and E_9. Patterns 1, 2 and 3 were very similar, differing only in the mobility of bands 1 and 2. Patterns 4 and 5 differed in the mobility of bands 3 and 4. The only band which showed no differences in any of the patterns was the fast one, the most distant from the origin in figure 3.

These results show that the detected variability in the EST system of *M. anisopliae* permits characterization of the different isolates. Characterization is also possible through the use of other biochemical methods such as immunoelectrophoresis, as shown by Fargues et al.[6]. The possibility of the electrophoretic characterization of different strains of *M. anisopliae* is particularly promising for the improvement of pest-control techniques, since some of these isolates may well exhibit different pathogenicity.

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Tetraploidy in *Protopterus* (Dipnoi)[1]

A. Vervoort

Institut Agronomique et Vétérinaire Hassan II (APESA), Département de Biologie Animale, 6, rue Mohammed Triki, Rabat-Agdal (Marocco), 17 May 1979

Summary. Comparative DNA values obtained by Feulgen cytophotometry for 3 species of *Protopterus,* as well as karyotype analysis, show the existence of a diploid-tetraploid relationship within the genus *Protopterus.*

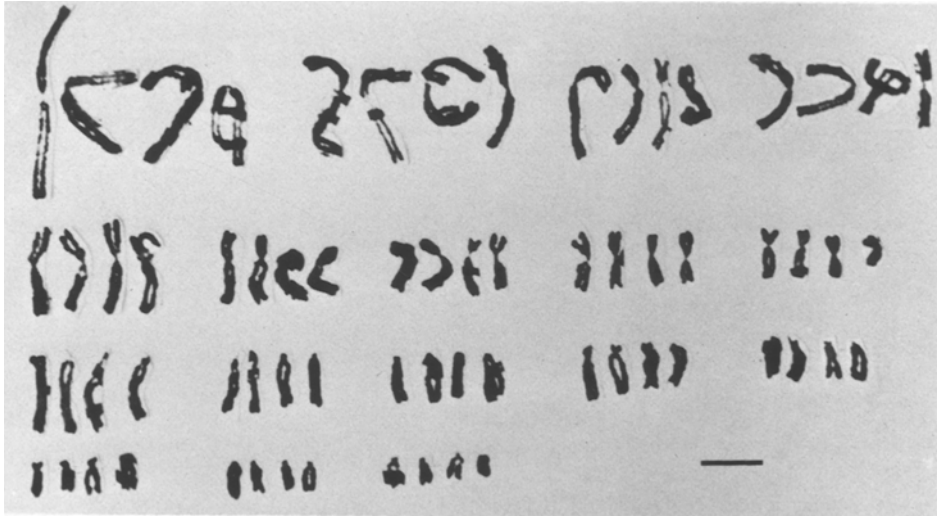
Lungfish (Dipnoi) today posses very large amounts of nuclear DNA (100–284 pg)[2–4] but rather few (32–38)[3,5,6] chromosomes. It has therefore been postulated[7] that their ancestors increased their genome size exclusively by tandem duplication, not by polyploidy.

In the case of the African genus *Protopterus,* with 4 species and 7 subspecies[8], 2 DNA values were reported: 100 pg for a non-identified species[2] and 284 pg for *P. aethiopicus*[4]. The question arises whether the discrepancy between the 2 values reflects biological variation or if it results from a difference in methods of measurement as was suggested[4].

In this study the DNA contents of *P. aethiopicus, P. dolloi* and *P. annectens* have been measured by Feulgen cytophotometry; in addition, the karyotype of *P. dolloi* has been studied for comparison with the previously reported[6] chromosome complement of *P. annectens.*

Materials and methods. 3 specimens of *P. dolloi* Boulenger, 1901, 3 specimens of *P. aethiopicus congicus* Poll, 1961, caught near Kinshasa, Zaire, and 4 specimens of *P. annectens annectens* (Owen), 1839, from the region of Dakar, Senegal, were used.

The Feulgen stain content of erythrocyte nuclei was measured with a Zeiss dual beam microspectrophotometer (UMSP I)[9] at 560 nm with a matched pair of 100× objectives. Blood samples which were to be compared in the same experiment were obtained simultaneously by heart-puncture from anesthetized fish and processed without storage. Drops of blood from 2 species (*P. dolloi* + *P. annectens* and *P. dolloi* + *P. aethiopicus*) were mixed on a slide before smearing; mixed cells could be distinguished as *P. dolloi* had much larger cells than the other species. Composite slides with chicken and *Protopterus* blood were also made. Slides were fixed with ethanol/acetic acid (3:1) for 30 min, hydrolysed with 5 N HCl at room temperature and Feulgen stained following the procedure of de Tomasi[10]. Because of the high density of *Protopterus* nuclei –



Karyotype of *Protopterus dolloi* (2n = 68); the bar equals 10 µm.

