PEPTIDASE-3 POLYMORPHISM IN POPULATIONS OF THE MADEIRAN LIZARD *LACERTA DUGESII*, FROM PORTO SANTO ISLAND

M. KHADEM [1,3], J. A. JESUS [2,3], A. BREHM [1,3,*]

With 2 figures and 2 tables

ABSTRACT. All populations of Madeira lizard, *Lacerta dugesii*, have two alleles for peptidase-3 enzyme system. However, the same locus has a third allele in some populations from Porto Santo Island. The relative frequencies of the three alleles were sureyed in 13 populations from this Islands. Using UPGMA method, with one exception, populations can be classified into two groups: group 1, includes populations from the north of the island that represents high altitude; group 2, includes central and southern populations that represents low altitude. The exceptional population is the one from Tanque that although belongs to the central and southern parts, shows a revers allelic frequencies. The possible action of human is discussed.

RESUMO. As populações da lagartixa da madeira, *Lacerta dugesii*, apresentam geralmente dois alelos no sistema enzimático da Pedtidase-3. No entanto, algumas das populações da ilha de Porto Santo apresentam um terceiro, raro alelo. Foi efectuado o levantamento das frequências relativas dos alelos deste sistema, nas populações desta ilha.

O método do UPGMA para construção de fenogramas foi aplicado aos dados obtidos, permitindo a distinção das diversas populações em dois grupos, com uma excepção. Um dos grupos compreende as populações da parte norte da ilha representando zonas de maior altitude. O outro representa as populações centrais e do sul, de mais baixa altitude. A excepção é a população de tanque a qual, fazendo parte do segundo grupo possuia frequências alélicas distintas e invertidas.

KEY WORDS: *Lacerta dugesii*, enzymatic polymorphism, population genetics.

[1] Unit of Cytogenetics and Molecular Biology, Department of Biology, University of Madeira, Colégio dos Jesuítas, 9000 Funchal, Portugal

[2] Unit of Ecology and Systematics, Department of Biology, University of Madeira, Colégio dos Jesuítas, 9000 Funchal, Portugal

[3] Centro de Ciências Biológicas e Geológicas da Universidade da Madeira

* Corresponding author: email brehm@dragoeiro.uma.pt

 ISSN 0870-3876

INTRODUCTION

The archipelago of Madeira is composed of four groups of islands: Madeira itself, the nearby Porto Santo, Desertas and the Selvagens, this last one located midway between Madeira and Canary islands. The geological history of the first three groups in relation with the Selvagens is not clearly known. According to TEIXEIRA (1949) and RIBEIRO *et al.* (1980) the Desertas group was united to Madeira itself, but how they were linked to Porto Santo is still a matter of discussion.

Lacerta dugesii MILNE-EDWARDS, 1829 (SAURIA: LACERTIDAE) is an endemic lizard of the archipelagos of Madeira. Its african or european origin remains unknown, which makes the species an interesting subject of biogeographic study.

BISCHOFF *et al.* (1989) studied the differentiation of *L. dugesii* on basis of morphological characters, as well as (very few) enzymatic systems. According to those authors, the species is subdivided into three subspecies, one from Porto Santo and the others from Madeira and Selvagens. We had undertake a large survey on th enzymatic polymorphism of this species (KHADEM *et al.* 1996) which is also in agreement with the distinctiveness of the lizards from Porto Santo. From all *loci* analyzed, one which contributes to the differentiation of this population is the peptidase-3 system.

We found that all populations of *L. dugesii* from the Archipelago of Madeira, have two alleles at the peptidase-3 locus, a slower and more common allele (designated as a) and a faster one (b). The population of Porto Santo we have used in our survey had a third and rare allele (designated as c). This allele was not found in other populations of the archipelago. For this reason we made a full survey of the peptidase-3 system in Porto Santo to determine its existence and frequencies in different populations of lizards collected from different parts of the island.

