to 60.9%. The similarity in these values is striking. The percentage value between *L. ocellatus* from San Luis and

*L. chaquensis* from Resistencia was a little lower, to 52.1%.

#### DISCUSSION

In spite of the natural variability of results when antisera from different rabbits are tested, the present data justify by their consistency the assumption that by their greater amount of common antigens, *Leptodactylus ocellatus* and *L. chaquensis* have a closer blood and genetic relationship than *Leptodactylus pentadactylus* does with either sibling species.

But the interspecific tests involving *L. ocellatus* and *L. chaquensis* populations indicate with significant uniformity that the similarity of the serum proteins of these forms does not warrant the conclusion that they are conspecific, as also indicated by other physiological observations and by their overlapping geographic distribution. Further, the few tests as yet carried out with antisera and heterologous antigens from different populations of the same species show a very high degree of serological affinity. Antiserum of *Leptodactylus ocellatus* from San Luis gave a value of 100% turbidity with the antigen from the

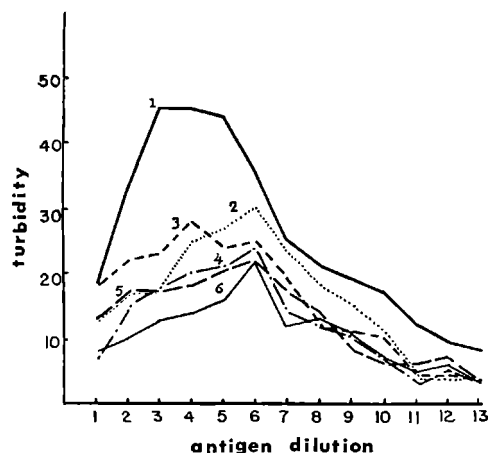


Fig. 3. Results of tests of antiserum of the *Leptodactylus pentadactylus*-Misiones population with homologous (curve 1) and heterologous (curves 2-6) antigens. Forms represented by the latter are: 2, *L. ocellatus*, Mendoza; 3, *L. chaquensis*, Resistencia; 4, *L. ocellatus*, San Luis; 5, *L. ocellatus*, Bahía Blanca; 6, *L. chaquensis*, Tucuman. See legend of Fig. 1 for further explanation.

Mendoza population of this species. Likewise, antiserum of *Leptodactylus chaquensis* from Tucuman showed a 97.3% reaction with antigen from *L. chaquensis* of Resistencia.

However, Resistencia and Tucuman populations of *L. chaquensis* do not react similarly either with anti-*ocellatus* or with anti-*pentadactylus* sera. The Resistencia population is the furthest from the *ocellatus* group, and its test with *ocellatus* antiserum was of the same order of magnitude as the test involving *L. pentadactylus* antigens. We have similarly demonstrated geographical variation in the pattern of relative concentration of globulins by means of paper electrophoresis in all-sympatric populations of *L. ocellatus* and *L. chaquensis* (Cei and Bertini 1961).

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