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PRECIPITIN TESTS AND TAXO-SEROLOGICAL
STATUS OF SOME EUROPEAN TOADS
OF *BUFO BUFO* GROUP

by

JOSÉ M. CEI *

ABSTRACT: Some preliminary precipitin tests were performed using specific seric antigens of European populations of the Common Toad (*Bufo bufo*). Photronreflectometric methods have been used. The serological distances between some subspecies and *B. regularis* and *B. mauritanicus* have been evaluated and discussed under an evolutionary point of view.

INTRODUCTION

The subspecific status of the European common toad *Bufo bufo* (Linné) is still somewhat unsettled. Some of its trinomial units, such as *Bufo bufo spinosus* (Daudin) have been discussed with uncertain conclusions when submitted to a careful morphological screening (PASTEUR and BONS, 1959; SERRA and ALBUQUERQUE, 1963). A revision of the geographical variation of this widespread toad is also needed, in view of its very extensive eurafrican and eurasiatic range. Despite Ladeira's opinion (1956), the existence of *Bufo bufo bufo* (Linné) in Portugal is improbable (PASTEUR et BONS, 1959). Both forms (*Bufo b. bufo* and *Bufo b. spinosus*) are reported, without Portuguese localities, by Serra and Albuquerque (1963). However their subspecific validity was questioned by these authors.

We performed some preliminary serological tests to contribute to the solution of the taxonomic problem of the European common toad. We

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used our standardized crossed precipitin reaction, using as specific antigens the whole albumin-globulin systems of the extracted sera. Samples of *Bufo bufo* were available from France (in the neighbourhood of Paris), from Italy

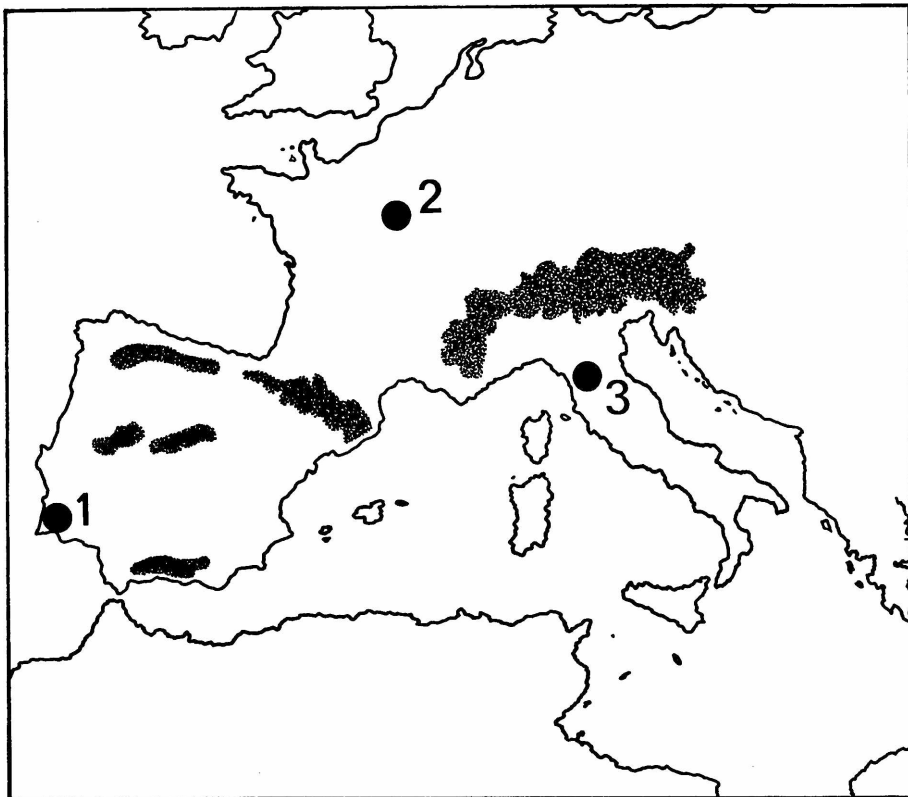


FIG. 1 — European localities of the *Bufo bufo* samples used in the serological tests. Stippled areas indicate the most important geographical barriers (Alpine, Pyrenean and Iberian mountains).