Materials and Methods

Samples of specimens were collected during the summer of 1995 in 13 localities, covering the whole area of the island of Porto Santo (Fig. 1). Tails of lizards were cut into small slides (<2mm) and manually smashed with a few drops of destilled water. After the homogenates were centrifuged 30 minutes at 10000 g the supernatant was collected and used in the subsequent electrophoretic analysis. The electrophoretic data were resolved using standard methods of horizontal starch gel electrophoresis and histochemical staining procedures (SELANDER *et al*. 1971). The buffer system used to detect Peptidase-3 activity was Tris-EDTA Borate pH 8,6 (0,18M Tris, 0,1M Boric Acid, 0,004M 2Na-EDTA).

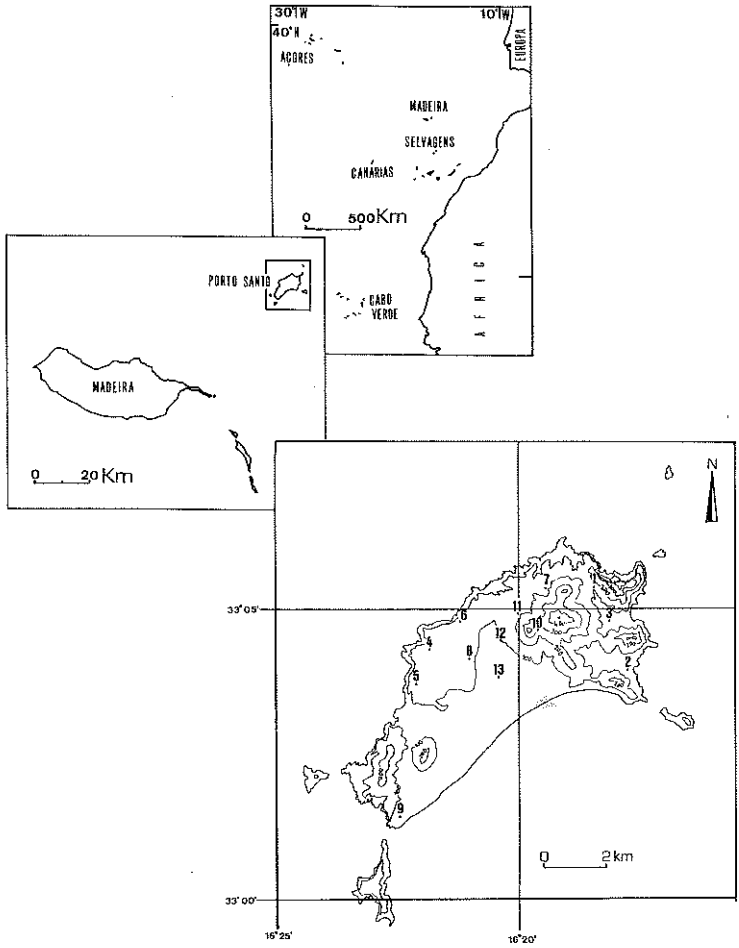

Fig. 1 - Collection localities of *L. dugesii* in Porto Santo island. Numbers represent the following sites: 1- Pico da Cabrita, altitude 265 meters; 2- Serra de Fora, alt 50 m; 3- Serra de Dentro, alt 50 m; 4- Barbara Gomes, alt 200 m; 5- Eiras, alt 160 m; 6- Fonte da Areia, alt 120 m; 7- Pedregal, alt 180 m; 8- Assoprões, alt 80 m; 9- Calheta, alt 20 m; 10- Pico do Castelo, alt 200 m; 11- Camacha, alt 160 m; 12- Farrobo, alt 80 m; 13- Tanque, alt 60 m.

Allelic frequencies were used to compute genetic distances between the populations, using Biosys-1 (SWOFFORD & SELANDER 1981). Genetic similarity between populations was estimated with the CAVALLI-SFORZA and EDWARDS (1967) chord distance algorithm, which was applied to produce a dendrogram with the unweighted pair group method using arithmetic average (UPGMA; SNEATH & SOKAL, 1973). For each population, the mean heterozigosity estimate was calculated as the ratio of observed

heterozygotic genotypes with the total number of individuals sampled.

