A preliminary biochemical genetic survey of four South African painted reed frog (*Hyperolius marmoratus*) populations

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Allozyme electrophoresis was used to evaluate genetic variability in painted reed frog *(Hyperolius marmoratus)* populations from the Northern Province and the Eastern Cape. Sixteen protein-encoding loci were resolved, of which seven displayed allelic polymorphism. Average heterozygosity (**H**) values in two permanent *H. m. taeniatus* populations from the Northern Province (7.9% and 6.3%) did not differ substantially from each other nor from the geographically distant Eastern Cape *H. m. verrucosus* (9.1%). An isolated Northern Province *H. m. taeniatus* population displayed more polymorphism than any other group with **H**=14.1%, which can probably be attributed to the periodic influx of reed frogs following dry periods. Genetic distances and gene diversity (F_{ST}) values conformed with expected values for conspecific populations. The results attained concur with the hypothesis of increased genetic heterogeneity among populations of small and relatively sedentary animals.

Painted reed frogs (*Hyperolius marmoratus taeniatus*) are comparatively widely distributed in the Eastern half of the Northern Province, South Africa. However, since large parts of the region can hardly be considered optimum reed frog habitat (water plants around permanent water bodies), the true nature of the distribution most likely comprise local islands in suitable habitat. There is widespread acceptance among evolutionary biologists of the hypothesis that such a distribution pattern can result in genetic divergence between populations. Numerous studies have demonstrated significant genetic heterogeneity between conspecific populations over relatively short geographic distances, even in large mammals with high motility. The question arises to what extent genetic dispersal is possible across the wider distribution area of *H. marmoratus*, considering the lower level of motility of reed frogs compared to large mammals. In this regard Ward, Woodwark & Skibinski (1994) suggested that amphibia show the highest degree of inter-population differentiation among vertebrates due to their relatively sedentary lifestyle. However, Hess *et al.* (1995) found identical rDNA restriction site maps for individuals from different localities within the distribution ranges for *H. m. verrucosus*, *H. m. broadleyi* and *H. m. angolensis*, which is somewhat contradictory to the prediction by Ward *et al.* (1994). How far can reed frogs migrate and maintain gene-flow when faced by barriers of unsuitable habitat and separated by considerable geographical distances? Apart from genetic divergence, it would also be informative to compare genetic diversity levels in temporary reed frog populations resultant from seasonal influxes with that in populations around more permanent water bodies. The objectives of the present study were thus (1) to determine genetic distances among reed frog populations with varying degrees of geographical separation and diverse life histories; and (2) to compare genetic diversities in such populations.

We sampled three *H. m. taeniatus* populations from the Northern Province. The first two groups originated from dams in the Hans Merensky Nature Reserve and in the Magoebaskloof area. Both of these localities are surrounded by large areas of natural habitat and host relatively permanent populations. A third population was collected from a seasonal pond within Pietersburg, completely surrounded by residential suburbs and industrial areas. A population of *H. m. verrucosus* from the Grahamstown botanical garden (Eastern Cape) was sampled as a reference group to provide a yardstick of genetic distances between sub-species or more widely separated reed frog populations. Sample sizes attainable from each population are presented in Table 1. Tissues samples were taken from the liver, heart and hind limb (skeletal muscle). Sample preparation, gel and buffer composition, electrophoretic separation of gene products and staining followed standard methods, as summarised in Murphy, Sites, Buth & Haufler (1990). Genetic interpretation of gels and nomenclature followed Murphy *et al.* (1990) and Shaklee, Allendorf, Morizot & Whitt (1990). We calculated average heterozygosity (**H**, Nei 1975), proportion of polymorphic loci (**PPL**) and number of alleles per locus (**A**) in each population, as well as Wright's (1978) F-statistic for gene diversity within and between populations (F_{ST}). Genetic distances (**D**) among populations was determined with the widely used coefficient method of Nei (1972).

