Belg. J. Zool., 133 (2) : 191-193 July 2003

Preliminary data on the genetic differentiation of populations of three frog species (Anura, Amphibia) from Cyprus and Greece

Pasqualina Kyriakopoulou-Sklavounou, George Xeros, Charis Charilaou and Anna Tsiora

Department of Zoology, Aristotle University of Thessaloniki, 540 06 Thessaloniki, Greece

Corresponding author : P. Kyriakopoulou-Sklavounou, e-mail : kyriakop@bio.auth.gr

Electrophoretic analysis was carried out on populations of *Rana ridibunda*, *Hyla savignyi* and *Bufo viridis* of Cyprus and compared with data from populations from Greece in an attempt to study their genetic variation. Enzymes and other proteins were separated using standard starch and polyacrylamide gel electrophoresis. Electrophoretic conditions were as described in our previous studies (1) (2) (3) and data analysed using the BIOSYS-1 computer package (4). Levene's (5) correction for small sample size was employed in chi-square analyses. The phylogenetic structure of the population was analysed with CONTML (PHYLIP 3.57c package) (6) based on allele frequencies. The loci, alleles and allele frequencies, degree of heterozygosity (H) and proportion of polymorphic loci (P) found in all Cypriot and Greek populations are shown in Table 1 (in appendix).

***Rana ridibunda* :** The eight enzyme and two protein systems investigated encode fifteen presumptive genetic loci. Many polymorphic loci had genotype frequencies not in good agreement with Hardy-Weinberg expectations (chi-square, $P < 0.05$). This may reflect mixing of different populations or sampling error. *R. ridibunda* populations from Greece and Cyprus differ mainly in two loci, LDH-2 and MProt. The phylogenetic analysis (Fig.1) showed two separate groups. *Rana epeirotica* (7) and *Rana cretensis* (8) form one group and *R. ridibunda* and *Rana balcanica* (9) the other one. The Cypriot *R. ridibunda* is closest to the Greek *R. balcanica.*

This tree is in agreement with a UPGMA tree constructed based on genetic identity values (not shown). From the allelic frequencies at the 15 loci tested, we calculated Nei's (10) values of genetic identity (I) and genetic distance (D). The values of D (0.202 and 0.223 respectively), found between both Cypriot populations and the Greek *R. ridibunda*, indicate that these populations are separated. On the other hand, the values of D (0.022 - 0.034, mean=0.028) between the Cypriot *R. ridibunda* and the Greek *R. balcanica* indicate that these populations are probably conspecifics. Eventually, the values of D between Cypriot *R. ridibunda* and both other species, *R. epeirotica* (D=1.152*)* and *R. cretensis* (D=1.023), demonstrate a higher differentiation. From all the above data we suggest that the water frogs of Cyprus are related to the Greek *R. balcanica*. The high degree of polymorphism found only in the Evros population has also been reported in other studies (1) (11). This region probably represents a hybrid zone between the Greek *R. ridibunda* and *Rana bedriagae* of Anatolia, and gene flow occurs between them. Further studies including populations of *R. bedriagae* from Anatolia would provide more accurate data for a more final conclusion.

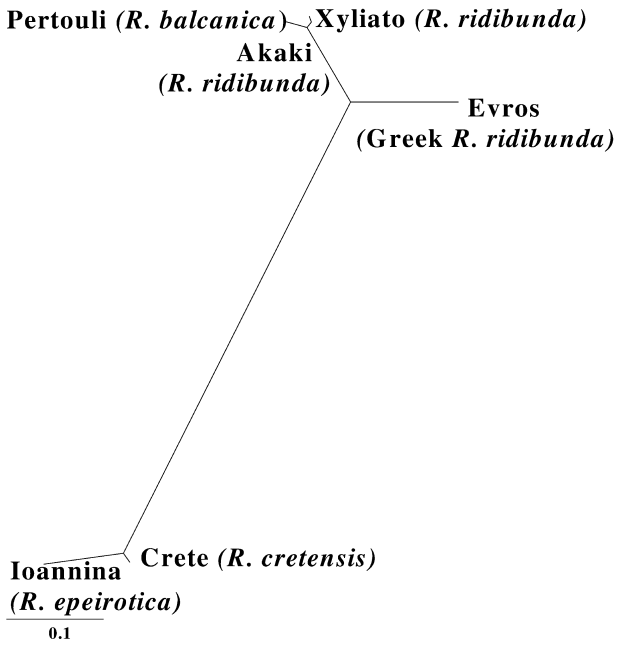

Fig. 1. – Maximum likelihood tree (CONTML, Felsenstein 1993) based on the allele frequencies of each population of water

***Hyla savignyi* :** The five enzyme and two protein systems investigated encode ten presumptive genetic loci. Most polymorphic loci had genotype frequencies in good agreement with Hardy-Weinberg expectations (chi-square, $P > 0.05$). Information on genetic structure of *H. savignyi* is very limited. A lower average heterozygosity (0.088) has been reported for *H. arborea savignyi* in Israel (12). The values of genetic identity (I) between the two populations of *H. savignyi* from Cyprus were 0.992 and between *H. savignyi* and Greek *H. arborea* 0.698 while values of D were 0.008 and 0.366 respectively. These results indicate that populations of *H. savignyi* of Askas and Sotira are the most closely related and differ substantially from the population of *H. arborea* from Greece. The high degree of genetic identity is typical in island populations (13).