Results and Discussion

Table 1 shows the allelic frequencies in the 13 populations sampled as well as their levels of heterozigosity. In all populations, Pep-3 was find to be polimorphic, i.e., at least two alleles were present. A general pattern that can be seen from the analysis of allele frequencies, is that adjacent populations tend to have similar values (populations 8, 10, 11 and 12). Five populations included in this study do not conform to the Hardy-Weinberg equilibrium (populations 2 to 5 and 13) with P values ranging from .002 (population of Tanque, x^2 14.560; df3) to .024 - Barbara Gomes, x^2 9.479; df3). All the others fall into P values varying from .288 (population 1) to .868 (population 12). Heterozigosity estimates are extremely variable with values as low as .100 (population 12) up to .937 (population 2). In this respect the ratio between observed homozygotes and heterozygotes and their expected values, follow normal values according to Hardy-Weinberg (P between .90 and .20), except for population 2 which is less then .01 and shows an excess of heterozygotes.

TABLE 1 - Allele frequencies, expressed as percentage in 13 populations of *Lacerta dugesii* for the Peptidase-3 system, and mean heterozigosity (H). Coefficients of CAVALLI-SFORZA and EDWARDS (1967) chord distances were calculated for all pairs of populations examined (Table 2).

Locus and aleles	Population												
	1	2	3	4	5	6	7	8	9	10	11	12	13
(N)	8	16	11	9	11	41	16	17	10	10	8	10	14
Pep-3													
allele a	.625	.531	.773	.772	.545	.768	.531	.824	.700	.850	.938	.950	.143
allele b	.188	.375	.136	.111	.182	.207	.406	.176	.300	.150	.063	.050	.714
allele c	.188	.094	.091	.167	.273	.024	.063	.000	.000	.000	.000	.000	.143
H	.500	.937	.272	.333	.545	.414	.437	.235	.400	.300	.125	.100	.285

The values depicted in Table 2 should be read simply as comparative measures of the peptidase-3 system among the populations surveyed. Caution should be taken when one tries to extrapolate these values to effective phylogenetic distances. The analysis of the data allowed us to detect the existence of two groups of populations (group I comprising populations 1 to 5 and population 7, group II comprising populations 6 and 8 to 12). Mean distances (D) within group I is similar to that of group II (.136 versus .121), but the

distance separating group I from group II is the double (D = .270). The lowest distance found is .017 between Farrobo and Camacha (population 11 and 12) and the highest is .599 between Farrobo and Tanque (population 12 and 13).

TABLE 2 - CAVALLI-SFORZA and EDWARDS (1967) chord distances for all 13 populations of *L. dugesii*. The population number key is the same given in Figure 1.

	Population											
	2	3	4	5	6	7	8	9	10	11	12	13
1	.145	.109	.075	.066	.185	.179	.286	.289	.290	.320	.328	.373
2	****	.182	.204	.182	.187	.039	.257	.211	.271	.338	.352	.272
3		****	.074	.170	.108	.198	.195	.225	.194	.214	.222	.443
4			****	.117	.179	.231	.268	.294	.266	.276	.281	.444
5				****	.250	.219	.349	.347	.353	.380	.387	.364
6					****	.161	.104	.118	.112	.174	.189	.427
7						****	.240	.182	.256	.331	.345	.272
8							****	.093	.023	.115	.132	.495
9								****	.116	.207	.224	.423
10									****	.092	.109	.513
11										****	.017	.585
12											****	.599

A second feature that can be seen from data of Table 2 is the clear separation of population 13 (Tanque) from all the others. This is the only site were allele b is in higher frequency than allele a. Apart from this grouping, one can say that the peptidase-3 system do not present any clear geographic cline in Porto Santo. It is interesting however, to note that group I includes northern peripheric populations, namely the ones from the highest points in the island. Group II represent central and south populations all with a strong influence of human activities.

Fig. 2 represent the topology constructed from data of Table 2 and applying UPGMA. The existence of the two groups of populations already detected from the analysis of the genetic distances is evident. Population 13 (Tanque) appears quite separated from the others due, in part, to the fact it is the only site were allele b is present in higher frequency than allele a. The explanation for the existence of these frequencies is not obvious. Human activities may be responsible for the displacement by chance of some individuals, which led to the distribution we see today.

