

## Protein Variation and Taxonomy in some Genera of the Family Scincidae (Reptilia) in Egypt

### Protein Polymorphism and Genetic Heterozygosity Among Populations of the Sandfish Skink; *Scincus scincus* L.

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**ABSTRACT.** Genetic variation among natural populations of *Scincus scincus* was demonstrated according to the electrophoretic analysis of 27 enzymatic and non-enzymatic proteins encoded by 40 structural gene loci. With respect to polymorphism and heterozygosity, the populations were moderately variable compared with other genera of skinks. The populations were polymorphic for only 7 loci and the overall mean proportions of polymorphic loci (P) and heterozygosity (H) were 7% and 3%. The mean values of the coefficients of genetic distance (D) and similarity (S) were 0.032 and 0.968. Significant geographic heterogeneity in allelic and genotypic frequencies was observed at several polymorphic loci over all populations. Factors affecting this heterogeneity are genetic drift, diversifying selection and mating behaviors.

Since the demonstrations that genetic polymorphism at structural gene loci encoding enzymatic and non-enzymatic proteins can easily be detected by gel electrophoresis (Hubby and Lewontin 1966 and Harris 1966), the efforts have been directed towards evaluating the causal factors of genic polymorphism in natural populations. In a series of papers prior to 1976, research workers have concluded that the typical natural populations of organisms are highly polymorphic for genes specifying the primary amino-acid sequence of enzymes and other proteins (Powell 1975). As a result of the explanation of this extraordinary genetic diversity within populations, population geneticists have been divided between those who believe that

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polymorphism is due to some form of balancing natural selection (the "selctionist school") and those who believe that it is the result of a stochastic equilibrium between the input of nonselected mutations and their loss by random genetic drift (the "neutralist school").

Since it is virtually impossible to separate selection at a certain locus from the effect of selection of linked blocks of genes that may happen to contain a single selected locus (the "hitch-hiking" effect, Ohta and Kimura 1971), it is difficult to measure selection directly at most loci. As a result, attempts to validate any theory have depended upon observations of the statistical distribution of allelic frequencies in different populations of a species or of closely related species. Moreover, these attempts are based on the use of specific predictions of allelic frequency distributions implied by neutral theory such as: the expected heterozygosity in a population (Kimura and Crow 1964), the relation between proportion of loci polymorphic and average heterozygosity (Kimura and Ohta 1971), the average amount of heterozygosity expected to be contributed by different allelic frequency classes (Maruyama 1972), the relationship between the number of alleles and the variation in frequency from allele to allele (Johnson and Feldman 1973, Ewens 1972) and variation among loci in the degree of genetic divergence between populations and species (Lewontin and Krakauer 1973). All these predictions use, as their basic data, the frequency distributions of alleles with populations. However, some depend upon the actual values of population size, migration between populations and mutation rates, while others are parameter-free.

Subsequently, it has been found that some of the observed allelic frequency distributions lead to contradictory results when substituted into the various predictions. For example, the relationship between number of loci and evenness of allelic frequency distribution, the results are at variance with the prediction of neutral theory (Johnson and Feldman 1973). However, the relationship between proportion of polymorphic loci and the average heterozygosity is in agreement with neutral theory (Kimura and Ohta 1971). Taken all together, the data on genic polymorphisms demonstrated by gel electrophoresis fit neither neutralist nor the selctionist hypothesis satisfactorily and contradict these hypotheses in one way or another (Lewontin 1974).

It has been recognized by Hubby and Lewontin (1966) that the electrophoretic method underestimates the actual amount of polymorphism because many gene substitutions would not differ from each other in charge. Also, there are two other generalizations that depend mainly on the assumption that the electromorphic classes are genetically homogeneous. One is the remarkable lack of allelic differentiation

between clearly distinct species even in their monomorphic genes (Lewontin 1974). Second, there is the remarkable similarity of allelic frequency distributions between widely separated populations of some species (Prakash *et al.* 1969) or even between closely related species (Ayala and Powell 1972). This similarity has been considered as strong evidence of the selective control of these polymorphisms.

For all these consideration, an actual estimation of allelic variation and genic heterozygosity among populations of the sand fishskink; *Scincus scincus* was undertaken in this study.

## Materials and Methods

### 1. Samples:

This study is based on adult individuals of *Scincus scincus* collected between August and September, 1990. Information on collecting sites and the corresponding sample sizes is given in Figure 1. Specimens were collected from five localities either by digging into their burrows in the loose sandy habitats or by direct live trapping. The animals were collected and transported alive to the laboratory in Germany, where this work was carried out.

### 2. Laboratory Techniques:

The procedures of preparation of samples and tissue homogenates are described by Gabri *et al.* 1994.

### 3. Electrophoretic Techniques:

A total of 27 enzymatic and non-enzymatic proteins controlled by 46 genetic loci were surveyed electrophoretically among populations by two systems: continuous (Stegemann 1977) and discontinuous (Maurer 1968) gel electrophoresis. These loci are listed below, in addition to the tissues in which they are investigated, with reference to the published allelic variation. The modes of biochemical staining which followed are also mentioned beside each protein locus.

### 1. Enzymatic Proteins.

#### a. Dehydrogenases.

1. Malate (NAD) dehydrogenase (Mdh-NAD); Ec 1.1.1.37 (supernatant form), examined in liver, heart, kidney and muscle (Modification of the method of Shows and Ruddle 1968).

