Cytogenetics of *Xenopus laevis*. I. G-banding pattern of *Xenopus laevis* chromosomes[1]

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Summary. Although karyotype analyses by G-band staining have been used routinely in mammals, birds and reptiles, few have been reported in amphibia. We succeeded, however, in differential chromosome staining of some chromosomes of the African clawed toad, *Xenopus laevis,* by using the trypsin technique.

The band-staining techniques which specifically stain some regions of chromosomes came rapidly into use following the development of the fluorescence staining method (Q-band) by Caspersson[3]. Various band-staining techniques have been reported by a number of investigators, and applications of these methods have become an essential part of cytogenetical and cell genetical analyses. Karyotype analysis by G-band staining is one which is used extensively for animal chromosomes. The G-band method has already been used in humans, other mammals, birds and reptiles, but few such studies have been reported in amphibia[4-6]. In order to analyze the chromosomes of *X. laevis,* karyotyping by G-band staining was attempted in the present study.

Metaphase chromosome preparations were made with primary and secondary cultures of adult renal cells or cells from tadpoles. The tissues were minced, trypsinized and then transferred to culture media. Leibovitz' medium was used diluted for amphibia, and supplemented with 10% fetal bovine serum. When primary culture was carried out, penicillin (250 unit/ml) and streptomycin (2 mg/ml) were added to the medium. Cultures were maintained at 25 °C. Chromosome preparation was performed according to the conventional air drying methods. Colchicine (0.01%) was added to the culture prior to chromosome preparations. For the hypotonic solution, either 0.03% sodium citrate or 0.03 M KCl was used. The G-banding techniques used in the present study were the trypsin method of Seabright[7], the AGS method of Sumner et al.[8], and the urea method of Kato et al.[9].

The diploid chromosome number of *X. laevis* is 36: all chromosomes are bi-armed, with no centromere at the distal end. Tymowska et al.[10] measured in detail the length and arm ratio of each chromosome, and classified them into 7 groups. They reported that the karyotypes of this species did not indicate any morphological difference between male and female. In our study, G-banding pattern was not observed with the AGS and urea methods, though the chromosomes were differentially stained with Giemsa after the pretreatment with 0.05% trypsin in phosphate buffer (pH 6.8) at room temperature.

The differential staining pattern obtained with *X. laevis* chromosomes seemed to show similar characteristics to those of other vertebrates (fig. 1). Although this is the first G-banding pattern ever reported for *Xenopus* chromosomes, it was not adequate enough to schematize all of the 18 chromosome pairs. A reproducible staining pattern was obtained in about half of the chromosome complement. In figure 2, the reproducible banded chromosome pairs are schematized. Differentially stained chromosome regions are classified into 2 categories. Giemsa positive regions are indicated as dark bands.

Various pretreatments for chromosome banding give rise to species specific chromatin structures, which in turn specifically combine with staining dyes in Giemsa solution under certain conditions. The reactions are variable according to the pretreatment, and staining condition. It has been reported that G-banding applied to *Rana ridibunda* chromosome resulted in C-band-like pattern, as regions of constitutive heterochromatin were stained differently[5]. Yet, Rauci et al. carried out trypsin pretreatment and then stained them with Giemsa solution at pH 9[11].

In our case, G-banding only appeared with trypsin pretreatment[7], whereas it did not appear clearly with other

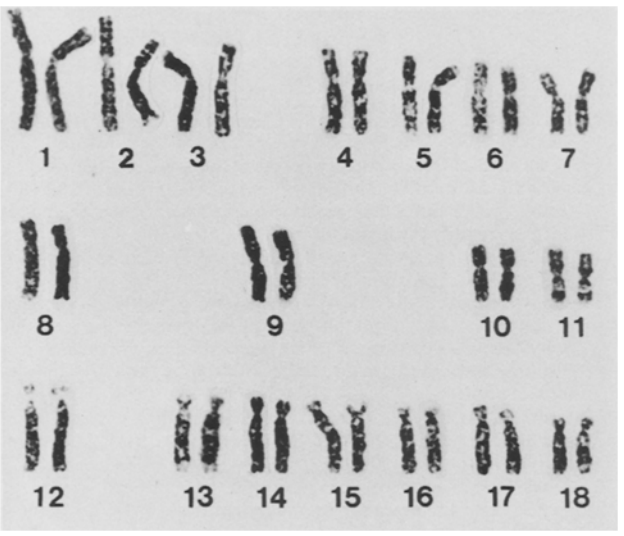

Figure 1. G-banding patterns of somatic metaphase chromosomes of *X. laevis* G-band staining was carried out following trypsin pretreatment[7]. Chromosome grouping is based on Tymowska et al.[10].