***Bufo viridis* :** The five enzyme and two protein systems investigated encode twelve presumptive genetic loci. Populations of *B. viridis* from Greece and Cyprus mostly

differ in two loci (ALB-1 and Hb-1). Most polymorphic loci had genotype frequencies in good agreement with Hardy-Weinberg expectations (chi-square, $P > 0.05$). Values of D and I between the two studied populations were 0.740 and 0.301 respectively. All genetic parameters showed that the population of *B. viridis* from Cyprus is more polymorphic than the Greek one. The results of the present study clearly showed that the populations of *B. viridis* from Cyprus and Greece are greatly differentiated.

Concluding this paper we have to underline that the studied frog species of Cyprus are genetically differentiated from the Greek ones. However, our data are preliminary and more detailed studies using additional methods, are needed, in order to elucidate the taxonomic status of these species and to give a global approach.

ACKNOWLEDGEMENTS

We are grateful to Dr. P. Lymberakis and to Mr. P. Maltopoulos for providing us with specimens from Crete and Evros. We also thank Dr.A.Triantafyllidis for his valuable help in data analysis.

REFERENCES

1. Kyriakopoulou-Sklavounou, P., P. Sogren & H. Jansson (1991). Discordant genetic and morphological differentiation between three *Rana ridibunda* populations from northern Greece. *Hereditas*, 115 : 51-61.
2. Kyriakopoulou-Sklavounou, P., Y. Karakousis & B. Alexiou (1992). A morphometric and electrophoretic study of two populations of *Hyla arborea* L. in Greece. *Comp. Biochem. Physiol*., 103B : 715-719.
3. Karakousis, Y.& P. Kyriakopoulou-Sklavounou (1995). Genetic and morphological differentation among populations of the green toad *Bufo viridis* from northern Greece. *Biochem. Syst. Ecol*., 23 : 39-45.
4. Swofford, D.L.& R.K. Selander (1989). BIOSYS-1, *a Computer Program for the Analysis of Allelic Variation in Population Genetics and Biochemical Systematics.* Release 1.7. Illinois *Natural Hist.* Survey, Champaign, Illinois.
5. Levene, H. (1949). On a matching problem arising in genetics.*Ann.Mathem.Stat*. 20 : 91-94.
6. Felsenstein, J. (1993). PHYLIP (Phylogeny Inference Package) version 3.57c. Computer program and manual distributed by the author. Department of Genetics, University of Washington Seattle.
7. Schneider, H., T.S. Sofianidou & P. Kyriakopoulou-Sklavounou (1984). Bioacoustic and morphometric studies of water frogs (genus Rana) of lake Ioannina in Greece, and description of a new species (Anura, Amphibia). *Z. zool. Syst. Evolutforsch*., 22 : 349-366.
8. Beerli, P., H Hotz & T. Uzzell (1994). Two new water frog species from the Aegian Islands Crete and Karpathos (Amphibia, Salientia, Ranidae). *Notulae Naturae. Acad. Nat. Sci., Philadelphia*, 470 : 1-9.
9. Schneider, H., U. Sinsch & T. S. Sofianidou (1993). The water frogs of Greece : Bioacoustic evidence for a new species. *Z. zool. Syst. Evolutforsch*., 31 : 47-63.
10. Nei, M. (1972). Genetic distance between populations. *Am. Nat*., 106 : 283-292.
11. Beerli, P., H. Hotz & T. Uzzell (1996). Geologically dated sea barriers calibrate a protein clock for Aegean water frogs. *Evolution*, 50 : 1676-1687.
12. Nevo, E. & S.Y. Yang (1979). Genetic diversity and climatic determinants of tree frogs in Israel. *Oecologia*, 41 : 47-63.
13. Gorman, G. C., Soule, M., S. Yang & E. Nevo (1974). Evolutionary genetics of insular Adriatic lizards. *Evolution* 29 **:**52..