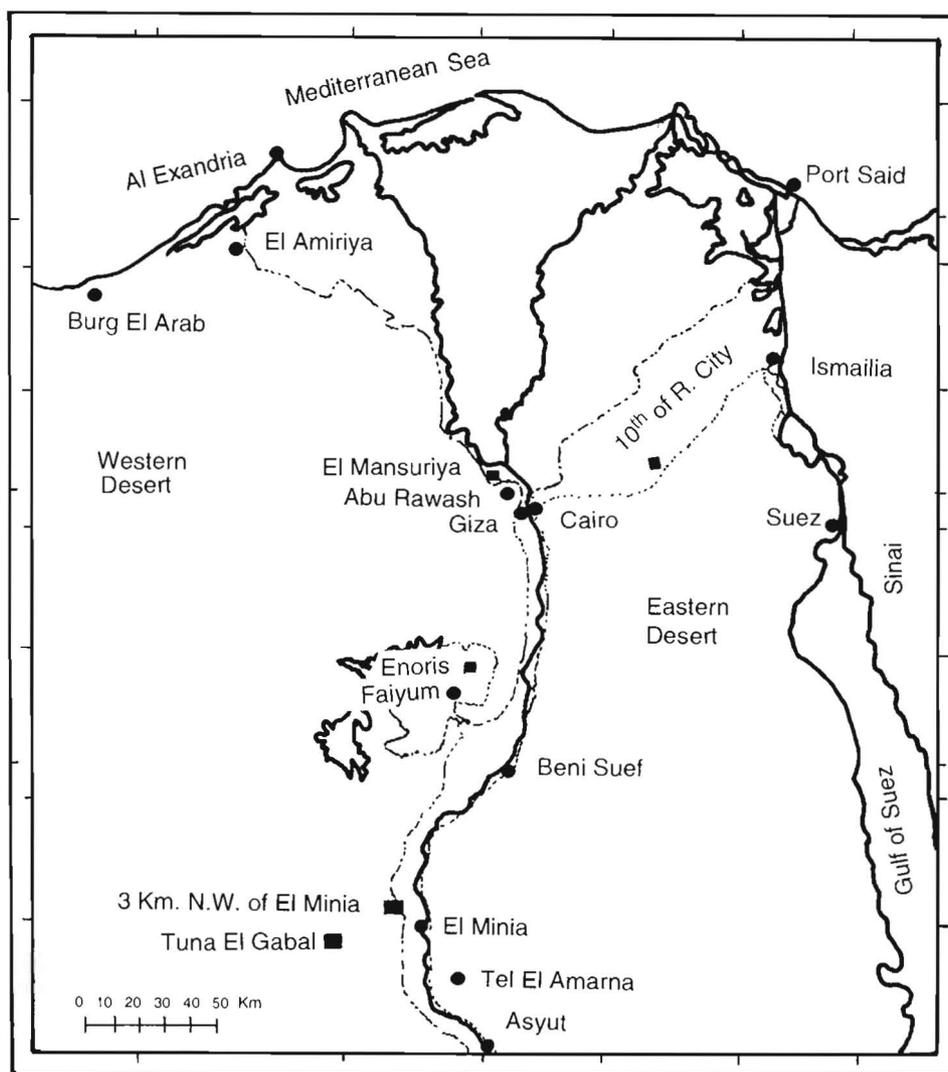


Fig. 1. The localities [■] from which specimens were collected. Sample sizes are: (1) El-Mansuriya (gizeh);  $n = 12$ . (2) Senoris (El-Faiyum);  $n = 10$  (3) 10<sup>th</sup> of Ramadan city (Cairo-Ismailia desert road);  $n = 11$  (4). Tuna El-Gabal (El-Minia);  $n = 10$ .

2. Malate (NAOP) dehydrogenase (Mdh-NADP); Ec 1.1.1.40 (supernatant form), examined in liver, kidney, heart and muscle (Modification of the method of Shows and Ruddle 1968).
3. Lactate dehydrogenases-1, -2, -3 (Ldh-1, -2 and -3); Ec 1.1.1.27, examined in liver, kidney, heart and muscle (Modification of the method of Markert and Massaro 1966).
4. Isocitrate (NADP) dehydrogenase-1 (Idh-1); Ec 1.1.1.42 (supernatant form), examined in kidney and liver (Modification of the method of Henderson, 1965).
5. Isocitrate (NADP) dehydrogenase-2 (Idh-2); Ec 1.1.1.42 (mitochondrial form), examined in heart (Modification of the method of Henderson 1965).
6. Alpha-Glycerophosphate dehydrogenase ( $\alpha$  - Gpd); Ec 1.1.1.6, examined in kidney (Modification of the method of Selander *et al.* 1971).
7. 6-Phosphogluconate dehydrogenase (6-Pgd); Ec 1.1.1.44, examined in heart (Modification of the method of Carter *et al.* 1968).
8. Xanthine dehydrogenase (Xdh); Ec 1.1.3.22, examined in kidney (Method of Selander *et al.* 1971).
9. Glutamate dehydrogenase (Gdh); Ec 1.4.1.2, examined in kidney (Method of Gemmeke 1980).
10. Glucose-6-phosphate dehydrogenases-1, -2 (G6-pd-1 and -2); Ec 1.1.1.49, examined in liver and kidney (Method of Gemmeke 1980).
11. Sorbitol dehydrogenase (Sdh); Ec 1.1.1.14, examined in kidney and liver (Method of Lin *et al.* 1969).
12. Alcohol-C6 (hexanol) dehydrogenase (Hdh); Ec 1.1.1.1, examined in liver (Method after Sherief 1990).

*b. Transaminases:*

1. Glutamate oxaloacetate transaminase-1 (Got-1); Ec 2.6.1.1, (supernatant form), examined in liver and heart (Modification of the method of Delorenzo and Ruddle 1970).
2. Glutamate oxaloacetate transaminase-2 (Got-2); Ec 2.6.1.1, (mitochondrial form), examined in liver (Modification of the method of Delorenzo and Ruddle 1970).

*c. Isomerases:*

1. Phosphoglucose isomerase (Pgi); Ec 5.3.1.9, examined in liver (Method of Delorenzo and Ruddle 1969).

*d. Transferases:*

1. Phosphoglucomutases-1, -2, -3 (Pgm-1, -2, -3); Ec 5.4.2.2, examined in muscle (Method of Spencer *et al.* 1964).
2. Hexokinases-1, -2 (Hk-1, -2)\*; Ec 2.7.1.1, examined in serum (Modification of the methods of Shaw and Prasad 1970 and Sherief 1990).

