Bonn. zool. Beitr.	Jg. 36	H. 3/4	S. 307—314	Bonn, Oktober 1985

Biochemical and immunological data on the systematic position of the endemic gecko of the Selvages Islands

by

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Introduction

The Selvages Islands, situated approximately 200 km north of Tenerife, have a local gecko population which was previously assigned to *Tarentola delalandii* (Dum. & Bib., 1836) (Bacallado & Oromi 1978). Recently these geckos were allied to *Tarentola boettgeri* Steindachner,1891, known from Gran Canaria and Hierro, because they share certain morphological characters, and described as *Tarentola boettgeri bischoffi* Joger, 1984.

Yet, unlike the other Makaronesian *Tarentola,* no live material of *bischoffi* was available previously. Thus the exact position of the Selvages gecko in the phylogram of the genus, based mainly on immunological comparisons of serum albumin (Joger 1984a), has remained uncertain.

Meanwhile Mr. G. Maul of Funchal, Madeira, had the great courtesy of sending 10 geckos alive from Selvagem Grande to the A. Koenig Museum, thus providing the material basis for additional biochemical-immunological investigations. The author feels extremely grateful to Mr. Maul for his kindness.

Methods

A) Serum Electrophoresis

Vertical polyacrylamid gel electrophoresis of blood serum was executed as described by Albrecht & von Hagen (1981). Three individuals of *bischoffi* were run simultaneously with the various Canary Island forms. Their plasma protein patterns could then be compared directly with each other.

B) Quantitative precipitin test

This test gives immunological distances which are roughly proportional to phylogenetic divergence times, as could be shown by Joger (1984a, 1985). Three monospecific rabbit antisera prepared against purified serum albumin were used: anti-*Tarentola a. annularis* (Egypt), anti-*Tarentola b. borneensis* (Branco, Cape Verde Islands), anti-*Tarentola delalandii* (Palma, Canaries). The quantitative precipitin test was executed as described by Prager & Wilson (1971), with the modifications of Joger (1984a). Nine heterologous probes (plus one homologous) could be tested at a time.

Immunological distances were expressed as reciprocal values of precent precipitation (100 % precipitation = homologous reaction = 0 % distance). Individual antisera showed considerable variation in their capability to differentiate between heterologous albumins. This was compensated by a reciprocity adjustment procedure. The reactivity of anti-*borneensis* serum was used as a standard; the other antisera were corrected by multiplying their distance values by a factor calculated to give equal values in reciprocal tests with *T. borneensis*.

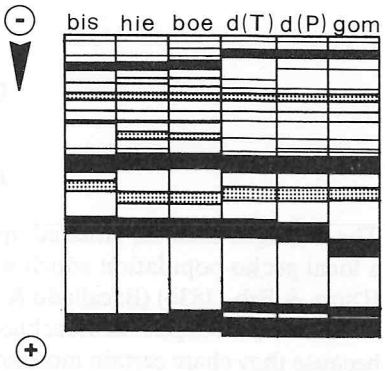

Fig. 1: Comparative PAGE serum protein pattern of *T. bischoffi* and the Canary geckos. Specific band patterns, combined from several individuals electrophoretic runs, are shown. bis = *bischoffi*, hie = *hierrensis*, boe = *boettgeri*, d (T) = *delalandii* (Tenerife), d (P) = *delalandii* (Palma), gom = *gomerensis*.

Results

Fig. 1 shows the electrophoretic pattern of *bischoffi* serum in comparsion with the Canary geckos. In spite of a high overall congruence, reflecting the relatively close affinities between the species concerned (all are members of the subgenus *Makariogecko* Joger, 1984b), the six populations can be subdivided into two electrophoretic pattern groups, one of them consisting of *bischoffi*, *hierrensis* and *boettgeri*, the other of *delalandii* and *gomerensis*. This does support the assignment of *bischoffi* to *boettgeri*, though the former shows more differences from the latter than *hierrensis* does.

The mean immunological distances derived from precipitin testing are summarized in Tab. 1. Some values gained with anti-*mauritanica* serum are also included, although this antiserum was no longer available for reaction with *bischoffi*.

T. boettgeri, both from Gran Canaria and from Hierro, generally show high immunological distances from the other species, indicating a large amount of amino acid differences. This is especially peculiar in the outgroup comparisons (*T. mauritanica* and *T. annularis*, which belong to different subgenera than the others). Theoretically, in these outgroup tests, all *Makariogecko* should share about the same distance from *annularis*, as they are considered offsprings of one common ancestor with *annularis* (the same applies to *mauritanica*).