Proteins stained for (with loci resolved) were creatine kinase (E.C. 2.7.3.2, **CK-1**); general (unspecified) proteins (**PRT-1,-2,-3,-4**); glucose-6-phosphate isomerase (E.C. 5.3.1.9, **GPI-1**); glycerol-3-phosphate dehydrogenase (E.C. 1.1.1.8 **GPD-1**); guanine deaminase (E.C. 3.5.4.3, **GDA-1,2**); hexokinase (E.C. 2.7.1.1, **HK-1**); isocitrate dehydrogenase (E.C. 1.1.1.42, **IDH-1**); $_L$-lactate dehydrogenase (E.C. 1.1.1.27, LDH-1,-2); malic enzyme (E.C. 1.1.1.40, **ME-1**); phosphoglucomutase (E.C. 2.7.5.1, **PGM-1**) and superoxide dismutase (E.C. 1.15.1.1, **SOD-1**). Seven out of the 16 loci resolved displaying allelic polymorphism. Polymorphic loci with diversity coefficients (**H**, **A** and **PPL**) are listed in Table 1. More gene diversity was observed within populations than between populations, with F_{ST}=0.187. Genetic distances (Nei 1972) among populations are presented in Table 2.

Table 1 Sample size, polymorphic loci, alleles resolved (with relative mobilities) and genetic diversity coefficients in four *H. marmoratus* populations

Population (with sample size):		Hans Merensky	Magoebas-kloof	Pieters-burg	Grahams-town
Locus:	Allele:	n=12	n=20	n=8	n=20
GPI-1	183	0.05	–	–	0.03
	100	0.68	0.48	0.62	0.86
	0	0.28	0.52	0.38	0.10
		h=46%	h=50%	h=47%	h=25%
PGM-1	100	1.00	1.00	0.94	0.85
	85	–	–	0.06	0.15
				h=11%	h=26%
LDH-2	150	0.05	–	–	–
	100	0.75	1.00	0.94	1.00
	50	0.25	–	0.06	–
		h=40%		h=11%	
CK-1	100	1.00	1.00	1.00	0.73
	63	–	–	–	0.27
					h=39%
HK-1	100	1.00	1.00	1.00	0.70
	57	–	–	–	0.30
					h=39%
GPD-1	100	1.00	0.95	0.31	0.50
	78	–	0.06	0.69	0.50
			h=10%	h=43%	h=50%
PRT-2	120	–	0.60	0.81	0.48
	100	1.00	0.40	0.19	0.52
			h=48%	h=31%	h=50%
A		1.3	1.2	1.3	1.4
PPL		12.5%	18.8%	31.3%	37.5%
H		7.9%	6.3%	14.1%	9.1%

Table 2 Genetic distances (Nei 1972) among four painted reed frog populations

	Hans Merensky	Magoebaskloof	Pietersburg
Magoebaskloof	0.019	–	–
Pietersburg	0.038	0.034	–
Grahamstown	0.056	0.040	0.027

Genetic diversity in reedfrog populations from diverse environments

The two stable populations from the Northern Province, Magoebaskloof and Hans Merensky, possess heterozygosity levels of comparable magnitude at 6.3% and 7.9%. *Hyperolius marmoratus verrucosus* from the Eastern Cape displayed a slightly higher level of heterozygosity at 9.1% and has more polymorphic loci and alleles than any of the *H. m. taeniatus* populations – Table 1. The level of diversity in the comparatively isolated Pietersburg reed frog population is almost twice as high compared to the other *H. m. taeniatus* populations from the Northern Province, with **H** of 14.1% *versus* 6.3–7.9%. This trend is confirmed by the **PPL** values for the respective populations (with **PPL**=31.3% as opposed to 12.5–18.8%). Although such a favourable level of genetic diversity is initially contradictory to the isolated state of the population, it can probably be accounted for by seasonal events. Since this is not a permanent habitat, periodic drying of the dam could lead to death or some migration of frogs from the area followed by an inflow after summer rain. This model is supported by the distribution of polymorphic loci among populations from the Northern Province (Table 1). The Hans Merensky and Magoebaskloof populations display allelic polymorphism at either the **LDH-2** or **GPD-1/PRT-2** loci,

whereas the Pietersburg group is polymorphic for all three. This could suggest a measure of pooling of diversity originating from separate and more limited gene-pools into the Pietersburg reed frog population. The high genetic heterogeneity in this population should then be seen as artificial and probably not representative for more stable *H. marmoratus* populations.