*e. Hydrolases:*

1. Esterases (Es-1, 2\*\*, 3, 4\*\*, 5, 6\*\*, 7); Ec 3.1.1.1, examined in liver, kidney and serum (Method of Shaw and Prasad 1970 or Selander *et al.* 1971).
2. Leucine amino-peptidases-1, -2, -3 (Lap-1, -2 and -3)\*\*; Ec 3.4.11.2, examined in serum (Modification of the method of Smith and Rutenberg 1966).
3. Glycyl leucine-peptidase (Glp)\*; Ec 3.4.11, examined in kidney (Modification of the method of Shaw and Prasad 1970).

*f. Lyases:*

1. Fumarase (Fum); Ec 4.2.1.2, examined in heart (Method of Shaw and Prasad 1970).
2. Aconitase (Acon)\*; Ec 4.2.1.3, examined in serum (Modification of the methods of Shaw and Prasad 1970 and Sherief 1990).
3. Aldolase (Ald); Ec 4.1.2.12, examined in serum (Method of Shaw and Prasad 1970).

**3.2 Non-enzymatic Proteins.**

Method of Stegemann 1977.

*a. Plasma:*

1. Albumin (Al), liver and serum.
2. Post-Albumin-1\*\*, 2 (PAI-1\*\* and -2), liver and serum.

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\* Enzymes that are assayed by the continuous system of electrophoresis.

\*\* Enzymes that are excluded from the analysis.

3. Transferrin (Trf), serum.
4. Plasma protein-A, B\*\* (Pp-A and -B\*\*).

*b. Hemolysate:*

1. Hemoglobin (Hb).
2. Erythrocytic protein-A, B\*\* (Etp-A and -B\*\*).

The enzymatic and non-enzymatic protein bands were designated according to the system of nomenclature proposed by Allendorf and Utter (1978).

The interpopulation heterogeneity in genotypic and allelic frequencies were also tested using the G-test and the genic contingency Chi-square test of Workman and Niswander (1970). For more information about the modes of designation of alleles at each locus and for the other methods employed in data analysis, see Gabri *et al.* (1994).

## Results

*Survey of Protein Variation:*

Of the 46 genetic loci analyzed in this study, only 7 loci were polymorphic among populations, 33 loci were monomorphic and 6 loci were shown to be polymorphic in one or more population but could not be scored in all with sufficient constancy and clarity in order to score their phenotypes and were, therefore, excluded from the analysis. The electrophoretic patterns of bands appearing on the gels were described for each protein locus. The allelic frequencies of the 7 polymorphic protein loci are given in Table 1.

### 1. Polymorphic Proteins.

*Post-Albumin-2 (PAI-2):*

At the PAI-2 locus, two alleles are detected (Fig. 2). Allele PAI-2<sup>a</sup> is fixed in three localities and has a frequency of 0.33 in El-Mansuriya, together with allele PAI-2<sup>b</sup>, which is restricted only to this locality and occurred at a frequency of 0.67.

*Isocitrate Dehydrogenases (Idh<sub>S</sub>):*

The mitochondrial form (Idh-2) is monomorphic, while the supernatant form (Idh-1) is polymorphic. Two alleles, Idh-1<sup>a</sup> and Idh-1<sup>b</sup>, are shown at this locus; allele Idh-1<sup>a</sup> is fixed in Tuna El-Gabal only, while allele Idh-1<sup>b</sup> is fixed in the other three localities.

**Table 1.** Allele frequencies and heterozygosity at 7 polymorphic loci

Protein Locus and Allele		Population and number of individuals (N)				
		El-Mansuriya (12)	Senoris (10)	Ismaelia (11)	Tuna El-Gabal (10)	$h_1^*$
PAI	-2 <sup>a</sup>	0.33	1.00	1.00	1.00	0.11
	-2 <sup>b</sup>	0.67	-	-	-	
$h_1$		0.44	0.00	0.00	0.00	0.00
$h_{obs}$		0.00	0.00	0.00	0.00	
IDH	-1 <sup>a</sup>	-	-	-	-	0.00
	-1 <sup>b</sup>	1.00	1.00	1.00	1.00	
$h_1$		0.00	0.00	0.00	0.00	0.00
$h_{obs}$		0.00	0.00	0.00	0.00	
PGI	-1 <sup>a</sup>	-	-	-	-	0.00
	-1 <sup>b</sup>	1.00	1.00	1.00	1.00	
$h_1$		0.00	0.00	0.00	0.00	0.00
$h_{obs}$		0.00	0.00	0.00	0.00	
ES	-1 <sup>a</sup>	0.33	0.70	-	0.30	0.32
	-1 <sup>b</sup>	0.67	0.30	1.00	0.70	
$h_1$		0.44	0.42	0.00	0.42	0.00
$h_{obs}$		0.00	0.00	0.00	0.00	
ES	-3 <sup>a</sup>	0.17	0.15	0.32	-	0.44
	-3 <sup>b</sup>	0.50	0.70	0.36	-	
$h_1$		0.33	0.15	0.32	1.00	0.49
$h_{obs}$		0.61	0.47	0.67	0.00	
Pp-A	-1 <sup>a</sup>	1.00	0.30	0.64	0.00	0.22
	-1 <sup>b</sup>	-	0.70	0.64	-	
$h_1$		0.00	0.42	0.46	0.00	0.00
$h_{obs}$		0.00	0.00	0.00	0.00	
HB	-1 <sup>a</sup>	1.00	0.30	1.00	0.40	0.23
	-1 <sup>b</sup>	-	0.70	-	0.60	
$h_1$		0.00	0.42	0.00	0.48	0.00
$h_{obs}$		0.00	0.00	0.00	0.00	

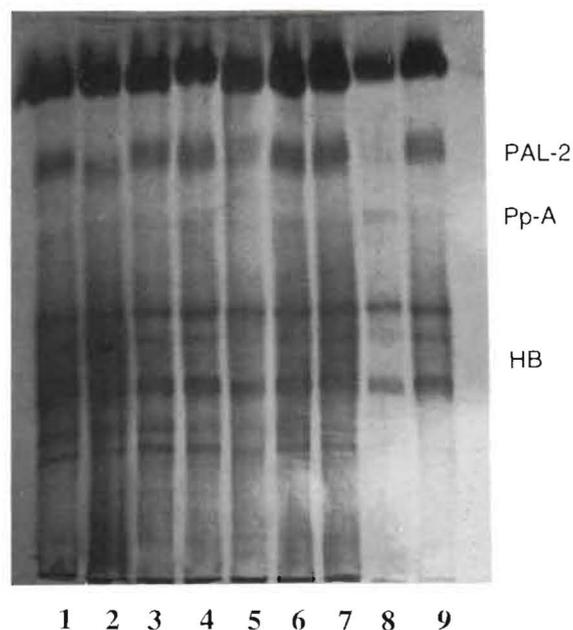
Trf. Serum (*Scincus scincus*)

Fig. 2. Variation in post - albumin-2, plasma protein - A and hemoglobin in serum. Sample designations are as follows: 1, 2 - El - Mansuriya; 3, 4, 5 - Senoris; 6, 7 = Ismailia; and 8, 9 = Tuna El-Gabal.