(Florence) and from Portugal (Serra de Monchique, Algarve), between May and July, 1971. The sera of the samples from the European localities (Fig. 1) were compared by crossed reciprocal tests. Also sera of some extra-european groups of toads have been added to increase the comparative significance of our observations: *Bufo mauritanicus* Schlegel from Algérie (Alger, Kabylie) and *Bufo regularis* Reuss from Southern Angola (Moçamedes).

MATERIAL AND METHODS

Blood samples were obtained by cardiac puncture, serum was pooled for any species and stored at -10°C . A simultaneous bleeding of the animals is very important, to assure a careful discriminating reaction and to avoid denaturation of the aged sera (FRAIR, 1969; CEI and CASTRO, 1970). Immune sera were prepared in rabbits, and FREUND'S adjuvant was utilized to strengthen antigenic power. Crossed precipitin reactions were carried out by means of the Photronreflectometric technique of LIBBY (1938), performed by BOYDEN *et al.* and by ourselves. The Aminco Photronreflectometer (Univ. Mod.) was stabilized on direct current. The total amount of precipitate was measured in galvanometric units and converted to a percentage of the value for the corresponding homologous reaction. Values below 100% correspond to homo-heterologous reactions. Decreasing amounts of scattering of the precipitating macromolecular systems indicate the lack of common seric antigens or their specific differentiation.

RESULTS

The results of the crossed reactions are given in the Tables I and II and exemplified graphically in Fig. 2. The serological relationship between the Florence and Paris populations is stronger than that between populations in Algarve and Paris or Florence and Algarve. That suggests that the antigenic difference between the Portuguese and French populations (25,5-27,5%) represents a difference at the specific level. Also the serological distance between the Algarve and Florence populations (19,7-20%) is still remarkable, followed by the shorter serological distance (14,2-15,2%) between the Paris and Florence populations. Antigen divergences are much greater (above 37%) in any reciprocal test of the European toads versus *Bufo mauritanicus* from North Africa, and highest (above 48%) in their reciprocal tests versus the western African toad *Bufo regularis*.

DISCUSSION

Serological relationships between European samples of *Bufo bufo* suggest very long periods of genetic isolation. Both the southern Portuguese and central Italian populations belong to the *spinus* subspecies of DAUDIN,

still checked in the recent lists of European batrachians (MERTENS and WERMOUTH, 1960). On the contrary, the population near Paris belongs to the nominal form *Bufo bufo bufo*, present in Italy only in the Alpine range (TORTONESE and LANZA, 1968). Therefore serological affinities seem not to agree well with the trinomial status of the toad. Crossed heterologous reactions between the Paris and Florence samples are stronger than crossed heterologous reactions between the Florence and Algarve samples, both from the reported Mediterranean range of the *spinosus* form. On the other hand ecological barriers and geographical segregation of the Portuguese toads from Algarve, at 900 km southwest of Paris, may play a role in their noticeable differentiation from the French toads. Common precipitating antigens are 72,5-74,5% in crossed heterologous reactions. Also the distance of some 1200 km between Florence and Algarve is very large, but only 20% of seric antigens do not react here in the reciprocal precipitin tests.

The *Bufo bufo* groups appears to be a very ancient stock, by inference from the widespread eurasian distribution of its recent forms and fossil remains. Genetic differentiation between populations indicated by the antigenic properties of sera are consistent with present and ancient geographical and ecological barriers: i.e. the Alpine and Pyrenean mountains or the Pleistocene events. Clinal trends cannot be easily identified in *Bufo bufo* by our limited preliminary tests. A disruption of a primitive ancestral stock may be probably assumed, from the present evolutionary patterns. By contrast, my conclusions concerning the serological features of *Bufo arenarum* could be taken here in account (CEI, 1969). This widespread South American species does not show any significant serological differentiation from its marginal populations of Porto Alegre (Brasil) to the farthest Patagonian populations. Percentages of its homo-heterologous crossed reactions range always above 90%. They are values of insignificant, intraspecific variation. Likewise clinal trends were easily demonstrated in *Bufo arenarum*, likely because of the absence of geographical barriers in its very extensive continental area, covering almost 1200 miles in latitude (from 30° to 43° South Lat.) and 850 miles in longitude.