Overall, the **H** values in the reed frog populations sampled (6.3–14.1%) are relatively high compared to levels recorded in physically larger species, for example average **H**=3.59% in large mammals (Nevo 1978). This is in accordance with the hypothesis of Selander & Kaufman (1973) that smaller animals perceive their environment as more course grained compared to larger ones and theoretically maintain higher levels of genetic variability as a result of varying selection pressures in different microhabitats. An even higher **H** value of 18.4% has been reported by Tawfik, Akef & Abdel-Mageid (1994) in the genus *Rana*. According to the latter authors, lack of heterozygosity in frogs may result in narrow habitat tolerance. Richards & Moore (1996) commented that evidence exists among hyperoliids of special adaptions for living in dry climates, such as increased resistance to dessication compared to other amphibians. The favourable genetic heterogeneity levels suggested by the results of the present study should therefore provide reed frogs with the ability to adapt to the array of environmental conditions from coastal regions (Eastern Cape) to the Lowveld (Northern Province) included in the present study.

Geographical differences

In contrast to results reported for *H. m. verrucosus*, *H. m. broadleyi* and *H. m. angolensis* (Hess *et al.* 1995), the current study showed substantial variation among *H. m. taeniatus* populations. Closest identity was found between the two stable *H. m. taeniatus* groups from Hans Merensky and Magoebaskloof, with **D**=0.019. Nevertheless, the Hans Merensky population lacks alleles present at the **GPD-1** and **PRT-2** loci in Magoebaskloof, while the latter lacks two **LDH-2** alleles compared to the Hans Merensky group. This suggests that gene flow is not maintained over large distances between the Northern Province *H. m. taeniatus* populations. Genetic distance between these two populations and the Eastern Cape subspecies is well correlated with the increased taxonomic and geographical distance, with **D**=0.04–0.056. Genetic distances between the Pietersburg group and the remaining populations are less informative. Distance between the Pietersburg frogs and the remaining *H. m. taeniatus* populations is 0.034–0.038 but, surprisingly, the Pietersburg group shows slightly more identity with *H. m. verrucosus* with a **D** value of only 0.027. Without sampling intermediate or transitional populations, it is not possible to assert whether this anomaly is due to any real geographical differences within the Northern Province or reflect the life-history of the Pietersburg reed frog population as described before. However, the physical distances involved and the known taxonomy of *H. marmoratus* suggest that the latter mechanism is more acceptable.

The magnitude of the **D** values calculated between reed frog populations (0.019–0.056) are relatively large and congruent with high **D** values of 0.016–0.194 reported for conspecific frog populations by Formas (1993). Although still within the expected range for conspecific populations (Thorpe 1982), such distances are essentially an order of magnitude higher than typical values calculated for conspecific ungulate populations (for example 0.0015–0.0088 by Grobler & Van der Bank 1994). This would confirm the hypothesis that genetic divergence in small and less motile animals would be more rapid compared to that in larger animals. Nevertheless, the F_{ST} value obtained suggests that most diversity is still resident within rather than between *H. marmoratus* populations, as expected for conspecific populations.

The results presented provide new data on the distribution of genetic diversity in a small southern African anuran. A question that remains to be solved is the scale on which geographical allozymic differences between reed frog populations are found. This can best be studied by sampling populations intermediate in their distribution between, for example, the Northern Province localities surveyed, or even on a microgeographical level by sampling several populations within the Pietersburg area. A priority during any follow-up study should also be to screen a greater number of loci.

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