*Phosphoglucose Isomerase (Pgi):*

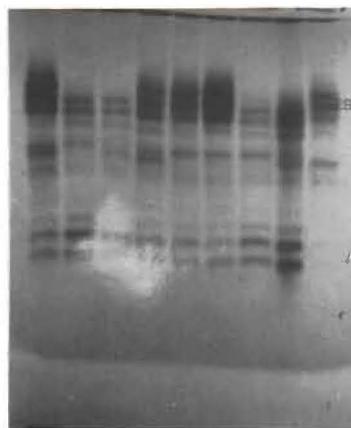
This locus has also two alleles; Pgi-1<sup>a</sup> and Pgi-1<sup>b</sup>. The distributions of these alleles among the four populations examined are closely similar to those present at Idh-1, as above.

*Esterases (ESs):*

Esterases were amongst the most variable proteins in this study. A total of seven separable zones of esterase activity were recognized on the polyacrylamide gels with  $\alpha$ -naphthyl acetate as substrate. Two of these zones, Es-1 and Es-3 are polymorphic and two, Es-5 and Es-7 are monomorphic in all populations. The others Es-2, Es-4 and Es-6 are not scored clearly in all populations and, therefore, are excluded from the analysis.

*Esterase-1 (Es-1):*

Two alleles were demonstrated for this esterase system (Fig. 3). Allele Es-1<sup>a</sup> is fixed in Ismailia only, while it has frequencies of 0.33 in El-Mansuriya, 0.70 in Senoris and 0.30 in Tuna El-Gabal. However, allele Es-1<sup>b</sup> is segregated at frequencies of 0.67, 0.30 and 0.70 in these latter localities.

**Es, Kidney (*Scincus scincus*)**

**Fig. 3.** Variation in esterase -1 in kidney extracts. Sample designations are: 1, 2, 3 = El-Mansuriya; 4, 7 = 4,7 = Senoris; 5, 6 - Ismailia; and 8,9 = Tuna El-Gabal.

1 2 3 4 5 6 7 8 9

*Esterase-3 (Es-3):*

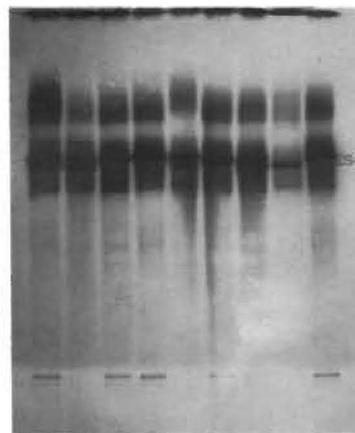
Three alleles were scored for this esterase system (Fig. 4). The three alleles are observed together in three of the four localities with frequencies shown in Table 1, while in the fourth locality (Tuna El-Gabal), allele Es-3<sup>c</sup> is fixed in all individuals.

*Plasma Protein-A (Pp-A):*

This type of plasma protein is a monomer, with two alleles (Fig. 2). Allele Pp-A<sup>a</sup> is fixed in El-Mansuriya and Tuna El-Gabal, but the two alleles, Pp-A<sup>a</sup> and Pp-A<sup>b</sup> have frequencies of 0.30 and 0.70, respectively, in individuals of Senoris and they have frequencies of 0.36 and 0.64 in Ismailia.

*Hemoglobin (Hb):*

Poymorphism in hemoglobin has been reported by Selander *et al.* (1971), Rasmussen *et al.* (1968). Foreman (1966) and by Ahl (1968), where they reported that two alleles are segregated at the Hb locus and encoded either the alpha or beta hemoglobin chain. Moreover, the configuration of the Hb molecule varied from two-

Esterases, Serum (*Scincus scincus*)

**Fig. 4.** Variation in esterase-3 in serum. Sample designations are: 1, 3, 6 - El-Mnsuriya; 2, 4 = Senoris; 5, 7, 9 = Ismailia; and 8 = Tuna El-Gabal.

to a single-banded pattern (Foreman 1968), however, Thompson *et al.* (1966) reported that a uniformly differing mobilities of single-banded hemoglobin pattern was recorded in *Peromyscus maniculatus* populations. This is consistent with the present findings here.

This hemolysate protein is a monomer among populations, with a wide darkly - stained band (Fig. 2). The Hb locus has two alleles, Hb-1<sup>a</sup> and Hb-1<sup>b</sup> which are observed with frequencies of 0.30 and 0.70, respectively, in Senoris and 0.40 and 0.60 in Tuna El-Gabal. However, allele Hb-1<sup>a</sup> is fixed in El-Mansuriya and Ismailia.

## 2. Monomorphic Proteins.

### *Xanthine Dehydrogenase (Xdh):*

This appeared as two bands, a cathodally - migrating wide darkly - stained band and an anodally - migrating faint secondary band, of constant mobility in all individuals of different localities.

### *6-Phosphogluconate Dehydrogenase (6-Pgd):*

This was scored in all populations as a single darkly - stained cathodally - migrating band.

### *Glucose-6-Phosphate Dehydrogenases (G6pd<sub>5</sub>):*

This enzyme was evident as two forms expressed in liver and kidney extracts and controlled by two genetic loci, G6pd-1 and G6pd-2. The two loci are

monomorphic in all populations. The G6pd-1 is represented by two bands, while G6pd-2 is represented by one band.

*Albumin (Al):*

This plasma protein is apparently monomorphic being observed as a single band in all samples.

