GEOGRAPHICAL BARRIERS AND SEROLOGICAL RELATIONSHIPS IN SOME AFRICAN TOADS OF THE *BUFO REGULARIS* COMPLEX

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GEOGRAPHICAL BARRIERS AND SEROLOGICAL RELATIONSHIPS IN SOME AFRICAN TOADS OF THE *BUFO REGULARIS* COMPLEX

J. M. Cei (*)

Many authors have recently called attention to the precarious taxonomic status of the widespread African species *Bufo regularis* Reuss (1943). Laurent (1952), Schmidt and Inger (1959), Poynton (1964), Keith (1968), and specially Blair and his collaborators (1969), have pointed out the inadequacy of the several diagnoses and definitions dealing with the mosaic of species and subspecies superficially assembled under the nominate form *regularis*. Although many of its morphological and osteological characters do not display greater variation than that displayed by species of more narrow range (Martin: in press), *Bufo regularis* appears to be a speciating group. That is stressed by very specialized derivatives such as *Bufo perreti* from Nigeria. The existence of cryptic species that replace one another allopatrically and that «have masqueraded under the name of *Bufo regularis*» has been claimed by Blair. In accordance with his experimental interpopulational crosses leading to high degrees of genetic incompatibility, two fundamental assemblies of populations seem to be recognizable from Egypt to South Africa. In Blair's opinion the break between the two comes at Lake Victoria and the Rift Valley.

This paper presents a number of observations, made by means of precipitin tests, on serological relationships between *regularis* toads throughout the African continent. This study may add a different kind of evidence for an objective discussion of the troublesome and evolutive problems of the super-specific features of the *regularis* complex.

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MATERIALS AND METHODS

Several samples of living toads *(B. regularis)* were received between October 71 and February 72. They were collected in the Saharian Ahaggar (Tassili n'Ajjer: Algeria); in the Uige forest (Carmona: Northern Angola); in the small streams from the desertic range of Moçamedes (Southern Angola); near Johannesburg and in the coastal territory of Durban, Natal (S. Africa). Fig. 1 gives a general sketch of the distribution and relative remoteness of the localities. Besides *regularis,* the following species belonging to the *regularis* complex were obtained and tested: *Bufo rangeri* from Johannesburg, *Bufo maculatus* from Uige and *Bufo pusillus* from Moçamedes, *Bufo pardalis* from Capetown (S. Africa). Moreover heterologous tests were carried out with different main lines of *Bufo* such as *B. mauritanicus* from Alger (Algeria), and *Bufo arenarum* from Río Negro (Argentina: S. América). They ennance the phyletic signification of our remarks.

Methods used in testing the precipitin crossed reactions have been formerly described by Boyden and De Falco (1943), later by Bolton, Leone and Boyden (1948); Boyden and Gemeroy (1950) and myself (1965). Specific sera were obtained simultaneously from cardiac bleeding, and kept at deep freezer temperature. Antisera were produced in rabbits by the usual procedure of spaced antigen injections strenghtened by Freund's coadjuvant, through 45 days. After the trial bleeding, the rabbits were bled by cardiac puncture. The blood was allowed to clot in centrifuge tubes, passed through Seitz filters and stored in deep freezer (-20°C). Photronreflectometric precipitin tests were carried out rapidly, by the Libby technique, to make the homo-heterologous cross reactions as suitable as possible.

RESULTS

Tables I and II show the results of the homo-heterologous tests, and Fig. 2 indicates the general serological relationships bearing upon the interrelations of the scattered populations of *regularis.* A representative selection of some of the curves showing the integrated areas, is given by Figs. 3 and 4.

The serological similarity, expressed as % of homo-heterologous precipitin reaction, between Angola and Djanet samples lies between 80,6 — 87,0. That range is indicative of a very recent or current speciative process. Based on former experiences, values above 90% in homo-heterologous precipitin reactions may represent only genetic and populational variations of the whole

