Indeed, 1 band of intermediate mobility, which is absent in the fresh sample, appears in the pattern of the samples stored at −20 °C and frozen and thawed. The pattern of the sample stored at −80 °C is indistinguishable from the pattern produced by the fresh ones.

Another enzyme, glucose-6-phosphate dehydrogenase (G6PD), which is monomorphic using the method[6] we have employed for its analysis has been studied in blood samples. In this case the sample showed the same electrophoretic pattern regardless of the storage conditions. Such a result suggests that in the PGM isozymic bands array, the slower isozyme is the primary form to be synthesized while the others derive from this form through secondary structural changes occurring during the sample ageing.

Our results on PGM show that if samples from different individuals are stored in different ways, such as those described above, and then compared by starch gel electrophoresis, a pattern is obtained that mimics a true genetic polymorphism. This effect is due to the appearance of a new sharp intermediate migrating band in the frozen and thawed homogenates that, together with the band given by the fresh preparation and still active, produces a phenotypic pattern which closely resembles the pattern one could expect from an heterozygote (figure 2).

Our observations indicate that some care should be used in the analysis of genetic polymorphisms, a topic which is at present acquiring increasing importance both in evolutionary and in population studies[7,8].

1 R. Scozzari, L. Ulizzi, A. Ferracin and M. Lunadei, Atti Ass. genet. ital., in press (1980).
2 R.K. Selander, in: Molecular Evolution, p.21. Ed. F.J. Ayala. Sinauer, Sunderland, Mass. 1976.
3 G.J. Brewer, An Introduction to isozyme Techniques. Academic Press, New York 1970.
4 N. Spencer, D.A. Hopkinson and H. Harris, Nature *204*, 742 (1964).
5 D. Hedgecock, Biochem. Gen. *14*, 561 (1976).
6 Tech. Rep. Ser. Wld. Hlth Org. No.366 (1967).
7 T. Dobzhansky, F.J. Ayala, G.L. Stebbins and J.W. Valentine, Evolution. Freeman, San Francisco 1975.
8 F.J. Ayala, ed. Molecular Evolution. Sinauer, Sunderland, Mass. 1976.

Karyotypes and nuclear DNA contents of Polypteridae (Osteichthyes)[1]

A. Vervoort

Institut Agronomique et Vétérinaire Hassan II (APESA), Département de Biologie Animale, 6, rue Mohammed Triki, Rabat-Agdal (Morocco), 26 July 1979

Summary. Calamoichthys calabaricus, Polypterus palmas, P. weeksii, P. delhezi and *P. ornatipinnis* have the same amount of DNA per erythrocyte nucleus. The karyotype of *P. weeksii* has 38 chromosomes and differs from the karyotypes of the other species, all with 36 chromosomes, by a Robertsonian chromosomal rearrangement. The karyotype condition is regarded as derived for vertebrates.

In recent work, *Polypterus* and *Calamoichthys* have been placed either in the Actinopterygii as derived chondrosteans[2,3] or outside the actinopterygians in a group of their own, the Brachiopterygii[4,5]. Papers on karyotypes[6-9] and DNA contents[10,11] of Polypteridae stressed the differences from actinopterygians - chondrosteans in particular - and noted some resemblances with Dipnoi and Amphibia.

The Polypteridae exhibit remarkable chromosomal homogeneity; the 6 species reported *(C. calabaricus, P. palmas, P. delhezi, P. ornatipinnis, P. senegalus* and *P. endlicheri congicus)* had 36 chromosomes, all with median (m) or submedian (sm) centromeres[7-9]; the papers, however, disagreed over the number of m and sm chromosomes. The karyotype similarity seems to be accompanied by marked differences in DNA contents: values of 8.54 pg *(C. calabaricus)*[10], 11.7 pg *(P. palmas)*[11] and 13.67 pg *(P. bichir)*[10] were reported.

Materials and methods. Relative DNA amounts of 5 species (table) were determined from erythrocytes by Feulgen cytophotometry in a Zeiss UMSP I[12]. Blood films were fixed in ethanol:acetic acid (3:1) for 30 min; 4 separate series, which included 2 slides from each species, were hydrolyzed with 1 N HCl at 60 °C for 6 min and stained following the procedure of de Tomasi[13]. 240 cells per species (30 cells from each slide) were measured.

The specimens of *C. calabaricus* (from Cameroun) and of *Polypterus* (from the Zaire river near Kinshasa) listed in the table as well as 3 males and 1 female of *P. delhezi* from lake Tumba (Zaire) were karyotyped. For each fish at least 4 metaphase spreads[7] were analyzed. Nomenclature for centromeric position, determined from chromosome arm ratios (r = long arm/short arm), follows Levan[14].

Results. The table shows the similarity between the DNA amounts of the 5 species studied here; no statistical difference (0.05 level) was found between *C. calabaricus* and each of the 4 *Polypterus* species.

