Thus 16 separate photometric measurements were made in an area of 3.75 micron square. We further programmed the photometer to read 10 times at 1/60 second intervals in each position. Thus the area of the "minimum effective aperture" was discriminated by an averaged relative density of 160 photometric measurements. All electrical power supply were line conditioned. The 450W xenon illumination source was operated at a stabilized 24V. The EMI9789B bialkali cathode photometer was operated at a typical 0.4% standard deviation of the measured value in 50% transmission level of the chromatid. The measuring beam was monochromatized to 602 nm.

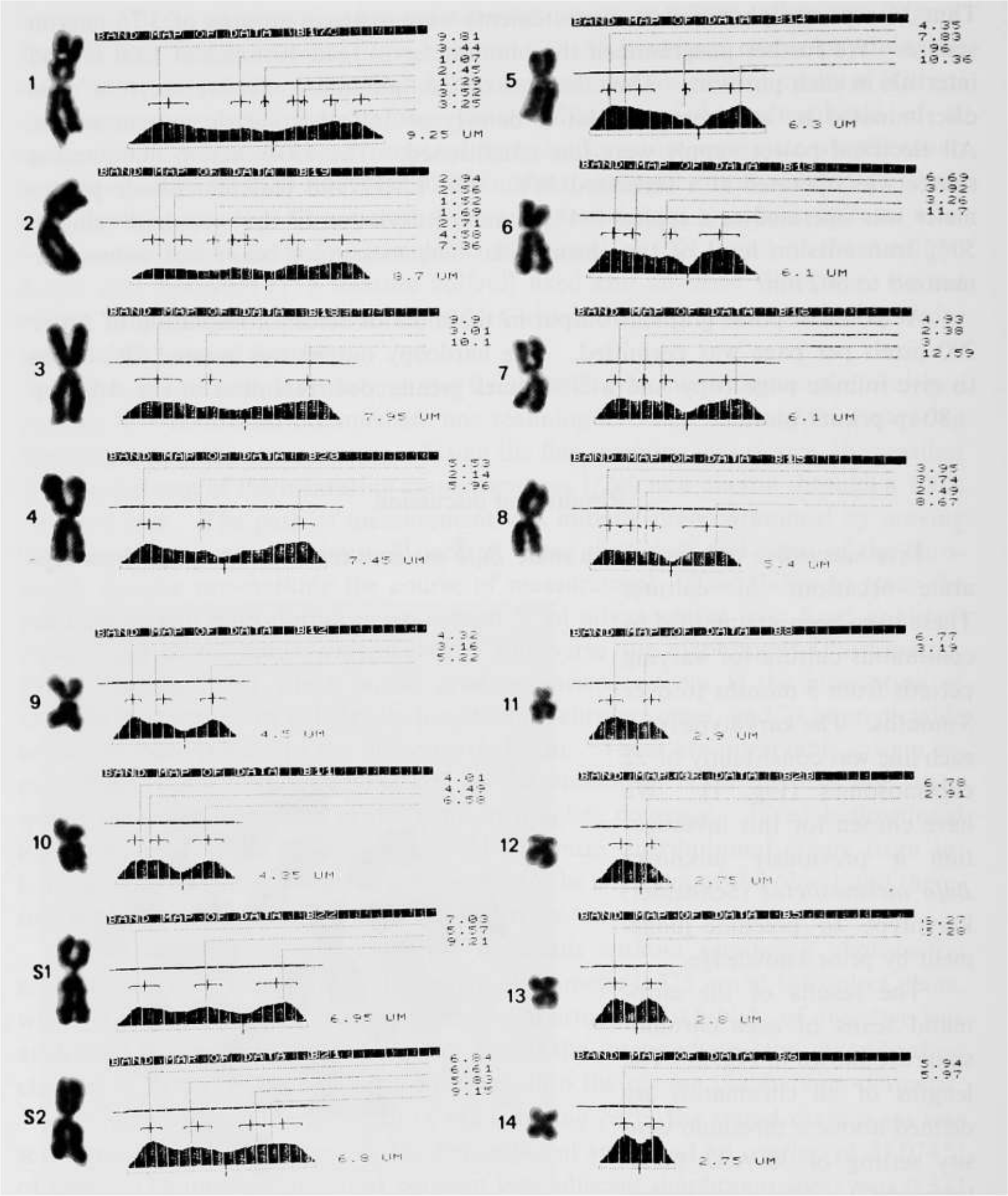
At point to point graphics output in the monitor screen a resolution of 320 × 200 pixels per page was permitted. The hardcopy output was rotated 90° so as to give infinite page copy and a 3.84 times greater dot resolution in the Adcomp ×80 sp printer/plotter.

Results and discussion

Five successive cell lines from male *Bufo melanostictus* were produced on separate occasions of culture. These have been maintained in continuous culture for varying periods from 3 months to over 8 months. The karyotype from each line was consistently of 22 chromosomes (Fig. 1). We have chosen for this investigation a previously unknown *Bufo melanostictus* (Schneider) karyotype to preclude judgement by prior knowledge.