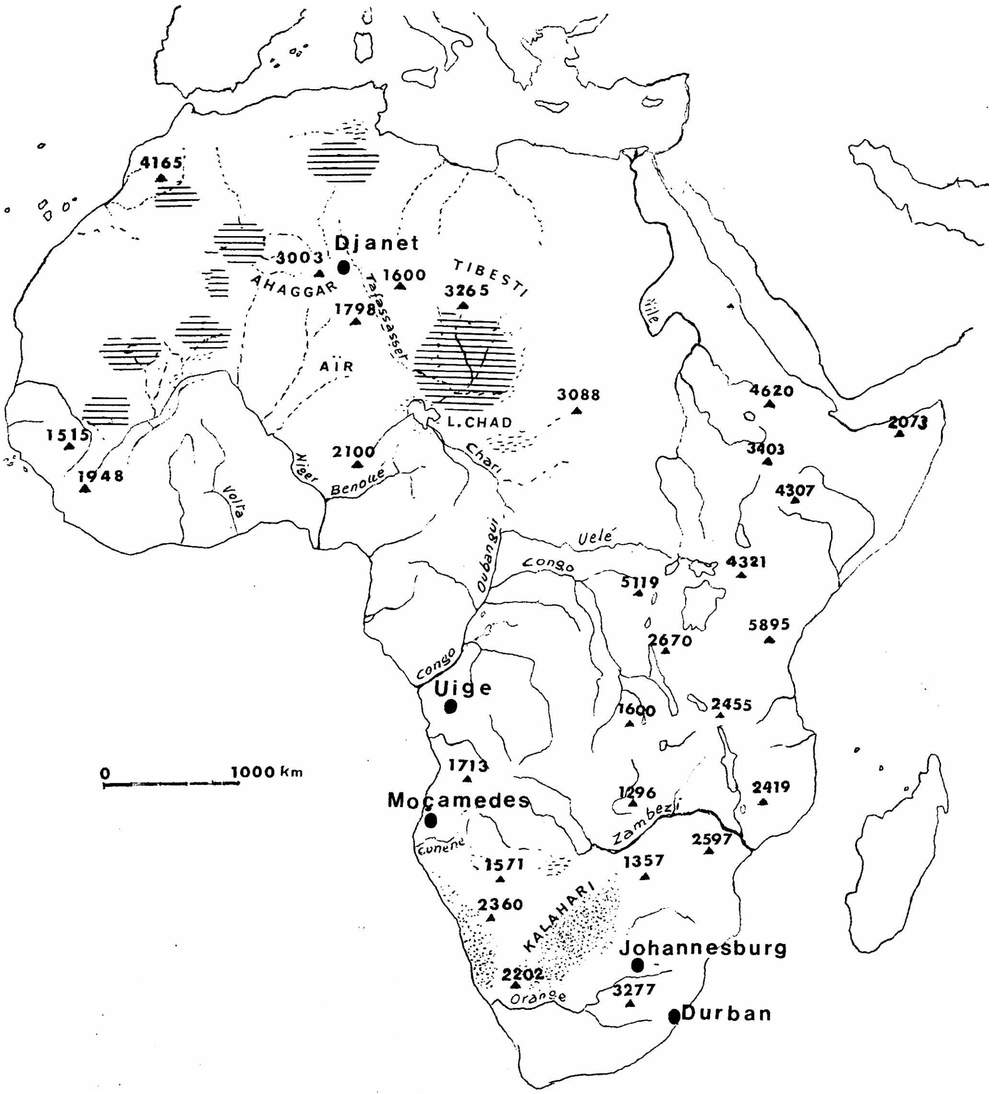


FIG. 1 — Geographical relationships of some African toads of the *Bufo regularis* complex. Black circles: localities of the studied *regularis* populations. Hatched areas: Pleistocene lakes and swamps in Sahara; the broken lines point out the dried pleistocenic main rivers, upper 5° North Lat. Scattered significant altitudes of the continental relief are expressed in m.a.s.l. Stippled areas indicate the approximate desert range of the Kalahari Basin. (Redrawn in accordance with Furon's general reports, 1941, 1960).