No differences were found between the karyotypes of *C. calabaricus, P. palmas, P. ornatipinnis* and *P. delhezi.* Figure 1 shows a karyotype of *P. delhezi* with the 36 chromosomes divided in 3 size groups: a) large chromosomes: 4 pairs were clearly m but the 5th pair, with r = 1.6 ± 0.23 (SD from 32 metaphases), varied around the m-sm borderline (r = 1.7); b) medium-sized chromosomes: 4 m and 4 sm pairs but 2 pairs classified as sm were also borderline cases; c) small chromosomes: 5 msm pairs, which arm ratios could not be determined more precisely. Only the large pairs 1 and 5 could be identified in all metaphases. The karyotype of *P. weeksii* (figure 2), with 2n = 38, differs from the karyotypes with 2n = 36 by the

DNA contents in Polypteridae

Species	Specimens	DNA content in % of *C. calabaricus*	SD in % of mean
C. calabaricus Smith, 1865	3♂, 1♀	100	6.8
P. palmas Ayres, 1850	2♂, 2♀	98	8.3
P. delhezi Boulenger, 1899	2♂, 2♀	107	7.9
P. ornatipinnis Boulenger, 1902	2♂, 1♀	105	10.1
P. weeksii Boulenger, 1898	2♂, 2♀	108	8.4
P. bichir[10]		164	

absence of 1 medium-sized m pair and the presence of 2 small pairs with nearly terminal (t) centromeres. No chromosomal sexual dimorphism was noted.

Discussion. Previous papers gave different DNA values for *C. calabaricus*[10] and *P. palmas*[11] whereas I found they had the same relative DNA content. The disparity may be accounted for by the choice of the standard values used to convert relative values into picograms. Indeed, Vialli[10] used the lowest of the values (4.9 pg[15], 5.6 pg[16], 6.3 pg[17]) reported for his standard species *S. irideus (= S. gairdneri);* on the contrary, Bachmann[11] used for his standard species much higher values (*B. bufo:* 15.1 pg, *R. sphenocephala:* 14.8 pg) than those reported elsewhere (e.g. 11.6 pg for *B. bufo*)[18,19]. But the difference between *P. bichir* and *C. calabaricus* (table) was found by comparison with the same standard[10].

The disagreement between previous descriptions of polypterid karyotypes, exemplified by the case of *P. delhezi,* concerns only the classification of chromosomes with r near the m-sm borderline: Cataudella[9] classified them all as m while Urushido[8] classified all except the small ones as sm.

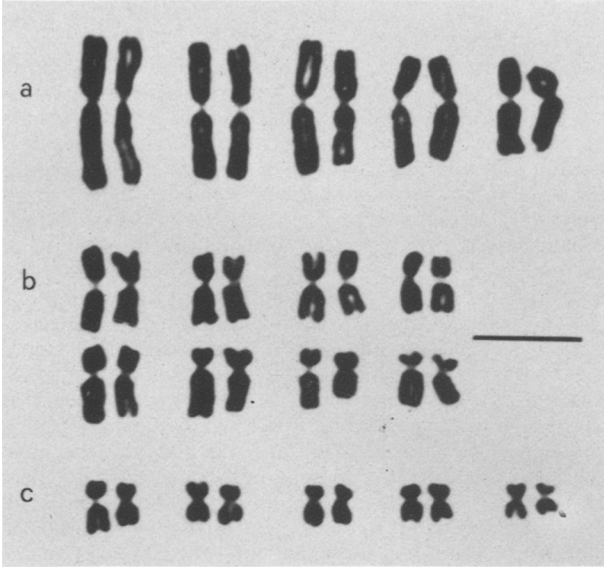

Fig. 1. Karyotype from gill epithelium of *Polypterus delhezi* (2n = 36); bar = 5 µm.

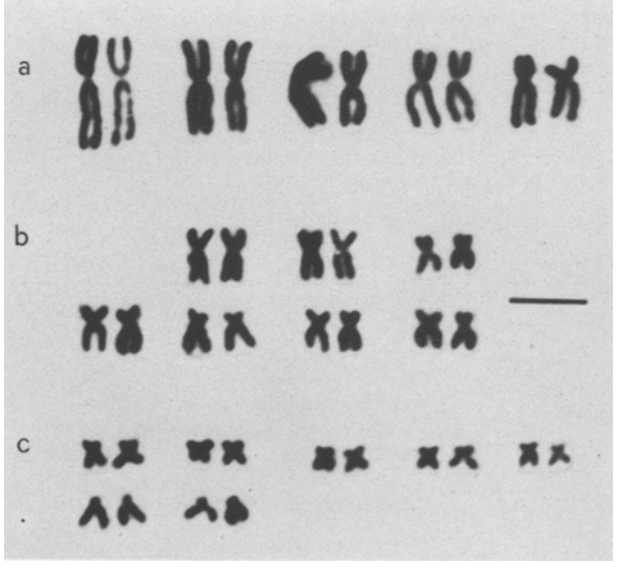

Fig. 2. Karyotype from gill epithelium of *Polypterus weeksii* (2n = 38); bar = 5 µm.

