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Serum Proteins in Allopatric and Sympatric Populations of
Leptodactylus ocellatus and *L. chaquensis*

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THE interrelation of two very similar neotropical frogs, *Leptodactylus ocellatus* and *L. chaquensis* was discussed in previous papers (Ceï, 1950, 1957). *Leptodactylus ocellatus* is a widespread leptodactylid that ranges from Venezuela to south of Bahia Blanca in Argentina. *Leptodactylus chaquensis* is a related species that appears to replace *ocellatus* ecologically in the Gran Chaco (Fig. 1). The two forms are sympatric only in a relatively narrow area along the Rio Paraguay and Rio Paraná, in Paraguay and in the Argentine provinces of Corrientes and Formosa (see Fig. 1). Morphologically few characters serve to distinguish the two species, but the physiology and sexual cycle of the two species are strikingly distinct (Ceï, 1948, 1949). The males of *L. chaquensis* are characterized by a discontinuous cycle of spermatogenesis in contrast to *ocellatus* which shows continuous spermatogenesis. The isolating mechanisms that function in the area of sympatry are unknown (Ceï, 1956). However, statistically significant differences in the seroproteins of *ocellatus* and *chaquensis* have been demonstrated by electrophoretic methods (Ceï and Bertini, 1959). At that time allopatric Argentine populations were compared: *ocellatus* from Córdoba and *chaquensis* from Tucumán. The serum protein patterns were similar in both species, but a significant difference in the ratio of two globulin fractions was obtained. The present paper is a more extensive discussion of the serum protein patterns of both species in sympatric as well as allopatric populations.

METHODS AND MATERIALS

Current methods of paper electrophoresis were utilized (Wunderly, 1954; Block, Durum, Zweig, 1955) with the following specific conditions: Whatman 3 MM paper was used and run for six hours; ionic strength 0.06; pH 8.6; buffer Veronal-Sodium Veronal; 1.5 milliamperes/centimeter. The blood was obtained by cardiac puncture, clotted, refrigerated and the sera of individual samples was usually utilized within a week. All hemolyzed samples were discarded. Samples of both species were employed in the same electrophoretic operation in order to compare the relative characteristics in the same run and electrophoretic field. The strips were treated with bromphenol blue and analyzed photocolometrically by elution. Samples and localities are tabulated in Table 1.

RESULTS AND DISCUSSION

The results, tabulated in Table 1, give the relative protein concentrations of the sera. In Fig. 2 the characteristic electrophoretic patterns of *ocellatus* and *chaquensis* are illustrated.

Four evident fractions are observed in the patterns. The fastest moving fraction (A) appears to correspond to the albumin-like protein. The other three fractions, probably globulins, are indicated as G₁, G₂, and G₃. The protein fraction G₃ exhibits cathodic mobility, particularly in *ocellatus* samples. The relative concentrations of the serum proteins are shown in Table 1 and demonstrate some of the differences between the