*Transferrin (Trf):*

This plasma B-globulin appeared as two darkly stained bands in all populations.

*Erythrocytic Protein-A (Etp-A):*

This hemolysate protein is monomorphic for two bands over all populations.

*Glutamate Oxaloacetate Transaminases (Got<sub>s</sub>):*

Electrophoretic patterns of variation were observed for this enzyme in most tissues. This variation is interpreted as two genetic loci Got-1 and Got-2 encoding two isoenzymes. The Got-1, the supernatant form, is anodally migrating and is monomorphic, being represented by three bands. The mitochondrial form (Got-2) is cathodally - migrating and is uniformly monomorphic for one band in all populations.

*Leucine Aminopeptidases (Lap<sub>s</sub>):*

Three Lap systems controlled by three genetic loci, Lap-1, Lap-2 and Lap-3, are demonstrable together in all populations. Moreover, all the three loci are monomorphic in all samples. The Lap-1 locus is represented by two bands, while each of the others, Lap-2 and Lap-3, are formed of one band.

*Sorbitol Dehydrogenase (Sdh):*

One prominent system appears in extracts of liver, heart and kidney. Its phenotype is a two-banded pattern, which is visible against a dark background.

*Lactate Dehydrogenases (Ldh<sub>s</sub>):*

Three Ldh systems controlled by three loci are demonstrated in all populations. The three loci, Ldh-1, Ldh-2 and Ldh-3, are expressed in liver, heart and muscles extracts. With the exception of kidney and testis, wherein the two loci, Ldh-1 and Ldh-2, are represented. At the Ldh-1 locus, only two polypeptide subunits are shown in all populations. At the Ldh-2 locus, three polypeptide subunits are observed. However, only a single polypeptide subunit appeared at the Ldh-3 locus over all individuals. Also, it was found that there is no combination between the polypeptide subunits of Ldh-1 and Ldh-2.

*Alpha-Glycerophosphate Dehydrogenase ( $\alpha$ -Gpd):*

Only one enzyme form controlled by one genetic locus ( $\alpha$ -Gpd-1) is observed in all individuals and its molecule is apparently a monomer, being represented by a single band.

*Malate Dehydrogenases (Mdh<sub>s</sub>):*

Two forms of Mdh are demonstrated in all populations. One form is NAD-dependent and termed Mdh-1 and the other is NADP-dependent and designated Mdh-2. The Mdh-1 locus is monomorphic for two bands in all individuals, while the Mdh-2 locus is monomorphic for one band.

*Isocitrate Dehydrogenases (Idh-2):*

This mitochondrial form migrates anodally and is best demonstrated in heart extracts, while the supernatant form (IDH-1) migrates cathodally, on the same buffer, and is clearly shown in kidney and liver extracts. The Idh-2 locus produces a slow-migrating darkly stained band of constant mobility in all individuals.

*Aldolase (Ald):*

This enzyme is represented by a single cathodally - migrating band in all populations.

*Glycyl Leucine Peptidase (Glp):*

The phenotypes of Glp, as appeared in this study, undergo changes during staining procedures, hence they must be scored as quickly as possible. These changes are apparently the result of dissociation of the enzyme polymers due to enzymatic action. The phenotypes scored here are obtained after processing for several times. From these phenotypes, it is evident that the protein molecule is a dimer in all populations.

*Hexokinases (Hk<sub>s</sub>):*

Two isoenzymes encoded by two gene loci are demonstrated at this protein. The Hk-1 molecule is composed of two subunits, while that of Hk-2 is monomorphic for only one band in all populations.

*Phosphoglucomutases (Pgm<sub>s</sub>):*

The electrophoretic variation at the phosphoglucomutases is controlled by three genetic loci; Pgm-1, Pgm-2 and Pgm-3. The Pgm-1 and Pgm-3 are monomorphic for one band, while Pgm-2 is monomorphic for two bands in all individuals.

*Hexanol Dehydrogenase (Hdh):*

This enzyme appeared as a single band in all populations and it migrates cathodally under the conditions employed.

*Glutamate Dehydrogenase (Gdh):*

This monomorphic protein appeared in kidney extracts as a single band of constant mobility in all populations.

*Aconitase (Acon):*

This enzyme is apparently monomorphic, being represented by two bands in all samples examined. Because the bands are stained darkly and fade rapidly during fixation, the possibility of polymorphism for bands of closely similar mobility cannot be excluded.

*Fumarase (Fum):*

The configuration of this enzyme is apparently a monomer in all individuals, being represented by two bands.

*Esterase-5 (Es-5):*

This locus appeared prominently in extracts of liver and kidney extracts. Phenotypes of Es-5 show that the three-banded form is distributed in all populations.

*Esterase-7 (Es-7):*

This cathodally - migrating locus is monomorphic for two bands.

## Discussion

In terms of the 40 genetic loci controlling the 27 proteins studied in estimating the overall genetic variation among populations, only 7 loci or 17.5% were polymorphic. The considered mean proportions of polymorphic loci (P), number of alleles per locus (A), calculated heterozygosity per locus ( $H_i$ ) and observed heterozygosity ( $H_{obs}$ ) are listed in Table 2.

*a. Proportion of Polymorphic Loci:*

The general mean of polymorphic loci (P) among populations (7%) was comparatively moderate among those of the other species of family Scincidae (see

**Table 2.** Estimates of degree of genetic variation among natural populations based on analysis of 27 proteins controlled by 40 loci

Population	Number of individuals	Mean Proportion of				
		P	A	H <sub>1</sub>	H <sub>0bc</sub>	H <sub>i</sub>
El-Mansuriya	12	0.08	1.10	0.04	0.03	0.03
Senoris	10	0.10	1.13	0.04	0.01	0.03
Ismailia	11	0.05	1.08	0.03	0.02	0.03
Tuna El-Gabal	10	0.05	1.05	0.02	0.00	0.00
Mean based on pooled samples	43	0.07	1.09	0.03	0.02	0.02

further comparison, p. 196). However, a considerable range of variation was apparent among the four populations. The value of P was 5% at the populations of Ismailia and Tuna El-Gabal, while it was 8% at that of El-Mansuriya and 10% at Senoris population.