The progressive diminution of common seroproteins evidently may have arisen from the late evolutionary processes which have led to the present species, superspecies or species groups, or to the extinction of many intermediate phyletic links. Certainly our serological yardstick indicates the longer periods of genetic isolation of the *Bufo bufo* group from *B. mauritanicus* and *B. regularis* groups. Within the *B. bufo* group differences range from 14,2 to 27,5%, whereas the differences are 37,9-43,2% between *B. bufo* and *B. mauritanicus*, but 48,1-50,9%, between *B. bufo* and *B. regularis*.

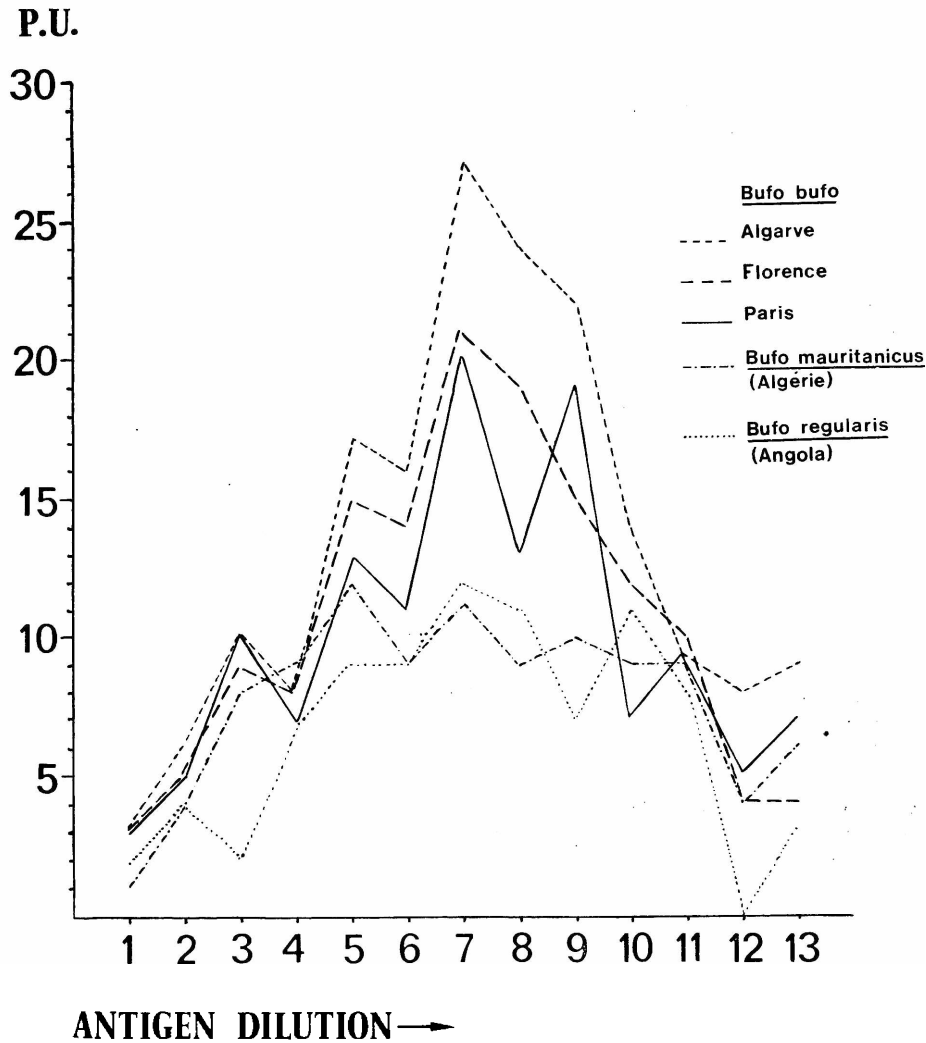


FIG. 2—Curves of turbidities and heterologous / homologous area ratios in precipitin tests with an anti-*Bufo bufo* ALGARVE serum and the following antigens: *Bufo bufo* FLORENCE, *Bufo bufo* PARIS, *Bufo mauritanicus* ALGÉRIE and *Bufo regularis* ANGOLA. The antigen dilution was begun at 1: 5 (Evans Buffer). The photronreflectometric measures are expressed as Photronreflectometric (galvanometric) Units (P.U.).