These discrepancies with the "albumin clock" hypothesis (see Maxson et al. 1975) were discussed previously (Joger 1984a) and led to the hypothesis that serum albumin of *boettgeri* had underwent a higher evolutionary rate than other *Tarentola.*

As can be seen from its distance to *annularis*, *bischoffi* does not show the higher evolutionary rate of *boettgeri*, but stays in the "normal" range of *Makariogecko.* The latter is shown in the fourth line of Tab. 1.

In the fifth line of Tab. 1, an attempt was made to compensate for the exceptional high rates of *boettgeri* (correction factor F). If *boettgeri* values are divided by 1.31 [1], the numerical values for anti-*mauritanica* serum (*T. b. boettgeri*), and of anti-*annularis* serum (mean of *T. b. boettgeri* and *T. b. hierrensis*), become virtually identical to those of the other *Makariogecko* tested. *T. boettgeri* values of anti-*delalandii* and anti-*borneensis* serum, if corrected likewise, get close to the distance values measured for *bischoffi.* However, the reaction of anti-*borneensis* serum against *bischoffi* is still far even from the corrected value.

An alternative adjustment was executed in the sixth line of Tab. 1, by subtracting 10 % of homologous reaction from each immunological distance that involves *boettgeri* (correction factor F'). The congruence with expected distance values is good, too, with the exception of anti-*delalandii* serum, where the distance of *bischoffi* appears too high, compared with the F' value of *boettgeri.*

Discussion

The results shown in Tab. 1 indicate that if *bischoffi* shares a common ancestor with *boettgeri* rather than with other Canary geckos — as is probable with regard to the electrophoretic pattern as well as to morphological characters [2] — the acceleration of the evolutionary rate observed in *boettgeri* must have occurred after its separation from *bischoffi.*

This acceleration deserves further discussion. It must be emphasized that it represents a unique case, at least within the genus *Tarentola*, in which a uniform evolutionary rate of albumin is fairly well documented (Joger 1984a).

Immunological distances are proportional to the number of amino acid differences between the species concerned (Prager & Wilson, 1971). A higher evolutionary rate, as suspected for *boettgeri* albumin, means that this species has accumulated an unusually large number of autapomorphic amino acids. This results in failure of a number of heterologous antibodies to react in precipitin reactions. However, that certain number of apomorphic amino acids

1) The justification of this correction was explained previously (Joger 1984a: 231f).

2) A unique character of *T. boettgeri* is its bluish-grey metallic eye colour (see Joger & Bischoff, 1983). This character state is not shared by *bischoffi.* The living specimens which became now available have dull greyish-brown eyes like other *Makariogecko.*

Table 1: Immunological distances (% of homologous reaction) of *Tarentola* albumins. Means of several precipitin tests (n in brackets). F and F': Correction factors for adjustment of *boettgeri* values; for explanation see text.

Antiserum against serum albumin of:	*T. borneensis*	*T. delalandii*	$\frac{T.\ bor. + T.\ del.}{2}$	*T. annularis*	*T. mauritanica*
Albumins in test:					
Gran Canaria	31.1 (3)	29.3 (5)	30.2 (8)	48.1 (2)	51.3 (1)
Hierro	30.6 (2)	31.0 (2)	30.8 (4)	36.3 (1)	—
Selvages	20.0 (1)	23.7 (3)	21.9 (4)	30.7 (2)	—
$\bar{x}$ (*bor./del.*)	(19.2)	(19.2)	—	32.2 (2)	39.2 (2)
F: $\frac{\bar{x}\ (G.C./Hierro)}{1.31}$	23.5 (5)	23.0 (7)	23.3 (12)	32.2 (3)	39.2 (1)
F': $\bar{x}$ (G.C./Hierro)−10	20.9 (5)	20.2 (7)	20.5 (12)	32.2 (3)	41.3 (1)