Moreover, a relative geographic variation in allelic frequencies at several loci was observed (Table 1). The populations shared in an average of 33.3% of all alleles and the percentage of polyallelic loci reached 14.3%. Among the four populations, the overall mean value of alleles number per locus (A) was 1.09 and it has a relatively wide range from 1.05 at Tuna El-Gabal to 1.13 at Senoris. In Ismailia and El-Amiriya populations, the values were 1.08 and 1.10, respectively.

Considering the deviation from Hardy-Weinberg equilibrium, only 5 of 7 polymorphic loci have more heterozygotes than expected, where  $X^2_{(1,3)}$ , ranged from 13.45 to 34.63, was significant at  $p < 0.005$ . Also, there was a significant interpopulation heterogeneity in genotypic frequencies at these 5 loci ( $\sum X^2_{(1,2,3,9)}$ ) ranged from 15.61 to 74.4;  $p < 0.005$ , Table 3.

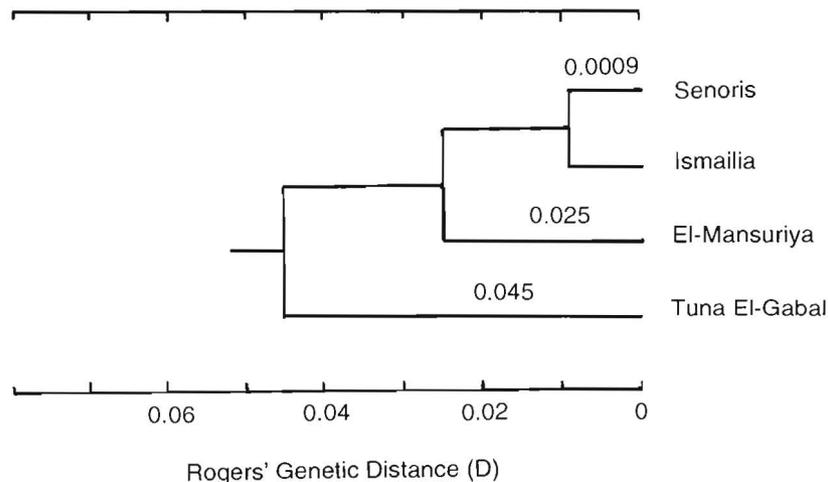
Additionally, the interpopulation heterogeneity in allelic frequencies was significant only at 6 loci, these were: PAI-2,  $X^2_{(3)} = 71.4$ ; Idh-1,  $X^2_{(3)} = 75.7$ ; Pgi,  $X^2_{(3)} = 75.7$ ; Es-1,  $X^2_{(3)} = 55.0$ ; Pp-A,  $X^2_{(3)} = 54.2$ ; Hb,  $X^2_{(3)} = 54.2$ , and  $p > 0.05$ . But at the 7<sup>th</sup> locus (Es-3), the heterogeneity was not significant at  $p < 0.995$  for  $X^2_{(3)} = 0.46$ .

*b. Genic Heterozygosity:*

As shown in Table 2, there was a relative difference between the calculated and observed heterozygosity, but the difference was statistically not significant at  $p < 0.5$ . The overall mean heterozygosity among populations was 3%; a value similar to that of *Chalcides ocellatus* (Soliman *et al.* 1994), lower than that of *Mabuya quinquetaeniata* (Gabri *et al.* 1994) and higher than that of *Chalcides sepsoides* (Shahin *et al.* in press) and *Eumeces schneideri* (Zahran *et al.* in publication). In comparing the values among the four populations: that of Tuna El-Gabal was relatively small (2%), moderate at Ismailia (3%) and slightly high at both El-Mansuriya and Senoris (4%).

*c. Phylogenetic Relationships:*

The means of genetic distance (D) and similarity (S) were 0.032 and 0.968, respectively. The matrices of these coefficients are given in Table 4. In view of the phenogram (Fig. 5), the populations of Senoris and Ismailia are genically the most alike (D = 0.009; S = 0.991). That of El-Mansuriya is more similar to the previous populations (D = 0.025; S = 0.975) than to that of Tuna El-Gabal, where D = 0.045; S = 0.955.



**Fig. 5.** Phenogram showing the genetic relationships among populations. Cophenetic correlation ( $r$ ) = 0.99.

**Table 3.** Interpopulation heterogeneity of genotypic proportions and Chi-Square test for 5 polymorphic loci deviating from Hardy-Weinberg equilibrium among populations

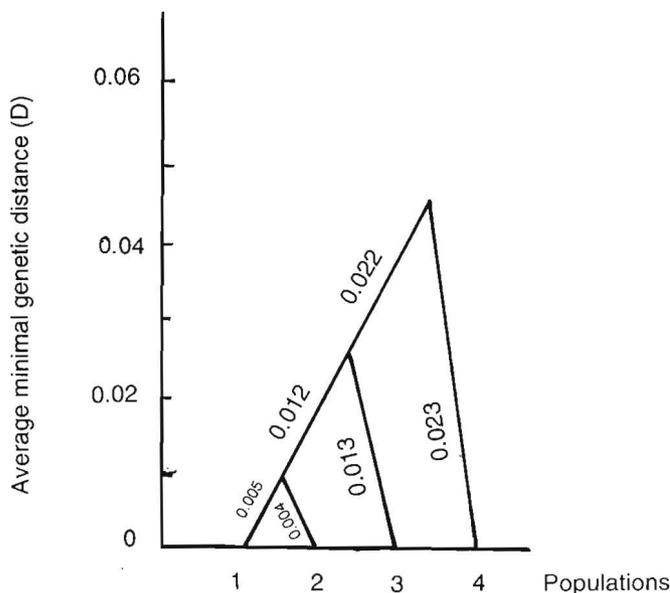
<b>1. Post-Albumin-2 (PAI-2)</b>						
<b>Genotype: Observed (expected)</b>						
				$X^2$ (d.f)	Sign.	
Population	No.	AI-1 <sup>a</sup> /2 <sup>a</sup>	PAL-2 <sup>b</sup> /2 <sup>b</sup>			
El-Mansuriya	12	4 (1.3)	8 (5.3)	15.61 (1)	p > 0.005	
				$\Sigma X^2_{(1)} = 15.61$	p > 0.005	
<b>2. Esterase-1 (ES-1)</b>						
<b>Genotype: Observed (expected)</b>						
				$X^2$ (d.f)	Sign.	
Population	No.	Es-1 <sup>a</sup> /1 <sup>a</sup>	Es-1 <sup>b</sup> /1 <sup>b</sup>			
El-Mansuriya	12	4 (1.3)	8 (5.3)	15.61 (1)	p > 0.005	
Senoris	10	7 (4.9)	3 (0.9)	24.46 (1)	p > 0.005	
T. El-Gabal	10	3 (0.9)	7 (4.9)	24.46 (1)	p > 0.005	
				$\Sigma X^2_{(3)} = 64.53$	p > 0.005	
<b>3. Esterase-3 (ES-3)</b>						
<b>Genotype: Observed (expected)</b>						
				$X^2$ (d.f)	Sign.	
Population	No.	Es-3 <sup>a</sup> /3 <sup>a</sup>	Es-3 <sup>b</sup> /3 <sup>b</sup>	Es-3 <sup>a</sup> /3 <sup>c</sup>		
El-Mansuriya	12	4 (2.0)	-	8 (1.3)	34.63 (3)	p > 0.005
Senoris	10	-	7 (4.9)	3 (0.5)	15.74 (3)	p > 0.005
Ismailia	11	-	4 (1.5)	7 (2.2)	24.03 (3)	p > 0.005
				$\Sigma X^2_{(9)} = 74.4$	p > 0.005	