The results of the chromatid scans of each chromosome are shown in Fig. 2. The lengths of all chromatids are defined above a threshold density setting of 30 A.U. (arbitrary units). This is our standard background elimination setting for most photometric scans of chromosomes in the laboratory. The longest chromosome was under 10 microns while the shortest was just over 2 microns, in mid-metaphase stage. Scans of sister chromatids produced similar profiles, and when the different chromosome profiles were placed in descending order of length ad-

Fig. 1. Typical metaphase chromosome spread of male common toad, *Bufo melanostictus*. 1 : 3 acetic methanol fixation and 10% aqueous Giemsa staining. 2n = 22.



jacent profiles look like those from sister chromatids with closely matched lengths, centromere indices and humps of higher densities. There were however two chromosomes which did not quite pair up. These were the sex chromosomes, labelled S1 and S2 which had quite different configurations and density profiles. According to the classification of centromere positions by Levan *et al.* (1964), S1 would be unique in being the only chromosome in the entire karyotype with a submetacentric configuration since its $p/(p+q)$ index is 34.3%. S2 with a $p/(p+q)$ index of 47.8% is like the rest of the chromosomes in the karyotype, in being metacentric in configuration. The chromosomes of *B. melanostictus* could be classified into two

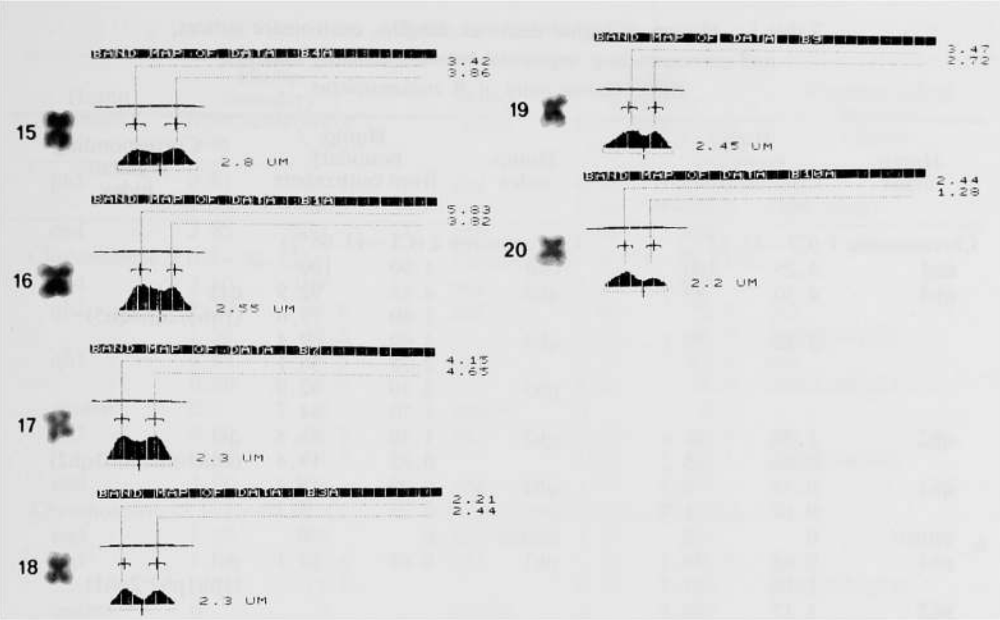


Fig. 2. Densitometric profiles from uniform path width direct tracing of the right chromatid of the chromosomes of Fig. 1. Dotted vertical lines lead from the peak density of each hump in the profile to the integrated density value (in arbitrary units/1000) of each hump. The boundary limits of each hump are defined by the horizontal brackets above the hump. The length of each chromatid is measured from the threshold setting of 30 arbitrary units. The centromere point was set by visual inspection before scanning, and indicated by the vertical bar beneath the profile. Chromatids were scanned at random sequence whereupon the data filenames, B1 to 22 are not chromosome numbers.

groups 1) the big chromosomes, numbered 1 to 10 including S1 and S2, and 2) the small chromosomes, numbered 11 to 20. The unique submetacentric S1 was not a female feature, and thus analogous to the Y chromosome of mammals.

An attempt was made to define the positions of the humps in the densitometric profiles relative to the centromere position, and compare them between chromosomes which were matched (Table 1). In this the computer was programmed to initially locate the peaks and antipeaks in the profiles, and then had the mid-density value between the peak and the adjacent antipeak derived. This mid-density position was arbitrarily taken as the boundary of each hump of higher density in the profile. By converting the distance of the hump boundaries into percentage distance from the centromere, compensation for length differences between pairs was not needed. When their hump boundaries overlapped then Table 1 considered the humps to be of the same 'segment' of the chromosome arm. Thus segmental homologies for all paired chromosomes could be defined. The inherent morphology of the untreated vertebrate chromatid is a uniform spiral of radiating chromonemata. Homologous humps or segments, of higher densities in chromatids that were stained simply with aqueous Giemsa had not been reported, yet often variegated Giemsa dye bindings were commonly observed in chromosome spreads. Without objective means of measurement, however, such observations could not be documented.