The substitution of 1 medium-sized m pair in the karyotype with 2n = 36 by 2 small t pairs in the karyotype of *P. weeksii,* with 2n = 38, clearly indicates a Robertsonian chromosomal rearrangement; the direction of the change, centric fission of the m pair or centric fusion of the 2 t pairs, is more difficult to evaluate. In favour of fission speaks the fact that the 38 chromosome karyotype seems to be exceptional for Polypteridae; however, the karyotypes of *P. bichir, P. retropinnis* and *P. ansorgei* (the most primitive member of the family)[20] have not yet been reported.

Comparison with other vertebrate groups indicates that the polypterid karyotypes could be the result of chromosome fusion on a larger scale. Indeed, Morescalchi's suggestion[21] that a karyotype with high 2n and with microchromosomes is primitive not only for tetrapods but for jawed vertebrates in general is supported by the finding of such a karyotype in sturgeons and a paddlefish (Chondrostei)[22-24], in ratfishes, rays and a shark (Chondrichthyes)[22,25], as well as in lampreys (Cyclostomata)[26]. It follows that the chromosome complements of the Polypteridae, irrespective of their placement within or outside the Actinopterygii, are the likely result of chromosome number reduction – not by loss but by fusion as indicated by their high DNA value. The karyotypes of polypterids, dipnoans[16] and modern groups of amphibians[21] resemble each other by low 2n and lack of microchromosomes; there are no indications that this resemblance has been inherited from a common ancestry.

1 Research supported by the Division of Human Genetics, K.U. Leuven, Belgium and the UNAZA, campus Kinshasa, Zaire. The Department of Human Genetics, University of Amsterdam, Netherlands, provided facilities for cytophotometry.

2 A.S. Romer, in: Notes and comments on vertebrate paleontology, p. 61. University Chicago Press, Chicago 1968.
3 B. Schaeffer, in: Interrelationships of fishes, p. 222. Ed. P.H. Greenwood, R.S. Miles and C. Patterson. Academic Press, London 1973.
4 M. Poll, Bull. Acad. r. Belg. Cl. Sci. *51,* 553 (1965).
5 E. Jarvik, in: Current problems of lower vertebrate phylogeny, p. 515. Ed. T. Ørvig. Almqvist and Wiksell, Stockholm 1968.
6 E. Capanna and S. Cataudella, Experientia *29,* 491 (1973).
7 T.E. Denton and W.M. Howell, Experientia *29,* 122 (1973).
8 T. Urushido, E. Takahashi, Y. Taki and N. Kondo, Proc. Jap. Acad. *53* Ser. B, 95 (1977).
9 S. Cataudella, L. Sola and E. Capanna, Experientia *34,* 999 (1978).
10 M. Vialli and P. Casonato, Rc. Ist. lomb. Sci. Lett. *106,* 50 (1972).
11 K. Bachmann, O.B. Goin and C.J. Goin, Copeia *1972,* 363.
12 H.G. Schiemer, Z. wiss. Mikrosk. *69,* 244 (1969).
13 A.G.E. Pearse, in: Histochemistry, 2nd edn, p. 201. J. and A. Churchill, London 1960.
14 A. Levan, K. Fredga and A.A. Sandberg, Hereditas *52,* 201 (1964).
15 R. Vendrely, in: The Nucleic Acids, p. 167. Ed. E. Chargaff and J.N. Davidson. Academic Press, New York 1955.
16 S. Ohno and N.B. Atkins, Chromosoma *18,* 455 (1966).
17 R.A. Pedersen, J. exp. Zool. *177,* 65 (1971).
18 E. Olmo, Caryologia *26,* 43 (1973).
19 E. Olmo and A. Morescalchi, Experientia *34,* 44 (1978).
20 M. Poll and C. Deswattines, Annls Mus. r. Afr. cent. Sér. 8vo *158,* 54 (1967).
21 A. Morescalchi, Boll. Zool. *37,* 13 (1970).
22 S. Ohno, J. Muramoto, C. Stenius, L. Christian, W.A. Kittrell and N.B. Atkin, Chromosoma *26,* 35 (1969).
23 F. Fontana and G. Colombo, Experientia *30,* 739 (1974).
24 G. Dingerkus and W.M. Howell, Science *194,* 842 (1976).
25 A. Nygren and M. Jahnke, Sw. J. agric. Res. *2,* 229 (1972).
26 I.C. Potter and E.S. Robinson, in: The biology of lampreys, p. 279. Ed. M.W. Hardisty and I.C. Potter. Academic Press, London 1971.