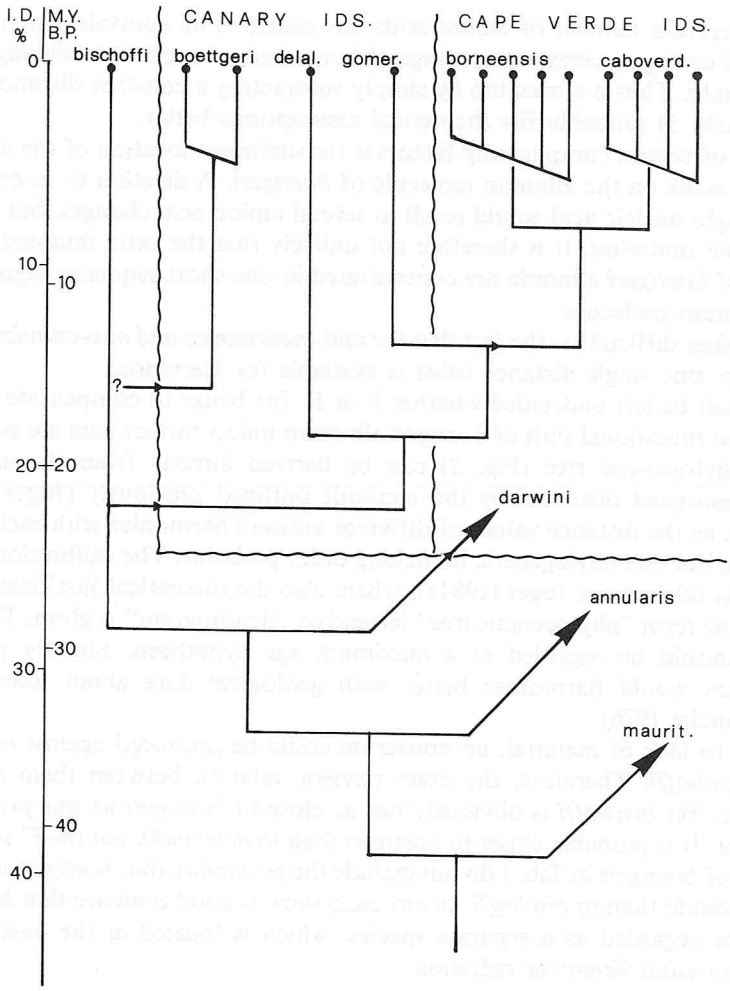

Fig. 2: Position of *T. bischoffi* in the phylogram of *Tarentola*. Divergence points between species are set according to their mean adjusted immunological distance values (I.D. in percent of homologous reaction). Subspecific levels are not differenciated inter se. The hypothetical time axis shows maximum divergence times according to previous investigations (million years before present). Small arrows indicate invasion events of Canarian resp. Cape Verde archipelagos.

would constitute a larger fraction of the relatively low immunological distance between closely related species, than the part it would take in the higher distance between more distant species. Therefore, a distance-proportional correction factor like F may not be adequate.

If a certain number of amino acids has changed, an equivalent number of antibodies (e.g., a certain percentage of homologous reaction) is missing in the precipitate. Thus a correction by simply subtracting a constant distance value (F' in tab. 1) probably fits theoretical assumptions better.

One of several complicating factors is the unknown location of the mutated amino acids on the albumin molecule of *boettgeri*. A deletion or insertion of one single nucleic acid would result in several amino acid changes, but require only one mutation. It is therefore not unlikely that the extra mutated amino acids of *boettgeri* albumin are concentrated in one short sequence segment on the protein molecule.

Another difficulty is the fact that for anti-*mauritanica* and anti-*annularis* sera, no resp. one single distance value is available for *hierrensis*.

It shall be left undecided whether F or F' fits better to compensate for the assumed mutational shift of *boettgeri* albumins unless further data are available.

A phylogenetic tree (Fig. 2) can be derived directly from the adjusted immunological distances by the methods outlined previously (Joger 1984a, 1985b), as the distance values of different antisera harmonize with each other, making but one phylogenetic branching order probable. The calibration of the tree was taken from Joger (1984a), where also the theoretical justification for using the term "phylogenetic tree" instead of "dendrogram" is given. The time scale should be regarded as a maximum age hypothesis. Slightly younger branches would harmonize better with geological data about island ages (Schmincke 1976).

Due to lack of material, no antiserum could be produced against *boettgeri* and *bischoffi*. Therefore, the exact phyletic relation between them remains obscure. Yet *bischoffi* is obviously not as close to *boettgeri* as was previously thought. It is probably closer to *boettgeri* than to *delalandii*, but the F' adjusted values of *boettgeri* in Tab. 1 do not exclude the possibility that *boettgeri* in closer to *delalandii* than to *bischoffi*. In any case, there is good evidence that *bischoffi* must be regarded as a separate species, which is located at the base of the Makaronesian *Tarentola* radiation.

T. bischoffi exhibits a number of character states regarded as being plesiomorphic within *Makariogecko* (see Joger 1984a):
— Eye colour greyish-brown[2)],
— "standard" dorsal pattern of five transverse patches (Fig. 3),
— keeled dorsal tubercles,
— low number of interorbital scales,
— low number of subdigital lamellae.

In general appearance, pholidosis and body size *bischoffi* is intermediate between *boettgeri* and *delalandi*, and could well be an ancestral form the more specialized ones are derived from.