<b>4. Plasma Protein-A (Pp-A)</b>					
<b>Genotype: Observed (expected)</b>					
				$\chi^2$ (d.f)	Sign.
Population	No.	Pp-A-1 <sup>a</sup> /1 <sup>a</sup>	Pp-A-1 <sup>b</sup> /1 <sup>b</sup>		
Senoris	10	3 (0.9)	7 (4.9)	24.46 (1)	p > 0.005
Ismailia	11	4 (1.5)	7 (4.5)	14.05 (1)	p > 0.005
				$\Sigma \chi^2_{(2)} = 38.51$	p > 0.005
<b>5. Hemoglobin-1 (HB-1)</b>					
<b>Genotype: Observed (expected)</b>					
				$\chi^2$ (d.f)	Sign.
Population	No.	Hb-1 <sup>a</sup> /1 <sup>a</sup>	Hb-1 <sup>b</sup> /1 <sup>b</sup>		
Senoris	10	3 (0.9)	7 (4.9)	24.46 (1)	p > 0.005
T. El-Gabal	10	4 (1.6)	6 (3.6)	13.45 (1)	p > 0.005
				$\Sigma \chi^2_{(2)} = 37.91$	p > 0.005

**Table 4.** Coefficients of Rogers' (1972) genetic distance (D) (above diagonal) and Rogers' (1971) genetic similarity (S) (below diagonal) for all paired populations

	<b>El-Mansuriya</b>	<b>Senoris</b>	<b>Ismailia</b>	<b>Tuna El-Gabal</b>
El-Mansuriya		0.027	0.023	0.0460
Senoris	0.973		0.009	0.042
Ismailia	0.977	0.991		0.0464
Tuna El-Gabal	0.9540	0.958	0.9536	

The phylogenetic relationship is further demonstrated in an unrooted tree (Fig. 6). As shown, the third branch which clusters the population of Tuna El-Gabal is the longest and consequently, is the most genically divergent. The second, the middle branch represents the population of El-Mansuriya and the first, the shortest branch gave off firstly that of Senoris and secondly, the population of Ismailia which is the nearest to the ancestor.



**Fig. 6.** Unrooted phylogenetic tree showing the relationships among populations. Average percent standard deviation (SD) = 0.033. Number designations are as follows: 1 = Senoris; 2 = Ismailia; 3 = El-Mansuriya; 4 = Tuna El-Gabal.

In summary, the natural populations of the sand fishskink in the sense of polymorphism and heterozygosity are among the genetically variable skins in Egypt. Comparable estimates of genic heterozygosity for other populations of several species of their continental lizards as well as those of other families occurring elsewhere are given in the following Table:

Family Genus	No. populations studied (No. species)	Range of heterozy- gosity	Mean heterozy- gosity (H)	Reference
<b>Lacertidae:</b>				
<i>Acanthodactylus</i>	6 (3)	0.00-0.14	0.11	Abd El-Megeid 1991
<i>Philochortus</i>	1 (1)	–	0.02	
<i>Eremias</i>	1 (1)	–	0.00	
<b>Mean</b>			<b>0.04</b>	
<i>Acanthodactylus</i>	6 (1)	0.14-0.25	0.19	Blanc and Cariou 1980
<b>Lacertidae:</b>				
<i>Acanthodactylus</i>	13 (1)	0.14-0.28	0.20	Blanc and Cariou 1987
<b>Amphisbaenidae:</b>				
<i>Bipes</i>	3 (3)	0.00-0.03	0.01	Kim <i>et al.</i> 1976
<b>Anniellidae:</b>				
<i>Anniella</i>	4 (2)	0.00-0.02	0.01	Bezy <i>et al.</i> 1977
<b>Crotaphytus:</b>				
<i>Uta</i>	17 (1)	0.00-0.10	0.05	McKinney <i>et al.</i> 1972
<i>Anolis</i>	3 (1)	0.04-0.06	0.05	Webster <i>et al.</i> 1972
<i>Sceloporus</i>	5 (2)	0.02-0.13	0.06	Hall and Selander 1973, Tinkle and Selander 1973
<b>Mean</b>			<b>0.04</b>	
<b>Lacertidae:</b>				
<i>Lacerta</i>	3 (1)	0.06-0.13	0.09	Gorman <i>et al.</i> 1975
<b>Teiidae:</b>				
<i>Cnemidophorus</i>	1 (1)	–	0.15	Gorman <i>et al.</i> 1977
<b>Scincidae:</b>				
<i>Chalcides ocellatus</i>	5 (1)	0.01-0.07	0.03	Soliman <i>et al.</i> 1994
<i>Chalcides sepsoides</i>	5 (1)	0.00-0.02	0.01	Shahin <i>et al.</i> 1994 *
<i>Eumeces schneideri</i>	2 (1)	0.00-0.02	0.01	Zahran <i>et al.</i> 1994*
<i>Mabuya quinquet</i>	5 (1)	0.04-0.07	0.06	Gabri <i>et al.</i> 1994
<i>Scincus scincus</i>	4 (1)	0.02-0.04	0.03	This study.