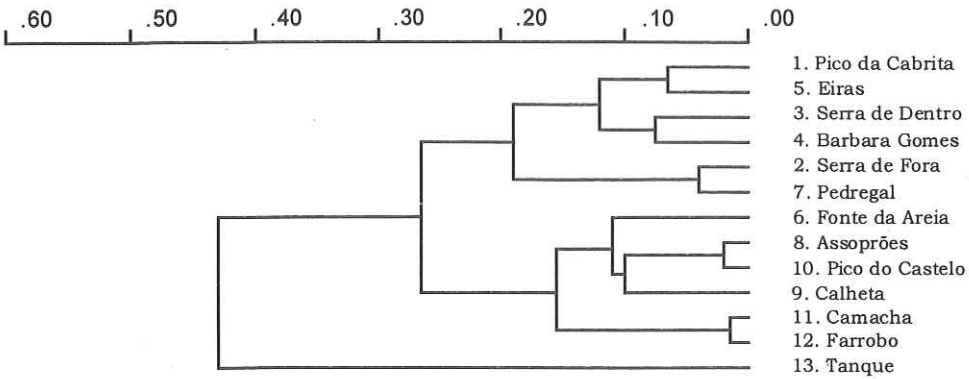

Fig. 2 - Cluster analysis using the UPGMA method, and the Cavalli-Sforza and EDWARDS (1967) chord distances. (Cophenetic correlation = .859).

It is also interesting to note that the rare allele c, is present, apart from Tanque and Fonte da Areia (populations 13 and 6), only in populations belonging to group I. Fonte da Areia, is a peripheric population, near the main waste deposit of the island. It is well possible that lizards belonging to other places have been introduced together with the deposit of waste.

The biochemical information gathered from this survey allowed us to detect a rare allele, which is present in just one island of the archipelagos of Madeira. Even within the island, this rare allele is not widespread but confined to the populations occupying sites with less human activity, namely the ones of higher altitude. The question to be asked is, what factors are contributing to the confinement of the rare allele to some localities. A further survey on the population dynamics of *L. dugesii* especially in what concerns its rate of dispersion and behaviour may provide an answer to this question.

REFERENCES

BISCHOFF, W.; OSENEGG, K. & MAYER, W.:
1989. Untersuchungen zur subspezifischen Gliderung der Madeira - Mauereidechse, *Podarcis dugesii* (MILNE-EDWARDS, 1829). Salamandra 25 (3/4): 237-259.

CAVALLI-SFORZA, L. & EDWARDS, A.:
1967. Phylogenetic analysis: models and estimation procedures. Evolution 32: 550-570.

KHADEM, M.; BREHM, A.; JESUS, J. & VICENTE, L.:
1997. Genetic variation of the endemic madeiran lizard *Lacerta dugesii* Milne-Edwards 1829 (Lacertidae). Submited.

RIBEIRO, A.; ANTUNES, M.; FERREIRA, P.; ROCHA, R.; SOARES, A.; ZBYSZEWSKI, G.; MONTEIRO DE ALMEIDA, F.; CARVALHO, A. & MONTEIRO, J.:
1980. Introduction à la géologie générale du Portugal. Serviços Geológicos de Portugal, Lisboa, 144 pp.

SELANDER, R.; SMITH, M.; YANG, S.; JOHNSON, W. & GENTRY, J.:
1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Stud. Genet. VI Univ. Texas Publ. , 7103: 49-90.

SNEATH, P. & SOKAL, R.:
1973. Numerical Taxonomy. The principles and practice of numerical classification. W. H. Freeman and Company, San Francisco, 573 pp.

SWOFFORD, D. & SELANDER, R.:
1981. Biosys-1: a FORTRAN program for the comprehensive analysis for electrophoretic data in population genetics and systematics. J. Hered. 72: 281-283.

TEIXEIRA, C.:
1949. Notas sobre a Geologia das Ilhas Atlântidas. Faculdade de Ciências de Lisboa.

Date Received: 15-3-96.