SUMMARY

Some preliminary precipitin tests were performed using specific seric antigens of European populations of the Common Toad (*Bufo bufo*). Photonreflectometric methods have been used. Serological relationships between *Bufo bufo spinosus* from Algarve (Portugal) and *Bufo bufo spinosus* from Florence (Italy) are somewhat stronger than that between Toads from Algarve and *Bufo bufo bufo* from Paris (France). However the serological distance is short enough between *Bufo bufo spinosus* from Florence and *Bufo bufo bufo* from Paris. The serological affinities seem not to agree exactly with the trinomial status of the Common Toad, and a very long period of genetic isolation may be suggested for the above mentioned populations. A longer period of genetic and specific isolation was also indicated by crossed reactions between *Bufo bufo* and *Bufo mauritanicus* from North Africa (Algérie) and furthermore by the significant high serological distance between *Bufo bufo* and *Bufo regularis* from Western Africa (Angola).

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TABLE I

Results of homo-heterologous precipitin reactions between sera and antisera of *Bufo bufo*, *Bufo mauritanicus* and *Bufo regularis*. Integrated areas are referred to the total amounts of Photronreflectometric units.

IMMUNE SERA	ANTIGENS	% OF INTEGRATED AREAS
anti- <i>Bufo bufo</i> Algarve (Portugal)	× <i>Bufo bufo</i> Algarve (Portugal)	100,0
	» » Florence (Italy)	80,3
	» » Paris (France)	74,5
	<i>Bufo mauritanicus</i> Kabylie (Algérie)	58,3
	<i>Bufo regularis</i> Moçamedes (Angola)	49,1
anti- <i>Bufo bufo</i> Florence (Italy)	× <i>Bufo bufo</i> Florence (Italy)	100,0
	» » Algarve (Portugal)	80,0
	» » Paris (France)	85,8
	<i>Bufo mauritanicus</i> Kabylie (Algérie)	60,4
	<i>Bufo regularis</i> Moçamedes (Angola)	49,6
anti- <i>Bufo bufo</i> Paris (France)	× <i>Bufo bufo</i> Paris (France)	100,0
	» » Algarve (Portugal)	72,5
	» » Florence (Italy)	84,8
	<i>Bufo mauritanicus</i> Kabylie (Algérie)	56,8
	<i>Bufo regularis</i> Moçamedes (Angola)	50,0
anti- <i>Bufo mauritanicus</i> Alger (Algérie) SERUM A	× <i>Bufo mauritanicus</i> Alger (Algérie)	100,0
	<i>Bufo bufo</i> Paris (France)	62,1
anti- <i>Bufo mauritanicus</i> Kabylie (Algérie) SERUM B	× <i>Bufo mauritanicus</i> Kabylie (Algérie)	100,0
	<i>Bufo bufo</i> Paris (France)	60,9
anti- <i>Bufo mauritanicus</i> Sahara (Algérie) SERUM C	× <i>Bufo mauritanicus</i> Sahara (Algérie)	100,0
	<i>Bufo bufo</i> Paris (France)	59,1
anti- <i>Bufo regularis</i> Moçamedes (Angola)	× <i>Bufo regularis</i> Moçamedes (Angola)	100,0
	<i>Bufo bufo</i> Paris (France)	51,9

TABLE II

Progressive serological distances (%) in the reciprocal precipitin tests between:

<i>Bufo bufo</i> from Florence (Italy) and Paris (France):	84,8 — 85,8
» » from Florence (Italy) and Algarve (Portugal):	80,0 — 80,3
» » from Algarve (Portugal) and Paris (France):	72,5 — 74,5
<i>Bufo bufo</i> from Florence, Paris, Algarve and <i>Bufo mauritanicus</i> from Algérie:	56,8 — 58,3 — 59,1 — 60,4 — 60,9 — 62,1
<i>Bufo bufo</i> from Florence, Paris, Algarve and <i>Bufo regularis</i> from Angola:	49,1 — 49,6 — 50,0 — 51,9

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