Table 1. Ratios of nuclear Feulgen stain content between 3 *Protopterus* species

Hydrolysis time (sec)[1]	*P. annectens/P. dolloi*			*P. aethiopicus/P. dolloi*		
	No. of slides	No. of determinations[2]	Ratio (mean ± SD)	No. of slides	No. of determinations[2]	Ratio (mean ± SD)
60	4	60	0.477 ± 0.019	4	60	0.508 ± 0.018
90	4	60	0.526 ± 0.024	4	60	0.476 ± 0.023
120	4	60	0.502 ± 0.015	4	60	0.524 ± 0.021
Average ratio: *P. annectens/P. dolloi:* 0.502				*P. aethiopicus/P. dolloi:* 0.496		

[1] Hydrolysis with 5 N HCl at room temperature.
[2] Each determination was obtained by measurement of 2 erythrocytes, 1 from each species, situated close to each other on the same slide.

Table 2. Ratios of nuclear Feulgen stain of 3 *Protopterus* species to chicken[1]

Protopterus species	No. of specimens	No. of slides	No. of determinations[2]	Ratio to chicken (mean ± SD)
P. annectens	4	8	200	32.37 ± 1.72
P. aethiopicus	3	6	150	32.06 ± 1.85
P. dolloi	3	6	150	65.28 ± 2.70

[1] Hydrolysis with 5 N HCl at room temperature for 120 sec.
[2] Each determination is the ratio of extinctions of 1 *Protopterus* erythrocyte to 1 chicken erythrocyte situated close to each other on the same slide.

comparable to that of Salamander nuclei[11,12] – short hydrolysis times had to be used. Ratios of extinctions were obtained for *P. annectens* to *P. dolloi* and for *P. aethiopicus* to *P. dolloi* after hydrolysis times of 60, 90 and 120 sec; the ratio of each *Protopterus* species to chicken was only determined after 120 sec hydrolysis, because, with the shorter times the staining of the chicken cells was too feeble.

Under these conditions extinctions of erythrocyte nuclei could be measured correctly; high background due to the presence of hemoglobin, as observed after fixation with alcohol or formol[12], did not occur. Mixed cells of 2 species showed sometimes significant but parallel variations in stain content between slides and between different parts of the same slide; each ratio therefore was calculated from the extinctions of 2 cells – 1 from each species – situated close to each other on the same slide. The means of these ratios did not show significant variations within or between slides.

Chromosome preparations were obtained by air-drying cells[13] from fin and tail-tip biopsies after colchicine treatment for 3 h in vitro.

Results. Table 1 shows that erythrocyte nuclei from *P. dolloi* take twice as much Feulgen stain as nuclei from *P. annectens* and *P. aethiopicus*; this indicates that *P. annectens* and *P. aethiopicus* have about the same DNA content, half as much as *P. dolloi*. The same conclusion can be drawn from table 2. In this table the measurements of different specimens from the same species were pooled as no significant differences within species were found. Accepting a nuclear DNA value of 2.5 pg for chicken erythrocytes[14], a value of about 80 pg was calculated for *P. annectens* and for *P. aethiopicus* and of 163 pg for *P. dolloi*.

15 metaphases from 3 specimens of *P. dolloi* produced counts ranging from 64 to 68 with the mode at 68. The chromosomes (figure) could arbitrarily be grouped in 17 quartets of morphologically similar elements: 9 quartets with median or submedian centromeres and 8 quartets with terminal or subterminal centromeres.

Discussion. The absolute DNA value calculated here for *P. annectens annectens* and for *P. aethiopicus congicus* (80 pg) is not too far from the value of 100 pg obtained nearly 30 years ago for a non-specified African lungfish by the Schmidt-Tannhauser method[2]. The much higher value, 284 pg, obtained by a Feulgen method for *P. aethiopicus* (subspecies not reported)[4] could indicate the existence of important intraspecific variation of DNA content in that species. However, differences in some details of the method applied, may also be responsible for discrepancies in the results.

