Chromosome Banding Patterns and the Nucleolar Organizer Region of the Eastern Mole (*Scalopus aquaticus*)

T. L. YATES[1], A. D. STOCK and D. J. SCHMIDLY

Department of Wildlife and Fisheries Sciences, Texas A & M University, College Station (Texas 77843, USA), and Department of Biology, The University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston (Texas 77025, USA), 1 March 1976.

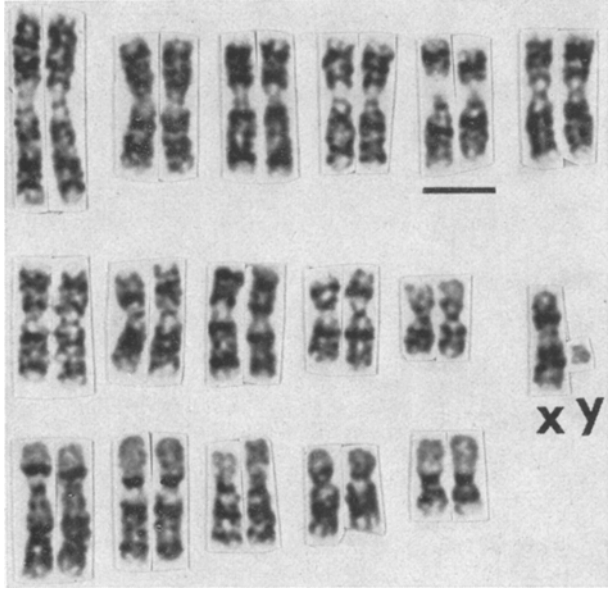
Summary. The C and G chromosome banding patterns and the AgAS positive sites (NOR regions) of cultured lung cells of the Eastern mole (*Scalopus aquaticus*) are presented. A distinctive secondary constriction is found on a pair of autosomes instead of on the *X*-chromosome as previously believed. The presence of a heterochromatic heteromorphism is noted and a large amount of constitutive heterochromatin is present in the karyotype.

Of the 15 currently recognized genera and 20 species in the family Talpidae, chromosomal information is available for only 8 species (representing 7 genera) and only 2 of these, *Talpa europea*[2] and *Scalopus aquaticus*[3], have been examined from numerous localities throughout their range. Although information on diploid number and gross chromosome morphology are available for all 5 genera of North American talpids (*Scapanus*[4], *Parascalops*[2], *Neurotrichus*[5], *Scalopus*[3], and *Condylura*[6]) to our knowledge there is no published data on the C-band and G-band patterns of the chromosomes of these taxa.

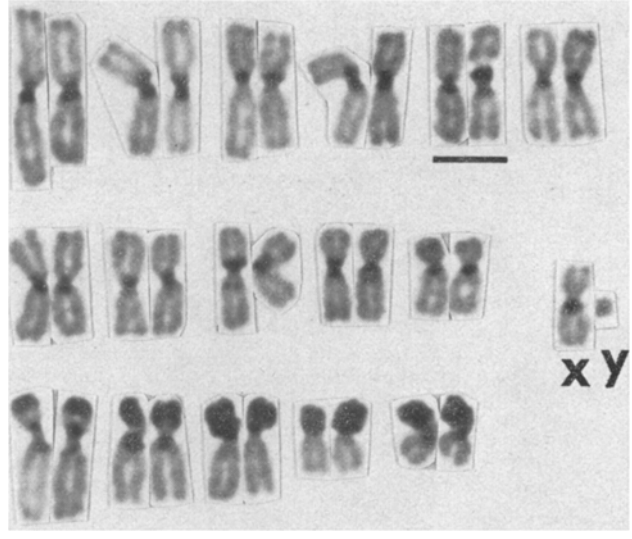
YATES and SCHMIDLY[3] after examination of the karyotypes of 23 specimens of *Scalopus aquaticus* from several localities throughout its range found the gross karyotype of all specimens examined to be uniform. They also reported the presence of a distinctive secondary constriction on what appeared to be the *X*-chromosome. Without the use of various banding techniques, however, the *X* could not be identified with certainty. The fact that other genera of talpids (*Parascalops*[2] and *Talpa*[3]) exhibited a similar constriction on a pair of autosomes raised a question as to the proper identification of the *X*-chromosome of *Scalopus*.