Received: November 25, 2001
Accepted after revision: April 24, 2003

APPENDIX : TABLE 1

Loci, alleles and allele frequencies, degree of heterozygosity (H), proportion of polymorphic loci (P) mean alleles per locus (M.N.A) found in frog species from Cyprus and Greece

Taxon		***R.ridibunda***		***R. balcanica***	***R. ridibunda***	***R. epeirotica***	***R. cretensis***	***H. savignyi***		***H. arborea***	***B. viridis***	
		Cyprus		**Greece**				**Cyprus**		**Greece**	**Cyprus**	**Greece**
Locality		**Xyliato**	**Akaki**	**Pertouli**	**Evros**	**Ioanninaa**	**Cretea**	**Askas**	**Sotira**	**Komotini**	**Ag. Dometios**	**Evros**
n		**25**	**7**	**6**	**10**	**5**	**4**	**9**	**34**	**9**	**8**	**5**
Locus	Allele											
AAT-1	A	1.00	1.00	1.00	1.00	1.00	1.00	0.88	0.76	1.00	1.00	1.00
	B	-	-	-	-	-	-	0.11	0.23	-	-	-
AAT-2	A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
ADH-1	A	1.00	1.00	1.00	0.40	-	-	-	-	-	-	-
	B	-	-	-	-	1.00	1.00	-	-	-	-	-
	C	-	-	-	0.30	-	-	-	-	-	-	
	D	-	-	-	0.20	-	-	-	-	-	-	-
	E	-	-	-	0.10	-	-	-	-	-	-	-
CK-1	A	1.00	1.00	1.00	0.40	-	-	1.00	1.00	1.00	1.00	1.00
	B	-	-	-	-	1.00	1.00	-	-	-	-	-
	C	-	-	-	0.60	-	-	-	-	-	-	-
EST-1	A	0.50	0.50	0.50	-	-	-	-	0.10	0.11	0.75	0.30
	B	0.50	0.50	0.50	-	-	-	0.27	-	0.33	0.25	0.70
	C	-	-	-	0.05	-	-	0.72	0.80	0.22	-	-
	D	-	-	-	0.80	-	-	-	0.08	0.33	-	-
	E	-	-	-	0.10	-	-	-	-	-	-	-
	F	-	-	-	0.05	-	-	-	-		-	-
EST-5	A	1.00	1.00	1.00	0.80	-	-	-	-	1.00	0.56	0.20
	B	-	-	-	0.20	-	-	1.00	1.00	-	0.44	-
	C	-	-	-	-	-	-	-	-	-	-	0.80
GPD-1	A	1.00	1.00	1.00	0.40	-	-	-	-	-	-	-
	B	-	-	-	-	1.00	1.00	-	-	-	-	-
	C	-	-	-	0.60	-	-					
LDH-1	A	1.00	1.00	1.00	0.40	-	-	0.16	0.25	0.50	0.31	-
	B	-	-	-	-	1.00	1.00	0.83	0.75	0.50	-	0.50
	C	-	-	-	0.30	-	-	-	-	-	0.69	0.50
	D	-	-	-	0.20	-	-	-	-	-	-	-
	E	-	-	-	0.10	-	-	-	-	-	-	-
LDH-2	A	0.64	0.93	1.00	1.00	1.00	1.00	-	-	-	1.00	1.00
	B	0.36	0.07	-	-	-	-	-	-	-	-	-
MDH-1	A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.69	-
	B	-	-	-	-	-	-	-	-	-	-	0.50
	C	-	-	-	-	-	-	-	-	-	0.31	0.50
MDH-2	A	1.00	1.00	1.00	1.00	-	-	-	-	0.61	0.44	0.20
	B	-	-	-	-	1.00	1.00	1.00	1.00	0.39	0.56	0.80
PGM-1	A	1.00	1.00	1.00	0.40	-	-	-	-	-	-	-
	B	-	-	-	-	1.00	1.00	-	-	-	-	-
	C	-	-	-	0.60	-	-	-	-	-	-	-
PGM-2	A	1.00	1.00	1.00	0.40	0.00	0.00	-	-	-	-	-
	B	-	-	-	-	1.00	1.00	-	-	-	-	-
	C	-	-	-	0.60	-	-	-	-	-	-	-
Hb-1	A	0.50	0.50	0.50	0.50	-	-	-	-	0.50	0.50	-
	B	0.50	0.50	0.50	0.50	-	-	1.00	1.00	0.50	0.50	1.00
ALB-1	A	-	-	-	-	-	-	-	-	0.50	0.44	-
	B	-	-	-	-	-	-	0.83	0.80	-	0.56	-
	C	-	-	-	-	-	-	0.80	0.19	0.50	-	1.00
ALB-2	A	-	-	-	-	-	-	-	-	-	0.94	0.70
	B	-	-	-	-	-	-	-	-	-	0.06	0.30
MProt	A	1.00	1.00	0.50	1.00	-	0.50	-	-	-	-	-
	B	-	-	0.50	-	-	0.50	-	-	-	-	-
	C	-	-	-	-	0.50	-	-	-	-	-	-
	D	-	-	-	-	0.50	-	-	-	-	-	-
		-	-	-	-	-	-	-	-	-	-	-
H		0.09	0.08	0.11	0.31	0.04	0.04	0.12	0.13	0.28	0.34	0.27
P		20.0	20.0	20.0	60.0	8.33	8.33	40	40	50	75.00	58.33
M.N.A.		1.20	1.20	1.20	2.07	1.08	1.08	1.40	1.50	1.70	1.75	1.58