The Chromosome Complement of the Aardvark, *Orycteropus afer*

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Abstract. The chromosome complement of the aardvark, *Orycteropus afer*, consists of 18 metacentric and submetacentric autosomes, a small metacentric X and a little acrocentric Y element. The aardvark is the only member of the Order *Tubulidentata*. Its relationship to any other taxa is not known, and its karyotype is unique and does not suggest any particular relationship. The DNA content of the nucleus is 1.67 × that of human lymphocytes, a fact not readily explicable by contemporary knowledge of phylogenetic relationships, but similar to the situation found in many *Marsupialia*. The findings are discussed with regard to evolutionary processes and the phylogenetic position of this species.

Introduction

Despite the relatively wide distribution of the aardvark (*Orycteropus afer*) throughout Africa south of the Sahara, its chromosomal complement has, to our knowledge, not been previously studied. Such a study might be useful in assessing the relationship of this species to other taxa. The aardvark is the only member of the Order *Tubulidentata* and, although it has been suggested in the past that the species is related to *Edentata*, there has been no formal support for this suggestion other than the specialization for subterranean life and diet (Morris, 1965; Romer, 1966).

We were fortunate to obtain skin biopsies from a male animal at the National Zoological Park, Washington, D. C. through the courtesy of Dr. C. Gray. Subsequently, Dr. R. Sampsell kindly allowed Dr. M. A. Grinberg to obtain a skin biopsy from the two year old female at Crandon Park Zoo, Miami, Florida. When this manuscript was prepared Dr. T. C. Hsu allowed us to quote his recent similar findings on a female at Point Defiance Zoo, Tacoma, Washington.

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Material and Methods

Skin biopsies, obtained after alcohol cleansing with the help of the most useful "Suture Removal Pak" (Sterilon Corporation, Braintree, Massachusetts) were shipped in Eagle's basal medium with 20% calf serum. The full thickness skin and subcutaneous tissue were finely minced, placed onto glass slides and cultured in large Leighton tubes according to the method described by Basrur et al. (1963). The nutrient used was Eagle's basal or McCoy's 5a modified medium to which 20% fetal calf serum, penicillin and streptomycin had been added. Although the latter medium is much superior in rodent cultures, the aardvark was equally well supported by both media. When fragments showed sufficient peripheral cell proliferation, the culture was subcultured in Carrel flasks, the original explants being propagated further. After partial sheeting, the subcultures were treated with colchicine, and the cells were trypsinized, exposed to hypotonic Earle's solution, spread onto slides, and air-dried, in the usual manner. The slides were stained with carbol fuchsin (Carr and Walker, 1961). One subculture of the male was exposed to tritiated thymidine 6 hours prior to harvesting and film emulsions exposed for 10 days. These preparations were photographed before and after radioautography and suitable spreads chosen for karyotyping.

Another subculture of the male was grown on coverslips in a large Leighton tube. After the coverslips were completely covered by cells they were removed and fixed in acetic acid-alcohol without hypotonic treatment. A suspension containing human lymphocytes in physiological saline was prepared from fresh tonsillar tissue obtained at tonsillectomy from a female patient; a small amount of the suspension was added to each coverslip, which was then put through the freeze-substitution fixation procedure as previously described (Atkin et al., 1965). After staining of the coverslips with Feulgen reagent, the microspectrophotometric measurements were made with a Deeley Integrating Microdensitometer. Thirty fibroblasts were measured and cells with what appeared to be tetraploid values or above were excluded; thirty control human lymphocytes were measured in the same manner.

The percentage of the haploid genome made up of X or Y was ascertained following the original method of Ohno et al. (1964) but modified by Galton et al. (1965). Briefly, metaphases were projected at 5000 × magnification onto F₅ Kodabromide photographic paper. Thirteen complete spreads were thus prepared, the chromosomes cut out uniformly and weighed on a torsion balance. The percentage was calculated according to the following formula:

\[
\frac{X \text{ (or } Y)}{(\text{autosomes/2}) + X} = \% \text{ X (or } Y).
\]

This procedure allows comparison with other mammalian species previously reported. The chromosomes of these thirteen spreads were then paired visually and their arm lengths measured with callipers. Using this data the idiogram of this unique species was constructed.

Results

The diploid number of chromosomes of the aardvark was found to be \(2n = 20\) in all three specimens examined. A few incomplete cells had chromosomes missing and the female specimen had several cells in endoreduplication. Cells in interphase had a single Barr body (sex chromatin) in the female; the endoreduplicated (unusually large) cells