In an attempt to solve this problem and obtain more detailed information on the morphology of mole chromosomes, we conducted C and G chromosome banding and AgAS staining[7] studies on the mitotic chromosomes of tissue cultured cells of the Eastern mole *Scalopus aquaticus*. G-banding patterns were induced by combining the trypsin[8] and the urea[9] techniques as described by STOCK et al[10]. C-bands were obtained with the alkaline SSC method of STEFOS and ARRIGHI[11]. Examination of the karyotype of *Scalopus aquaticus* after C and G banding reveals that the secondary constriction is on a pair of autosomes and not on the *X* (Figure). The reason for the appearance of the constriction on only 1 chromosome in conventional cytological preparations and thus mis-

[1] Current address of YATES: The Museum, Texas Tech University, Lubbock (Texas 79409, USA).
[2] A. M. GROPP, *Comparative Mammalian Cytogenetics* (Ed. K. BENIRSCHKE; Springer, New York 1969), p. 247.
[3] T. L. YATES and D. J. SCHMIDLY, J. Mammal. *56*, 902 (1975).
[4] J. F. LYNCH, Mammal. Chrom. Newsl. *12*, 83 (1971).
[5] R. M. BROWN and A. M. WATERBURY, Mammal. Chrom. Newsl. *12*, 45 (1971).
[6] A. MEYLAN, Rev. Suisse Zool. *75*, 691 (1968).
[7] C. GOODPASTURE and S. BLOOM, Chromosoma *53*, 37 (1975).
[8] M. SEABRIGHT, Chromosome *36*, 204 (1972).
[9] Y. SHIRAISHI and T. H. YOSIDA, Chromosome *37*, 75 (1972).
[10] A. D. STOCK, F. E. ARRIGHI and K. STEFOS, Cytogenet. Cell Genet. *13*, 410 (1974).
[11] K. STEFOS and F. E. ARRIGHI, Expl. Cell Res. *68*, 228 (1971).



a) G-banded karyotype of a male *Scalopus aquaticus* from Tyler Co., Texas, showing secondary constriction in chromosome pair 5 (underlined).



b) C-banded karyotype of a male *Scalopus aquaticus* from Tyler Co., Texas, showing heterochromatic polymorphism in chromosome pair 5 (underlined).

identification of the *X*-chromosome is readily apparent when chromosome pair 5 (underlined) in Figure b is examined. A large segment of constitutive heterochromatin is present near the centromere in one homologue effectively masking the secondary constriction in that chromosome in the conventional karyotype. The constriction is evident in both homologues in the G-banded karyotype (Figure a) if the chromosomes are not over contracted.

Further examination of Figure b reveals a considerable amount of constitutive heterochromatin in the karyotype of *Scalopus aquaticus*. The short arms of 4 pairs of autosomes are comprised almost entirely of constitutive heterochromatin. At present, we are unable to account for the apparent pattern of inheritence of the heteromorphic C-band regions wherein all individuals possess the heteromorphic pair of autosomes.

The secondary constriction region in this species corresponds to the nucleolar organizer region (NOR) as indicated by the AgAS techniques. We have not attempted in situ hybridization studies but in other species, the AgAS technique stains the sites of known 18s and 28s ribosomal cistrons. No difference in the size of the NOR region between homologues is indicated in our material.

Acid Phosphatases in *Drosophila auraria*

C. D. Triantaphyllidis and C. D. Kastritsis[1]

Laboratory of General Biology, Aristotelian University of Thessaloniki, Thessaloniki (Greece), 29 December 1975.

Summary. A study was carried out dealing with the acid phosphatases, in larvae, pupae and adults of *Drosophila auraria*. The most interesting finding is that the acid phosphatases are observed in 3 different phenotypes appearing as groups of 3 bands of different mobilities in the homozygotes and giving a 5 band phenotype in the heterozygote. A hypothesis is discussed in an attempt to explain the phenotypes observed and their variation during the development of the species.