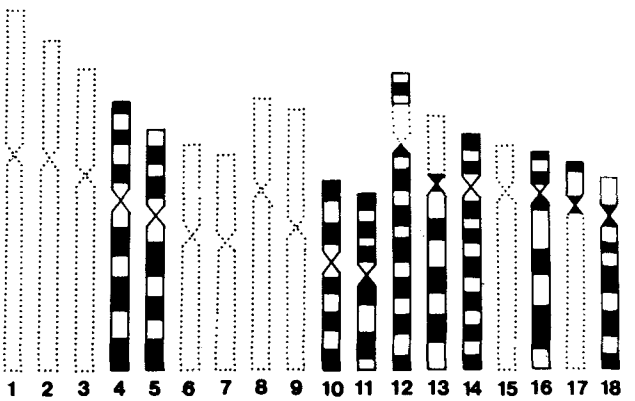

Figure 2. Diagrammatic representation of the maximum number of G-bands determined in the somatic metaphase chromosomes of *X. laevis.* Giemsa positive bands are shown as dark bands. No. 5 chromosome has 2 positive bands on the short arm and 3 positive bands on the long arm. One distinct pale band on the long arm adjacent to the centromere. No. 12 chromosome has 1 positive band at the center of the satellite, and 7 positive bands on the long arm. Secondary constriction, which is the nucleolar organizer region, was not stained. Nos 1–3, Nos 6–9, and No. 15 chromosomes are not schematizable. The short arm of No. 13 chromosome and the long arm of No. 17 chromosome are not schematizable.

methods. It seems likely that the trypsin method is useful for karyotype analysis in other amphibia. Recently, sex chromosomes were discovered in 2 anuran species by using other banding techniques[12,13]. Investigation is under way in our laboratory on sexual differences in the bandings.

1 We thank Dr T. Takeuchi of Tohoku University for critical reading of the manuscript.
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0014-4754/83/070786-02$1.50 + 0.20/0

Clinal variation at the peptidase-1 (*Pept*-1) locus in natural populations of *Drosophila subobscura*[1]

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Summary. The allele frequencies for the peptidase-1 (*Pept*-1) gene in 8 natural populations of *Drosophila subobscura* situated along a north-south transect through the distribution range of the species were determined. The occurrence of a cline along the transect studied is discussed in relation to the mechanisms of maintenance of the *Pept*-1 genetic polymorphism.

The adaptive significance of protein polymorphisms in natural populations of diploid organisms is still a much-debated point in evolutionary genetics[3,4]. It is not clear whether such genetic variability is maintained by some kind of balancing selection or is completely neutral and only subject to random genetic drift. Moreover, there are now numerous data available showing that simple electrophoretic techniques reveal much less of the genetic variability at the protein level than actually exists[5].

The present paper deals with the allele frequency variation of the peptidase-1 (*Pept*-1) locus in 8 natural populations of *Drosophila subobscura*. The gene coding for *Pept*-1 was found to be on chromosome 0 of *D. subobscura*[6]. The different collection sites are located approximately along a north-south line across the distribution range of the species (see table). The present investigation is part of a more extensive project for the study of the genetic composition of natural populations of *D. subobscura*[7,8].

The 14% starch horizontal gel electrophoresis conditions used were as follows. Electrode buffer: 0.18 M Tris, 0.1 M boric acid, 0.004 M EDTA 2 Na; Gel buffer: 1 in 4 dilution of bridge buffer. The final pH was 8.6. The run was at 250 V/70 mA/5 h. The staining mixture, modified by Harris and Hopkinson[9], utilized for 1 gel contained 0.1 M Na_2HPO_4 adjusted to pH 7.5 with 5 ml 0.1 M KH_2PO_4; 10 mg O-dianisidine; 10 mg Snake venom L-aminoacid oxidase; 10 mg peroxidase grade II (100 U/mg); 20 mg L-phenylalanyl-L-leucine; 10 mg $MnCl_2$; 20 ml agar (approximately 0.8% in 0.1 M phosphate buffer). The gels were stained at 37 °C. Enzyme activity was indicated by dark yellow-brown staining bands. The allozyme pattern of the *Pept*-1 locus found in the present study can easily be explained by the presence of 4 alleles (0.20, 0.40, 1.00, and 1.60). Homozygotes exhibit only single bands, heterozygotes 3 bands, as previously demonstrated for the dipeptidase-A locus in *D. melanogaster*[10].

The alleles found, their frequencies and the degrees of heterozygosity in the population samples studied are given in the table. Three common (0.40, 1.00, and 1.60) and 1 rare (0.20) active *Pept*-1 alleles were observed. No inactive

Allele frequencies (%) at the *Pept*-1 locus in 8 populations of *Drosophila subobscura*

Population	Location[a] Lat. (N)	Long.	Sample size	Allele 0.20	0.40	1.00	1.60	Degree of heterozygosity
1. Sunne (Sweden)[b]	59.50	13.09 E	9	0	33.3±16	66.7±16	0	44.4
2. F. Augustus (Scotland)[c]	57.10	4.41 W	191	0.6±1	48.3± 4	47.2± 4	3.9±2	53.9
3. Tübingen (Germany)[d]	48.31	9.02 E	215	0.4±1	43.1± 3	51.6± 2	4.9±1	54.6
4. Zernez (Switzerland)[b]	46.43	10.05 E	12	0	66.7±14	25.0± 3	8.3±8	48.6
5. Formia (Italy)[c]	41.15	13.37 E	157	0	70.8± 4	27.1± 4	2.1±1	42.5
6. Ponza (Italy, Island)[b]	40.54	12.58 E	19	0	78.9± 9	21.1± 9	0	33.3
7. Cinisi (Italy, Sicily)[b]	38.08	13.18 E	14	0	64.3±13	35.7±13	0	45.9
8. Bizerte (Tunesia)[b]	37.17	9.52 E	11	0	81.8±12	18.2±12	0	29.8
							Average:	44.6

[a]For further details see Pinsker and Sperlich[7] and Sperlich et al.[8]. Seasonal and altitudinal differences can be neglected since they are only weakly expressed in *D. subobscura;* [b]data from balanced lethal stocks; [c]data from balanced lethal stocks and isofemale lines; [d]data from balanced lethal stocks (14), isofemale lines (82), and a natural population (119).