The most probable hypothesis for the spreading of *Makariogecko* within Makaronesia, which was already outlined previously (Joger 1984c), is thus

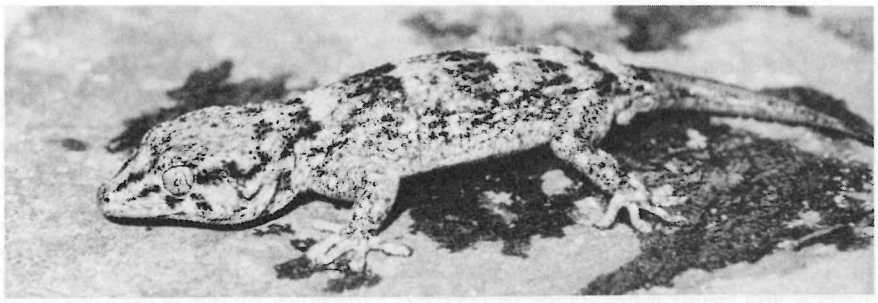

Fig. 3: Living *Tarentola bischoffi.*

confirmed. The origin of *Makariogecko* (except the relictary Cape Verdian species *T. darwini* Joger, 1984c) must have been the northernmost islands, viz. Madeira[3] and the Selvages. From there the prevailing southerly Canary Current could have carried at least two successful founder populations of geckos to the Canaries, the first of which gave rise to the *T. delalandii* / *T. gomerensis* lineage, the second to *T. boettgeri.* A predecessor of *gomerensis* likewise sent out colonist geckos which drifted southwards to reach the Cape Verde archipelago, where they happened to radiate into the polymorphic species *T. borneensis* and *T. caboverdiana.*

Zusammenfassung

Der Gecko der zwischen Madeira und den Kanaren gelegenen Selvages-Inselgruppe, beschrieben unter dem Namen *Tarentola (Makariogecko) boettgeri bischoffi* Joger, 1984, wurde einer vergleichend serologisch-immunologischen Analyse unterzogen. Die Platten-PAA-Gelelektrophorese bestätigte, daß *bischoffi* eine hohe Affinität zu *boettgeri* von den Inseln Gran Canaria und Hierro hat. Jedoch zeigte die quantitative Immunpräzipitation des Serumalbumins, daß *bischoffi* nicht die bei *boettgeri* beobachtete erhöhte Albumin-Evolutionsrate aufweist, sondern seine Immundistanzen im „normalen" Bereich von *Makariogecko* liegen. Er sollte daher artlich von boettgeri unterschieden werden. Zwei alternative Korrekturfaktoren der Immundistanzwerte von *boettgeri* werden diskutiert. Nach den korrigierten Werten erscheint ein gemeinsamer Abzweig von *boettgeri* und *bischoffi* möglich, doch bleibt die exakte Position von *boettgeri* im Phylogramm unklar. *T. bischoffi* jedoch stellt mit hoher Wahrscheinlichkeit die ursprünglichste lebende Art (mit Ausnahme von *T. darwini*) der kanarisch-kapverdischen *Tarentola*-Radiation dar, womit die Hypothese bestätigt ist, daß *Makariogecko* die makaronesischen Archipele sukzessiv von Norden nach Süden besiedelt hat. Die immunologische Datierung läßt auf eine Trennung der beiden Stammbaumäste *bischoffi-boettgeri* und *delalandii-borneensis* vor maximal 23 Millionen Jahren schließen.

3) The existence of an indigenous gecko population on Madeira is far from certain (Maul, pers. comm.). It is, however, possible that such a population was extermined there by the cool climatic conditions in the Pleistocene.

Resumen

La salamanquesa de las Islas Salvajes, que se encuentran entre Madeira y las Islas Canarias, la cual había sido descrita como *Tarentola (Makariogecko) boettgeri bischoffi* Joger 1984, fué sometida a un estudio serológico e imunológico. La Electroforesis en placas de gel PAA confirmó que *bischoffi* tiene gran afinidad con *boettgeri* de las islas Gran Canaria y Hierro. Sin embargo la precipitación imunológica cuantitativa de la albúmina del suero demostró que *bischoffi* no posee la tasa acelerada de evolución de las albúminas de *boettgeri* sino que se encuentran dentro del rango "normal" de Makariogecko. Por esto fué separada de *boettgeri* como especie aparte. Se discuten dos factores de corrección de las distancias imunológicas de *boettgeri*. Al corregir los valores se hace probable una ramificación común de *boettgeri* y *bischoffi*, sin que la posición exacta de *boettgeri* en el filograma quede esclarecida, pero se puede afirmar como altamente probable, que (a excepción de *T. darwini*) *T. bischoffi* sea la especie viviente más primitiva de la radiación del género *Tarentola* de las Islas Canarias y del Cabo Verde. Esto afirma la hipótesis que *Makariogecko* ha invadido los Archipiélagos de Macaronesia sucesivamente de Norte a Sur. El método imunológico conduce a la conclusión de que ambas ramas, *bischoffi-boettgeri* por un lado y *delalandii-borneensis* por el otro se han bifurcado hace máximo 23 millones de años.

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