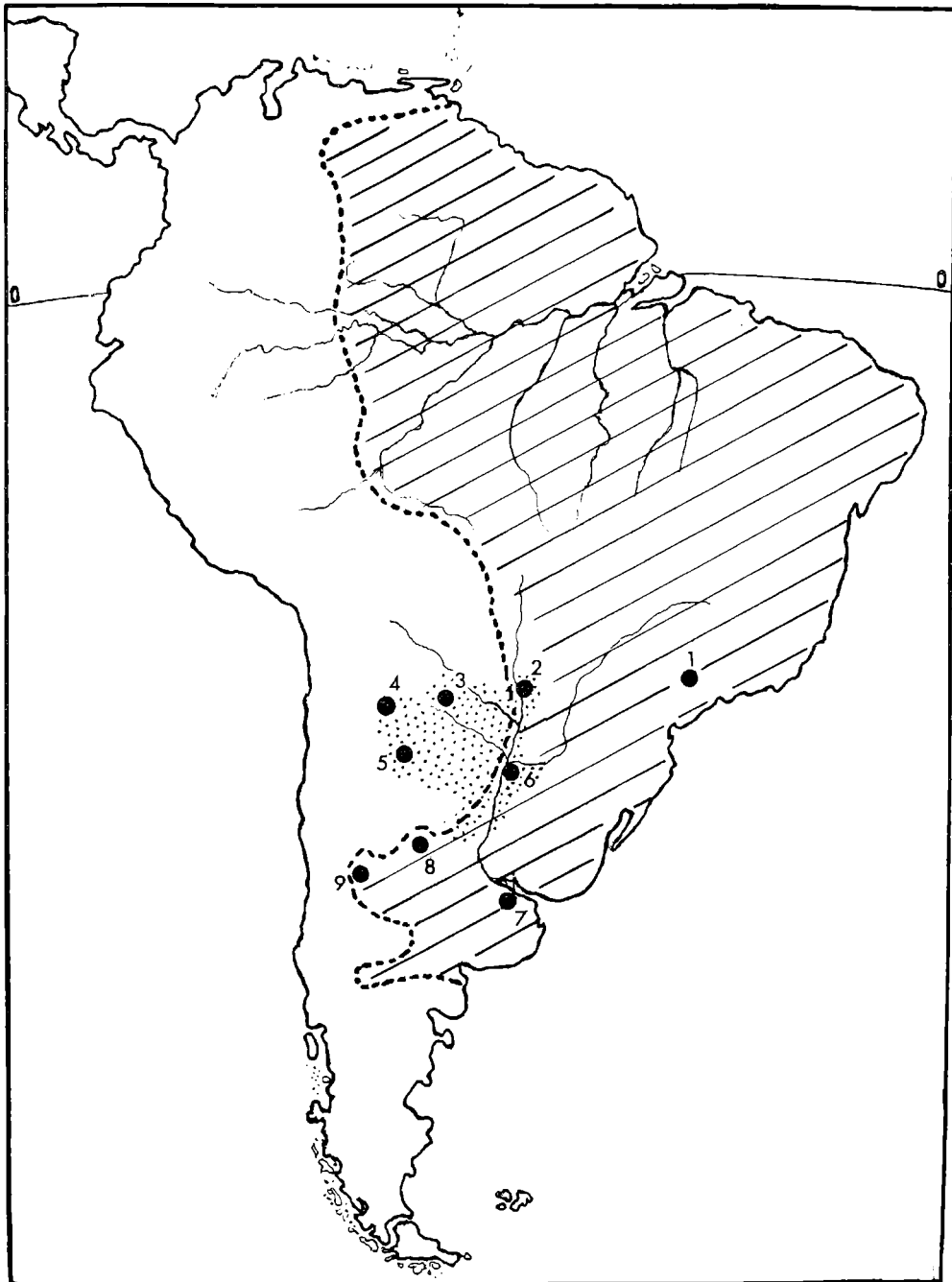


Fig. 1. Map showing the range of *L. ocellatus* (hatched area) and *L. chaquensis* (stippled). The localities are: 1. Sao Paulo; 2. Alto Paraguay; 3. Formosa; 4. Jujuy; 5. Tucumán; 6. Corrientes; 7. Buenos Aires; 8. Cordoba; 9. Mendoza.

TABLE 1. RELATIVE CONCENTRATION OF SERUM PROTEIN FRACTIONS OF THE TWO SPECIES OF *Leptodactylus* FROM VARIOUS LOCALITIES

Locality	Fractions				
	A	G1	G2	G3	G1/G2
<i>Leptodactylus ocellatus</i>					
Brazil (Sao Paulo)					
Nov. '59 (5 ♂)	15.0 ± 1.3* (10.8-18.0)	29.4 ± 1.0 (25.2-35.7)	27.1 ± 0.0 (26.9-27.2)	28.3 ± 2.0 (22.0-34.3)	1.08
Paraguay (Alto Paraguay: Primavera)					
Nov. '59 (1 ♂)	17.6	19.4	31.7	31.3	0.61
Corrientes (near to the town)					
Aug.-Sept. '60 (2 ♂, 3 ♀)	16.5 ± 1.4 (11.3-20.1)	23.1 ± 2.7 (18.5-33.5)	23.6 ± 1.6 (20.0-27.8)	36.9 ± 3.8 (24.3-45.7)	0.97
Buenos Aires (La Plata)					
Aug. '60 (8 ♂, 4 ♀)	14.8 ± 0.7 (10.3-18.5)	20.9 ± 0.8 (16.9-26.1)	23.8 ± 0.6 (20.2-26.2)	40.4 ± 1.2 (34.7-47.1)	0.87
Oct. '60 (1 ♂, 1 ♀)	14.7	19.1	23.3	42.9	0.82
Cordoba (Sierras)					
Aug. '59 (10 ♂)	16.2 ± 0.6 (12.2-19.3)	33.2 ± 1.1 (24.6-35.7)	21.1 ± 0.6 (18.1-23.8)	30.5 ± 1.8 (25.3-42.6)	1.52
Aug. '60 (4 ♂)	12.8 ± 1.5 (8.7-15.8)	20.8 ± 2.9 (23.1-36.7)	19.7 ± 0.6 (18.4-21.3)	37.7 ± 3.3 (31.5-46.9)	1.51
Mendoza (near the town)					
Aug. '60 (1 ♂)	15.1	28.1	20.7	31.1	1.35
<i>Leptodactylus chaquensis</i>					
Paraguay (Alto Paraguay: Primavera)					
Nov. '59 (4 ♂)	21.2 ± 3.5 (13.8-29.0)	30.6 ± 2.2 (26.8-33.8)	23.0 ± 0.9 (21.7-25.6)	25.1 ± 3.3 (18.3-33.5)	1.33
Corrientes (near the town)					
Aug.-Sept. '60 (16 ♂, 7 ♀)	17.1 ± 0.8 (9.7-24.3)	28.6 ± 0.8 (20.5-35.4)	19.0 ± 0.4 (14.9-23.3)	35.3 ± 1.3 (24.3-51.0)	1.50
Formosa (Central Chaco) Ing. Jaurez)					
March '60 (7 ♂)	23.8 ± 1.1 (21.1-29.6)	32.3 ± 1.2 (25.9-35.5)	18.9 ± 0.6 (16.8-21.2)	24.9 ± 1.0 (22.4-30.4)	1.70
Jujuy (near the town)					
March '60 (4 ♂)	14.1 ± 2.2 (10.0-19.1)	38.5 ± 3.7 (30.3-48.1)	19.0 ± 0.9 (16.7-21.4)	28.4 ± 3.3 (22.6-36.5)	2.02
Tucuman (San Miguel)					
Aug. '59 (6 ♂, 1 ♀)	18.2 ± 0.79 (16.4-21.4)	37.3 ± 0.4 (36.4-39.5)	15.7 ± 0.4 (14.2-17.2)	28.5 ± 0.7 (25.4-30.2)	2.37
Nov. '60 (3 ♂)	19.9 (17.3-24.1)	28.3 (27.4-29.3)	19.2 (17.3-21.0)	32.5 (29.2-35.8)	1.47