The relative DNA values obtained here show that *P. dolloi* has twice as much nuclear DNA as *P. aethiopicus* or *P. annectens*. *P. annectens* from Gambia, belonging to the same subspecies as the specimens from Senegal studied here, has 2n = 34 chromosomes[6], half as many as *P. dolloi* (2n = 68). Thus both DNA content and 2n number indicate the existence of a diploid-tetraploid relationship within *Protopterus*. The complement that was doubled to give the present tetraploid complement of *P. dolloi* probably was not very different from that of *P. annectens*; indeed, the idiogram of *P. annectens*[6] shows a majority of (sub)metacentric chromosomes and at least 5 pairs of (sub)telocentric chromosomes. In vertebrates, increase in DNA content is correlated with increase in cell size, decrease in metabolic level and decrease of embryonic development rate[15]. *P. dolloi* does indeed have larger cells (personal observation), larger eggs, embryos and larvae[16] but a much slower rate of embryonic development[17] than *P. annectens*. Polyploidy, at present well documented among lower vertebrates but still an exceptional evolutionary event, is apparently compatible with the genetic system of *Protopterus*, in spite of the excessively high amounts of DNA carried by the representatives of this ancient group extant today. It is interesting to note that tandem duplications, bound to induce continuous changes in the amount of DNA, were of little importance in the evolution of this group, if they occurred at all, since the DNA values of *P. annectens*, *P. aethiopicus* and *P. dolloi* show a clear cut 1:1:2 relation. However, the extent of possible intraspecific variation in DNA amount needs to be further investigated.

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Chromosome fragility and susceptibility of Bloom's syndrome fibroblasts to SV40 transformation[1]

M.S. Lin[2] and O.S. Alfi

Division of Medical Genetics, Children's Hospital of Los Angeles and Department of Pediatrics, University of Southern California, Los Angeles (California 90027, USA), 11 April 1979

Summary. A comparison of the frequencies of chromosomal aberrations and the rates of SV40 transformation was made using fibroblasts obtained from 2 patients with Bloom's syndrome (BS) and from a normal individual. BS cells were found to be more susceptible to chromosome damage, in confirmation of earlier reports, but surprisingly, BS cells were distinctly less prone to transformation.

It has been suggested that cultured fibroblasts from patients with genetic disorders associated with chromosomal abnormalities, such as Fanconi's anaemia and Down's syndrome, which also have a high incidence of malignancy, are more susceptible than normal fibroblasts to in vitro transformation by Sendai virus (SV40)[3-6]. Bloom's syndrome (BS) is a rare autosomal recessive disorder characterized by growth retardation, light sensitivity, defective immune response, and predisposition to cancer[7,8]. In addition, BS cells show a high frequency of sister chromatid exchanges (SCE), chromosome breakage and symmetrical chromosome exchanges[7-9]. These particular cytological characteristics prompted us to investigate the relationship between the high risk fo cancer in BS and its sensitivity to transformation by SV40.

Materials and methods. Skin fibroblasts were cultivated in vitro from 1 normal individual and 2 patients with BS (GM 811 and GM 1493, obtained from the Institute for Medical Research, Camden, N.J.). Cultures were maintained in Eagle's minimum essential medium supplemented with 20% fetal calf serum. Fibroblasts during 7-12 passages were used. In order to differentiate sister chromatids, the cells were incubated in the dark in medium containing 10^{-5} M 5-bromodeoxyuridine (BrdU) for 2 cycles of DNA replication. Q-banding and BrdU-DAPI fluorescences were used for the staining of chromosomes[10,11]. The transformation procedure described by Todaro et al.[4] was followed.

Results and discussion. The results of the cytogenetic findings are presented in table 1. The frequency of spontaneous chromosome aberrations in BS fibroblasts was high, compared with that of the control. This result is consistent with earlier reports on BS lymphocytes and bone marrow cells in which the values for chromosomal aberrations were higher than those of normal cells[8,9,12,13].

As shown in table 1, the fibroblasts from a normal control showed a mean of 8.2 SCEs/cell. In contrast, the frequency of SCE in BS fibroblasts was about 10-fold greater than that of the normal control. A high incidence was observed in all metaphases after BrdU treatment, although SCE per BS cell varied (table 1). This is in agreement with the previous reports on lymphocytes and bone marrow cells from BS patients[8,9,12].

The percentages of BS cells surviving after infection with SV40 were comparable with those found for normal cells. Results of the transformation studies are shown in table 2. Only a slight difference was observed in the transformation frequency of cells (either normal or BS cells) between the 2 experiments. The transformation frequencies of cells from a normal individual are in good agreement with those reported by Todaro et al.[5]. The transformation rate was lower in cells from the 2 BS patients than in those from the control. This difference was even more pronounced in the repeated experiment in cells from 1 of the 2 BS patients (table 2). This clearly indicates that our fibroblasts from BS

Table 1. Chromosomal aberrations and sister chromatid exchanges in fibroblasts from 1 normal individual and from 1 patient (GM 1493) with Bloom's syndrome

Cell strain	Chromosomal aberrations						Sister chromatid exchanges		
	No. of cells examined	No. of abnormal cells (%)	No. of breaks per cell	No. of interchanges per cell	No. of dicentrics per cell	No. of fragments per cell	No. of cells examined	Mean	Range
Normal	35	2 (5.7)	0.08	0	0	0	32	8.2	3-16
GM 1493	70	24 (34.3)	0.37	0.09	0.13	0.16	20	81.7	55-125