\* These papers are under publication.

Factors influencing the extent of genic variability among populations have been discussed by Ferguson (1980). The significant difference in allelic and, consequently, in genotypic frequencies could be due to selection for different homozygotes under varying environmental conditions (diversifying selection) or to genetic drift that increases homozygosity in geographically isolated populations (Selander *et al.* 1971). Moreover, under conditions of diversifying selection, heterozygotes may be produced by migration between populations, but such heterozygotes have a lower fitness than the homozygotes and this negative heterosis may promote speciation (Manwell and Baker 1970). In an outbreeding sexual population which is panmictic, mating choice was the affecting factor. Additionally, mutation introduces new variation into populations and brings about changes in allelic frequencies. However, the rate of mutation is sufficiently low to be neglected as a modifier of allelic frequencies. These five forces were causing populations to deviate significantly from Hardy-Weinberg equilibrium.

These factors among the populations of *Scincus scincus*, it could be concluded that factors of mating choice and genetic drift have a great effect in populations. Moreover, in those populations which showed a significant deficit in heterozygotes, selection at some loci (Christiansen 1977) or perhaps mating behaviors that promote local inbreeding (Porter and Geiger 1988) was the effective factor.

### Conclusions

Among *Scincus scincus* populations, only 7 loci (17.5%) of the 40 loci were polymorphic. The value of P ranged from 5 to 10%, with overall mean of 7% and that of H ranged from 2 to 4%, with general mean of 3%. The population of Senoris was the highest in level of polymorphism (P = 10%) and that of El-Mansuriya were the highest in heterozygosity (H = 4%). Those of Ismailia and Tuna El-Gabal were the lowest in level of polymorphism (P = 5%), while the latter only was the lowest in heterozygosity (H = 2%). The means of D and S were 0.032 and 0.968. The populations of Senoris and Ismailia are genically the most similar (d = 0.009; S = 0.991). The population of Ismailia is phylogenetically the nearest to the ancestor, while that of Tuna El-Gabal is the most divergent.

Significant geographic heterogeneity in allelic and genotypic frequencies was observed at several polymorphic loci over all the conspecific populations. The causal factors for this heterogeneity appear to be the genetic drift, diversifying selection and mating behaviors.

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## التغير البروتيني وعلاقته بالتقسيم في بعض أجناس فصيلة السقنقوريات (الزواحف) في مصر

التباين البروتيني وعدم التجانس الوراثي بين المجتمعات الطبيعية لجنس  
السقنقور من نوع سقنقور سمك الرمل (*Scincus Scincus L.*)

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قام هذا البحث بدراسة التغير الوراثي بين المجتمعات الطبيعية لجنس السقنقور من نوع سقنقور سمك الرمل (*Scincus Scincus L.*) من خلال دراسة نتائج التحليل الكهربائي لعدد ٢٧ من الأنزيمات والبروتينات المرتبطة بعدد ٤٠ شفرة جينية . وقد وجد أن عدد ٧ شفرات منها فقط (أو ٥, ١٧٪) غير متجانس (بوليمورفيك) بين الأربع مجتمعات التي شملتها الدراسة . وفي ضوء عدم التجانس (الهتيروزيجوستي H) وتعدد التشكيل (البوليمورفيزم P) فقد انفردت مجتمعات سقنقور سمك الرمل بنسب متوسطة بمقارنتها بمجتمعات الأجناس الأخرى من نفس فصيلة السقنقوريات . حيث وجد أن متوسط نسب عدم التجانس (الهتيروزيجوستي H) وتعدد التشكيل (البوليمورفيزم P) هي ٣٪، ٧٪ على الترتيب . وقد أوضحت مجتمعات منطقة الاسماعيلية وتونة الجبل بالمنيا المستوى الأقل في التغير الوراثي (بلغت نسبة تعدد التشكيل ٥٪) ، في حين أن المنطقة الأخيرة

قد أوضحت أقل نسبة في عدم التجانس ٢٪ . هذا وقد أبرزت مجتمعات منطقة سنورس بالفيوم المستوى الأعلى في تعدد التشكل حيث بلغت النسبة ١٠٪ ، وقد أظهرت مجتمعات منطقة المنصورية بالجيزة النسبة الأعلى في عدم التجانس (٤٪) . وقد بلغ متوسط قيم ثوابت التباعد الوراثي (D) والتشابه الوراثي (S) بين المجتمعات ٠,٠٣٢ و ٠,٩٦٨ ، على الترتيب . ومن الناحية التطورية فقد وجد أن مجتمعات منطقة الاسماعيلية هي الأكثر قربا من سلف السقنقور ، بينما وجد أن مجتمعات منطقة تونة الجبل بالمنيا هي الأكثر بعدا من السلف . هذا وقد وجد تباين ملحوظ في التكرار الوراثي والمظهري في غالبية الشفرات الجينية المرتبطة بالانزيمات والبروتينات بين الشعوب ، وقد أرجع ذلك إلى عدة أسباب منها : الانتخاب الطبيعي للأفراد المتجانسة تحت ظروف بيئية مختلفة (أي الانتخاب المتباين) مع أن هذا يؤدي أحيانا إلى ظهور أفراد غير متجانسة عن طريق الهجرة ولكنها لا يكون لها القدرة على الاستمرار وهو ما يسمى بعدم التجانس السلبي الذي ينشط عملية ظهور أنواع جديدة ، أو ربما إلى التراكم الوراثي الذي يزيد نسبة التجانس بين الأفراد المنعزلة جغرافيا ، أو إلى الاختلاف في درجات التكاثر وفرص التزاوج بين الأفراد خصوصا في الشعوب ذات التكاثر الخلطي .