*Mean ± S.E.

two species. No sex difference was found in either species.

The fraction A is usually relatively more concentrated in *chaquensis* than in *ocellatus*. The averages of this protein for the various

geographic samples range from 14.1 percent to 23.8 percent in *chaquensis* (with an individual range of 9.7 percent to 29.6 percent) while in *ocellatus* the samples range from 12.8 percent to 17.6 percent (the individual

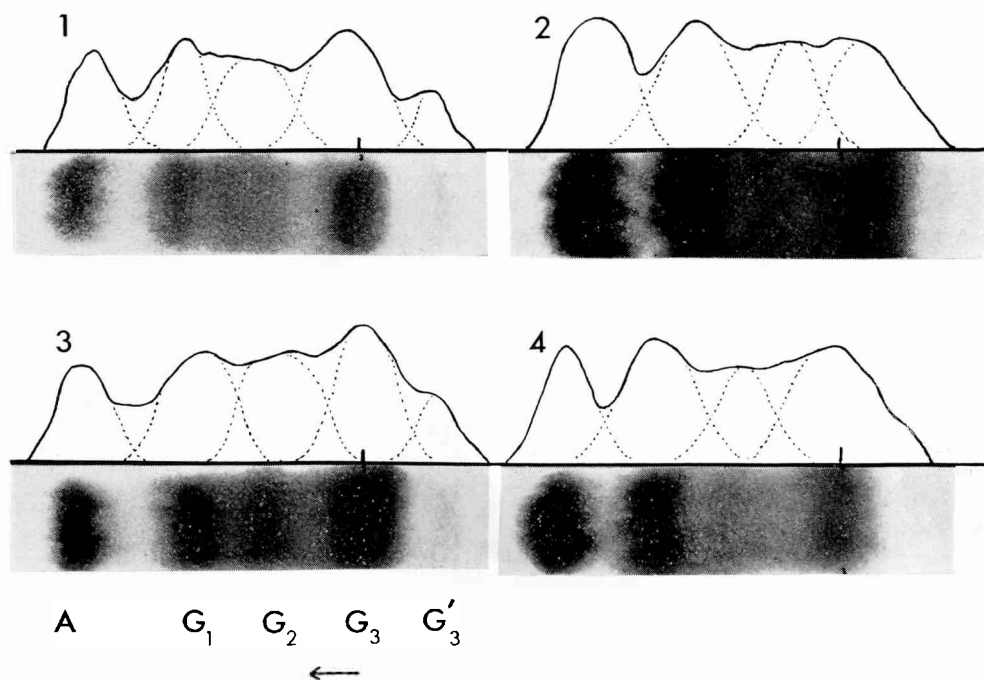


Fig. 2. Electrophoretic patterns of two sympatric and allopatric populations of *L. ocellatus* and *L. chaquensis*: 1. *ocellatus* from Corrientes; 2. *chaquensis* from Corrientes; 3. *ocellatus* from La Plata; 4. *chaquensis* from Tucumán.

values range from 8.7 percent to 20.1 percent).