TABLE I

PERCENTAGES OF CROSSED HOMO-HETEROLOGOUS PRECIPITIN REACTIONS IN *Bufo regularis* GROUP

SERA	ANTIGENS	%
anti-*regularis* Djanet (Sahara)	*regularis* Djanet (Sahara)	100.0
	» Uige (Carmona) (Angola)	87.0
	» Moçamedes (Angola)	80.6
	» Durban (S. Africa)	70.4
	pardalis Capetown (S. Africa)	67.2
	mauritanicus Alger (Algeria)	44.0 (*)
anti-*regularis* Uige (Angola)	*regularis* Uige (Angola)	100.0
	» Djanet (Sahara)	86.6
	» Moçamedes (Angola)	85.9
	» Durban (S. Africa)	70.7
	maculatus Uige (Carmona: Angola)	64.3
	pardalis Capetown (S. Africa)	66.2
	mauritanicus Alger (Algeria)	47.2
	arenarum Río Negro (Argentina-S. America)	46.1
anti-*regularis* Moçamedes (Angola) Serum — A	*regularis* Moçamedes (Angola)	100.0
	» Uige (Carmona) (Angola)	87.0
	» Djanet (Sahara)	81.4
	» Durban (S. Africa)	71.4
	pardalis Capetown (S. Africa)	67.3
	mauritanicus Alger (Algeria)	42.6
	arenarum Río Negro (Argentina)	46.3
anti-*regularis* Moçamedes (Angola) Serum — B	*regularis* Moçamedes (Angola)	100.00
	» Durban (S. Africa)	72.9
	pardalis Capetown (S. Africa)	69.0
	rangeri Johannesburg (S. Africa)	68.8
	pusillus Moçamedes (Angola)	68.5
	mauritanicus Alger (Algeria)	44.4
	bufo Paris (France)	51.9

(*) In three former tests with anti-*mauritanicus* sera and *regularis* antigens from Djanet (Sahara), the homo-heterologous percentages have been: 42.4 — 45.8 — 49.4.

TABLE II

PERCENTAGES OF CROSSED HOMO-HETEROLOGOUS PRECIPITIN REACTIONS IN *Bufo regularis* GROUP

SERA	ANTIGENS	%
anti-*regularis* Durban (S. Africa)	*regularis* Durban (S. Africa)	100.0
	» Johannesburg (S. Africa)	79.3
	» Moçamedes (Angola)	71.6
	pardalis (Capetown (S. Africa)	68.1
	rangeri Johannesburg (S. Africa)	60.5
	pusillus Moçamedes (Angola)	65.5
anti-*rangeri* Johannesburg (S. Africa)	*rangeri* Johannesburg (S. Africa)	100.0
	regularis Johannesburg (S. Africa)	65.4
	» Durban (S. Africa)	62.4
	» Moçamedes (Angola)	69.1
	pardalis Capetown (S. Africa)	83.7
	mauritanicus Alger (Algeria)	46.1
anti-*maculatus* Uige (Carmona) (Angola)	*maculatus* Uige (Carmona) (Angola)	100.0
Serum — A	*regularis* Uige (Carmona) (Angola)	62.3
	» Djanet (Sahara)	67.8
	pardalis Capetown (S. Africa)	76.9
	mauritanicus Alger (Algeria)	36.5
	arenarum Río Negro (Argentina)	39.6
anti-*maculatus* Uige (Carmona) (Angola)	*maculatus* Uige (Carmona) (Angola)	100.0
Serum — B	*regularis* Uige (Carmona) (Angola)	64.2
	» Djanet (Sahara)	68.8
	pardalis Capetown (S. Africa)	78.1
	mauritanicus Alger (Algeria)	41.7
	arenarum Río Negro (Argentina)	38.9
anti-*pusillus* Moçamedes (Angola)	*pusillus* Moçamedes (Angola)	100.0
	regularis Moçamedes (Angola)	67.9
	» Durban (S. Africa)	67.0
	pardalis Capetown (S. Africa)	79.0
anti-*pardalis* Capetown (S. Africa)	*pardalis* Capetown (S. Africa)	100.0
	rangeri Johannesburg (S. Africa)	84.7
	regularis Durban (S. Africa)	67.3
	» Moçamedes (Angola)	68.6
	mauritanicus Alger (Algeria)	49.1