In the literature of the last decade, one finds many references to enzyme polymorphisms of various *Drosophila* and other species. These studies are quite often extended to cover developmental variations of the various enzymatic patterns as well as species comparisons[2]. Since Kastritsis and Grossfield[3] have described the existence of 2 Balbiani rings in one of the chromosomes of *D. auraria*, our laboratory has undertaken a study of various aspects of the development and the genetics of this species.

Materials and methods. The laboratory stocks of *D. auraria* used in this investigation were obtained from Dr. J. Grossfield of New York University. As a reference we used stocks of *D. melanogaster* and *D. simulans* kept in our laboratory. Larvae of 1st, 2nd or 3rd instar, prepupae and pupae (early and late), as well as adults (♂ and ♀) of 3 h, 1, 2, 3, 4, 6, 8, 10 and 22 days of age were used to perform horizontal starch gel electrophoresis. For the 1st and 2nd larval instars, 4 individuals per sample were homogenized on glass microscope slides, as described by Beckman and Johnson[4]. In subsequent experiments with animals of the remaining ages, single individuals were utilized. The electrophoresis was performed using a discontinuous buffer system[5] with a voltage gradient of 8–10 V per centimeter. Assays were performed by using the techniques of MacIntyre[6].

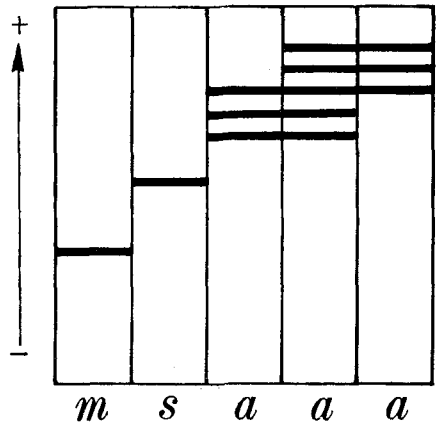


Fig. 1. Schematic representation of the relative positions of acid phosphatases in *D. melanogaster* (m. genotype Acph–$1^{1.00}$ / Acph–$1^{1.00}$), *D. simulans* (s, genotype Acph–$1^{1.51}$ / Acph–$1^{1.51}$), and the 3 *D. auraria* phenotypes (a, A/A, A/B and B/B). The lines represent only the position of each band and not their developmental variation.

In order to facilitate species comparisons, the allozyme controlled by the most common allele of *D. melanogaster* was assigned a mobility of 1.00; all other allozymes of the 3 species used were compared to that 'standard' and named accordingly. To make comparisons with other reports[6] possible, it should be mentioned that Acph-1^F of *D. melanogaster* is designated here as Acph-$1^{1.00}$ while the relative mobility of the Acph-1^F of *D. simulans* in reference to Acph-1^F of *D. melanogaster* is Acph-$1^{1.51}$.

Results and discussion. In the zymograms of *D. auraria*, 3 different phenotypes of Acph have been found, i.e. phenotype A (slow), AB, and B (fast) respectively, as shown in Figures 1 and 2. Phenotype A consists of 3 bands (1, 2, 3 in the direction of migration), phenotype AB of 5 bands (1, 2, 3, 4, 5) and phenotype B of 3 bands (3, 4, 5). The relative mobilities of these bands in reference to Acph-$1^{1.00}$ of *D. melanogaster* are Acph-$1^{1.90}$, Acph-$1^{2.06}$, Acph-$1^{2.22}$, Acph-$1^{2.38}$, and Acph-$1^{2.54}$.

[1] We want to thank Drs. R. P. Wagner and R. H. Richardson of the University of Texas at Austin for reading the manuscript and making helpful comments and suggestions. The technical help of Mr. A. Svinios and the secretarial help of Miss E. Pitia are greatly appreciated. Research supported by the National Research Institute of Greece.

[2] W. J. Dickinson and D. T. Sullivan, *Gene Enzyme Systems in Drosophila* (Springer-Verlag, New York, Heidelberg, Berlin 1975).

[3] C. D. Kastritsis and J. Grossfield, Drosoph. Inf. Serv. *47*, 123 (1971).

[4] L. Beckman and F. M. Johnson, Hereditas *51*, 212 (1964).

[5] G. C. Ashton and A. H. Braden, J. exp. Med. Sci. *14*, 248 (1961).

[6] R. MacIntyre, Genetics *53*, 461 (1966).