The G_3 fraction in *ocellatus* very often demonstrates evidence of a fourth globulin fraction (G_3'). The values of G_3 in Table 1 include the G_3' fraction. No such fraction is usually evident in *chaquensis*. The G_3' fraction is easily detectable by elution in *ocellatus*, but not in *chaquensis*.

The ratio G_1/G_2 is a measure of the relative concentrations of these bands of lower mobility. This ratio, like fraction A, illustrates the considerable geographic variation that exists in both species. In *chaquensis* the population averages range from 1.33–2.37, while in *ocellatus* they range from .61–1.52. There is, therefore, overlap of the index values between the two species. However, it is of interest to note that sympatric populations of the two species are sharply distinguishable by their G_1/G_2 value: in Corrientes, *ocellatus* 0.97, while *chaquensis* 1.50; in Alto Paraguay, *ocellatus* 0.61, while *chaquensis* 1.33. Similar ratios have been obtained only between allopatric populations of both species.

There is also evidence of seasonal variation in the G_1/G_2 ratio for *chaquensis* in Tucumán. The sharp difference between

the August and November values is probably attributable to the pronounced seasonal sexual activity characteristic of *chaquensis* (Cei, 1948, 1949). The sexual endocrine activity may cause variations in the lipoprotein level in the blood, which would affect the relative concentrations of the fractions. In *ocellatus* samples from Cordoba in two different years (August 1959 and 1960) show sharp constancy in the G_1/G_2 ratio. Similarly, samples from Buenos Aires taken two months apart gave equivalent ratios. As a consequence of the continuous sexual cycle of *ocellatus* there is apparently less seasonal variation in the serum protein concentrations than in *chaquensis*.

The characteristic seroprotein patterns of the two species in the sympatric zone supports the interpretation that *chaquensis* and *ocellatus* are two separate species. This case is similar to the discussion of the status of the turtles *Pseudemys floridana floridana* and *P. f. suwaniensis* by Zweig and Crenshaw (1957). These forms exhibit an analogous variation in the protein concentrations in two fractions of their electrophoretic patterns and also demonstrate reproductive isolation in the microgeographic zone of sympatry (Crenshaw, 1955). The results obtained

in comparing two closely related species such *chaquensis* and *ocellatus* confirm Zweig and Crenshaw's contention that paper electrophoresis is a useful tool in determining infra-generic relationships.

SUMMARY

Leptodactylus ocellatus and *L. chaquensis*, two closely related species of Argentine anurans, were compared by an electrophoretic study of the serum protein patterns. Significant differences were obtained between the two species, both in the comparison of allopatric as well as sympatric populations.

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LITERATURE CITED

- BLOCK, R. J., DURRUM, E. L. AND ZWEIG, G. 1955. Manual of Paper Chromatography and Paper Electrophoresis. Academic Press Inc., New York.
- CEL, J. M. 1948. El ritmo estacional en los fenomenos ciclicos endocrinosexuales de la Rana Criolla (*Leptodactylus ocellatus* L.) del Norte Argentino. *Acta Zool. Lilloana* 6:283-331.
- . 1949. Factores geneticos raciales que diferencian la regulacion hormonal del ciclo sexual en *Leptodactylus ocellatus* de la Argentina. *Ibid.* 7:113-134.
- . 1950. *Leptodactylus chaquensis* n. sp. y el valor sistemático real de la especie lincana *Leptodactylus ocellatus* en la Argentina. *Ibid.* 9:395-423.
- . 1956. Observaciones geneticas preliminares en poblaciones de Anfibios argentinos. *Biologica, Santiago de Chile* 22:15-19.
- . 1954-57. Notes batracologicas y biogeograficas argentinas. III. *An. Depto. Invest. Cient., Univ. Nac. Cuyo, Mendoza* 2(2):5-9.
- AND F. BERTINI. 1959. Diferencias entre *Leptodactylus ocellatus* y *L. chaquensis* reveladas por via electroforetica en suero. *Acta 1º Congr. Sudamer. Zool. Nov. 1959, La Plata.* (in press).
- CRENSHAW, J. W. 1955. Ph. D. Dissertation, Univ. of Florida.
- WUNDERLY, CH. 1954. Die Papierelectrophorese. Sauerlander & Co., Aarau.
- ZWEIG, G. AND J. M. CRENSHAW. 1957. Differentiation of species by paper electrophoresis of serum proteins of *Pseudemys* turtles. *Science* 126:1065-1067.

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