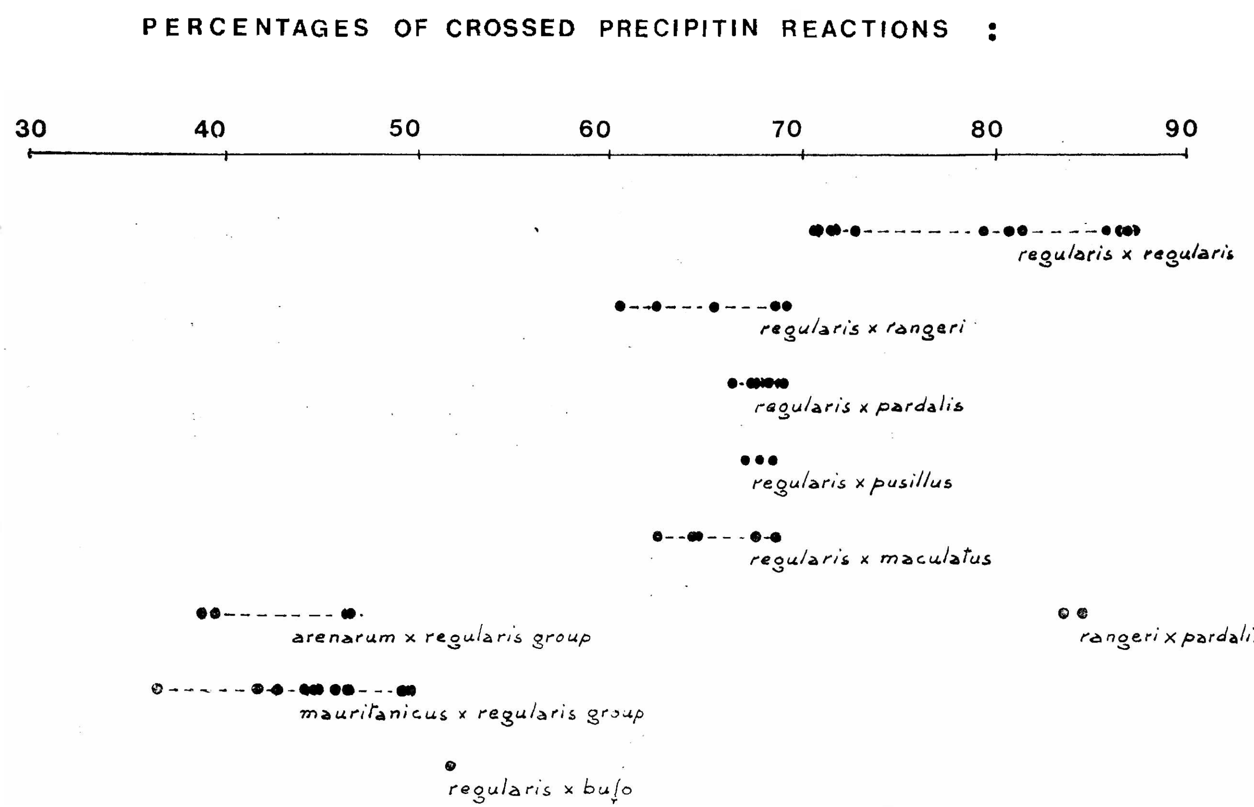
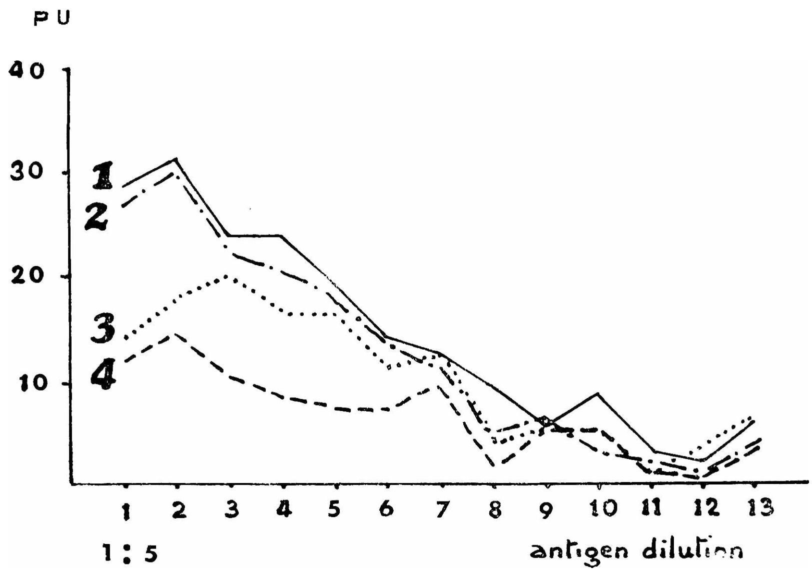


FIG. 2 — General serological relationships bearing upon the homo-heterologous crossed precipitin reactions between scattered populations of *Bufo regularis* complex.



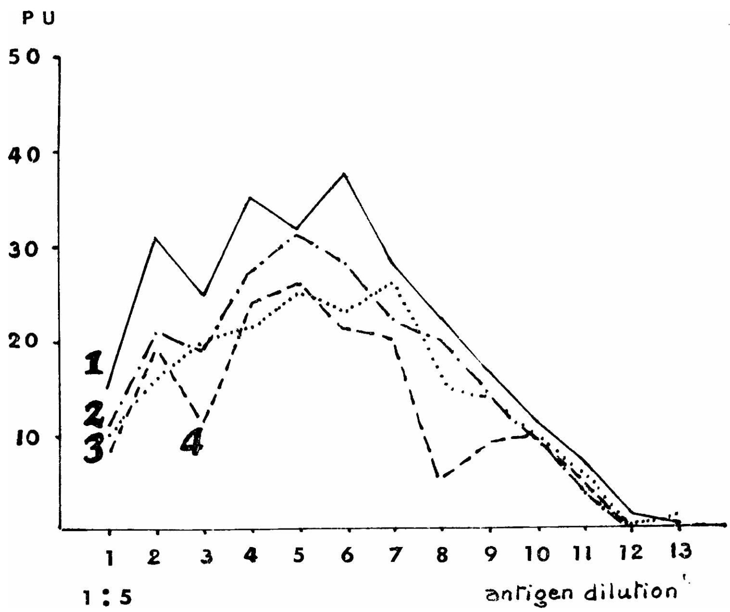


FIG. 3 — Curves showing the integrated areas concerning the homo-heterologous precipitin reactions between anti-sera of *Bufo regularis* from Durban and Djanet and some antigens of *regularis*, *rangeri* and *mauritanicus* populations.

Top: serum anti-*regularis*, DJANET x antigens, 1 — *regularis*, DJANET; 2 — *regularis* UIGE (CARMONA); 3 — *regularis* DURBAN; 4 — *mauritanicus*.
Bottom: serum anti-*regularis*, DURBAN x antigens, 1 — *regularis*, DURBAN; 2 — *regularis*, JOHANNESBURG; 3 — *regularis*, MOÇAMEDES; 4 — *rangeri*.
(PU: Photronreflectometric units)

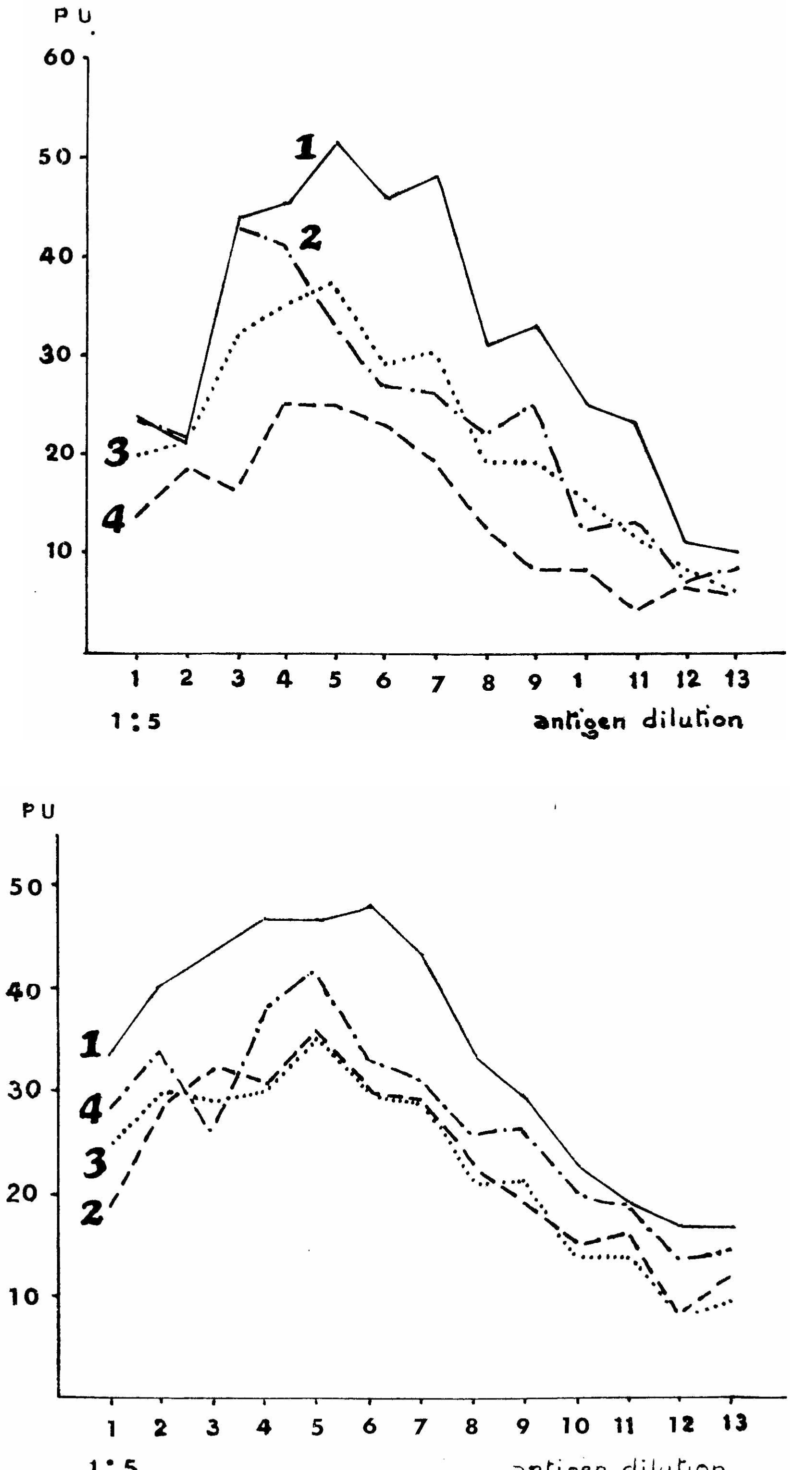


FIG. 4 — Curves showing the integrated areas concerning the homo-heterologous precipitin reactions between anti-sera of *Bufo regularis* and *Bufo pusillus* from Moçamedes and some antigens of *regularis*, *pardalis* and *mauritanicus* populations.

Top: serum anti-*regularis*, MOÇAMEDES x antigens, 1 — *regularis*, MOÇAMEDES; 2 — *regularis*, DURBAN; 3 — *pusillus*; 4 — *mauritanicus*.

Bottom: serum anti-*pusillus* x antigens, 1 — *pusillus*; 2 — *regularis*, MOÇAMEDES; 3 — *regularis*, DURBAN; 4 — *pardalis*.

antigen systems of specific sera. On the other hand, the percent values involving both the Angola and Djanet samples, and the Durban population, range between 70,4 — 72,9. In accordance with our serological yardstick, they correspond to a clear cut serological distance, at a true specific level. The only available percent value between *regularis* from Durban, and *regularis* from Johannesburg, increased to 79,3.

All the homo-heterologous reactions between the above mentioned populations of *regularis* and the related species of the complex, *pardalis, rangeri* and *maculatus-pusillus* are unquestionably lower than any reported values of *regularis* samples inter-se. These values range between 60.5 — 69.0%, for 21 cross reactions. That seems to point out a stronger or more ancient step of speciation and genetic isolation between these allopatric or sympatric forms. Also noteworthy is the relatively high similarity between *rangeri* and *pardalis* (83.7 — 84.7%). Moreover the available data for *pusillus* or *maculatus* versus *pardalis* are reasonably high (76.9 — 79.0%). We must emphasize finally the very low similarity between *regularis* from any locality, *pardalis, maculatus* or *rangeri,* versus *mauritanicus* (36.5 — 49.1%) or a South American representative of the broadskulled line of toads such as *Bufo arenarum* (39.6 — 46.3%). There was general uniformity of the protein content found in all the above mentioned forms of the *regularis* complex. Albumin--globulin in sera ranged from 2.6 to 3.5% in all *regularis* samples, and from 2.5 to 2.8% in *pardalis, pusillus, maculatus* and *rangeri.*

DISCUSSION

The present data make evident the striking serological diversity of the several African populations of *Bufo regularis* usually assembled under the same specific taxon. We are apparently dealing with a population chain, probably in process of genetic isolation. It spreads from the northern, now lonely oases of the Sahara Desert, to the Southern countries of Angola, through the central subtropical and tropical territories. On the other hand, a number of more or less isolated populations of *regularis,* South of the very arid Kalahari basin, must be considered.

The reported tests therefore indicate the close serological relationship between the toads from Djanet and the Angola samples from Uige and Moçamedes, in spite of the wide geographical intervals of some 3200 and 4100 km respectively. Conversely the serological distance increases between the Angola and Durban *regularis* toads, in spite of a relatively smaller geographical separation (2900 and 2250 km.). Percent values of homo-heterologous precipitin reactions between Uige or Moçamedes and Durban are, in fact, very similar (70.7 — 71.4 — 71.6 — 72.9%) to the value obtained between the Djanet and Durban toads (70.4), at some 6000 km of distance!. The present results appear to be in striking agreement with Blair's above mentioned assumption of two fundamental African groups of *Bufo regularis* populations. He has named them «Western» and «Eastern» groups, in accordance with their very low rate of reciprocal genetic compatibility, expressed by the low percentage of cross fertilization and hatching, they very low or no survival beyond larval stage and metamorphosis, the chromosomal differences, etc. Our data emphasize a parallelism between processes of speciation involved with segregative mechanisms (inter-specific fertiliy) and secondary differences reflected in structural changes in protein sequences of the antigen systems of specific sera.

Significantly greater serological distances pertain to the crossed reactions between *regularis* toads from any locality reported in the present paper, and the related species belonging to the 2n = 20 chromosomic complex as defined by Bogart (1968). Our serological data also agree well with Poynton's arrangement of the South-African toads (1964). The range of percent values between *regularis* from «Western» and «Eastern» groups, and *rangeri, pardalis* and *pusillus-maculatus* (60,5 — 69.0) stress the unquestionable position of good allo-sympatric species of these morphological forms, suggesting their probable and ancient speciative features. Furthermore the impressive serological affinity shown by *rangeri* and *pardalis* is remarkable. In Poynton's arrangement both species of toads have been considered as derivated from the same evolutive branch, in the general line of the broadskulled toads leading to the actual *regularis* complex (cfr. Inger, 1959). Fig. 1 gives a general sketch concerning the geographical situation of the present distributional and speciative patterns of the *regularis* groups. Recent studies by Hecht, Hoffstetter and Vergnaud (1961) and Vergnaud-Grazzini (1966) suggest that forms closely related to the modern *regularis* toads were already in existence by the upper Miocene (Morocco). Unfortunately no fossil *Bufo* are reported from the Pliocene and Pleistocene of Africa, probably due to lack of apropiate collecting techniques in this area. At any rate the Quartary paleoclimatic and biocenotic features of the Sahara and neighbouring countries must be kept in mind when the present distribution of the *regularis* populations is considered (Siboulet, 1970).

Attending the very humid climate prevailing during the pleistocenic stages, and the last arid cycle forthcoming gradually only 5000 years ago, it is understandable that no barriers have prevent the probable genetic interchange between continous populations of *regularis* toads, north of Cunene river up to Sahara. It is easily noticeable, for example, the likely hydrographical connection, as far as a relatively recent time, of the remote oases of Djanet with the ancient great lacustrine basin of Chad and the Congo basin by means of the now dried «uidian» and the deep valleys of the Oubangui and Chari rivers. On the other hand, the Kalahari basin and the coastal Namib Desert seems to have been a geographical barrier since the Lower Pleistocene. This is shown by the geological features of its eremic environment from the Great Namaland relief to the high Zambezi valley. Thus, any genetic flux between bordering Anuran populations could have been broken for a longer period, in order to the extended isolating mechanisms of genetic incompatibility and allo-sympatric speciation. It would be emphazised that in the careful checking of Poynton (1964) practically no sampling of frogs and toads — inclosed *Bufo regularis* — has been reported for the widely spread territories of Botswana, north of Gaberones and south of Okawango Swamps.

SUMMARY

Serological relationships of several African toads of the *Bufo regularis* group have been studied. Two fundamental assemblies of populations were recognizable by crossed precipitin tests. They seem correpond to the Blair's «Western» and «Eastern» groups in accordance with their reciprocal rate of genetic compatibility. Between the *regularis* toads North and South of Cunene river percent values of homo-heterologous precipitin reactions range between 70.4 — 72.9%. On the other hand the Angolan populations and the isolated *regularis* populations from Djanet (Sahara: Algeria) show values ranging between 80.6 — 87.0%, in spite of the wide geographical intervals. Serological distance increases between *regularis* toads and the several allo--sympatric species belonging to the same *regularis* complex, such as *pardalis, rangeri* or *maculatus-pusillus,* (range of percent values between 60.5 — 69.0%). Furthermore a noticeable similarity is remarkable between *rangeri* and *pardalis* (83.7 — 84.7%) in accordance with Poynton's systematic statements (1964). Evolutive features of this troublesome group of toads were briefly discussed. The paleogeographic interest of the pleistocenic climatic cycles of the present Saharian Desert and the ancient Kalahari Basin were also emphasized as very important ecological barriers.

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