Se reportant à ma révision de 1966, le lecteur verra que les Leggada (14 individus) de Bangui appartiennent à la série polymorphe 34/33/32/31 de *musculoides* alors que le seul exemplaire obtenu de Bangassou était doté de 22 chromosomes (*N.F.* = 36, chromosomes sexuels *TR*) et s'incorporait donc à la même série robertsonienne. Ceci dit, voici une présentation préliminaire des 4 sujets d'Ippy, lesquels, selon le Dr. PETTER, sont morphologiquement tous semblables et semblables aussi à leurs congénères classés comme *musculoides*.

♂/259. $2N = 28$, *N.F.* = 36, chromosomes sexuels *TR*. Une nouvelle branche s'ajuste à l'éventail robertsonien qui, pour un *N.F.* de 36, montre des nombres $2N$ de 34, 33, 32, 31, . . . 28, . . . 22, . . . 19, 18.

♀/260. $2N = 32$, *N.F.* = 36, chromosomes sexuels *TR*. Par la présence de deux grands métacentriques à bras égaux, cet individu diffère des *musculoides* à 32 chromosomes où les deux autosomes dibrachiaux sont des submétacentriques à index centromérique voisin de 0,25. Par ce caractère, il se rapproche du ♂/91 de Côte d'Ivoire, unique dans un échantillon de 30 Leggada de ce pays et auquel[2] j'ai attribué la dénomination de *f.c.* 4 (*f,c,* = formule chromosomique). La ♀/260 présente en outre l'un de ses *X* avec une délétion partielle d'un bras.

♂/261. $2N = 19$, *N.F.* = 36, chromosomes sexuels X/Y_1Y_2. Ce type de chromosomes sexuels multiples apparait pour la première fois chez les Leggada alors que j'ai décrit[6] un type X_1X_2/Y chez les *musculoides* de Rhodésie. Y_1 et Y_2 correspondent certainement aux deux bras de l'*Y* submétacentrique des *Mus f.c. 6* d'Angola[7] alors que chez *minutoides* d'Afrique du Sud, l'*Y* est acrocentrique, ces 3 formes possédant 18 chromosomes.

♀/262. $2N = 18$, *N.F.* = 36, chromosomes sexuels *TR* (X/X). Il s'agit certainement de la femelle du précédent.

Conclusions. L'impuissance du Taxonomiste à distinguer selon les critères morphologiques classiques (pelage, crâne, denture) des formes dont les caryotypes sont, dans beaucoup de cas, suffisamment diversifiés pour exclure l'interfécondité, nous confirme dans l'idée que les Leggada à *N.F.* de 36 et chromosomes sexuels *TR* forment une énorme collection d'espèces cryptiques définies par des caryotypes divers, chacun d'entre eux ayant une valeur sélective déterminée à l'égard d'un certain biotope. L'isolement reproductif est certainement effectif entre quelques formes, en voie de réalisation pour d'autres, ou non encore établi. Si les mécanismes impliqués dans la dislocation de ce grand collectif sont de nature principalement robertsonienne, j'ai montré que les inversions péricentriques et les délétions sont également à l'œuvre comme l'est aussi l'apparition de chromosomes sexuels multiples. L'isolement par mutations chromosomiques préludant aux transformations du «pool» génétique apparait, chez les Leggada, comme un facteur primaire de spéciation.

Summary. To the 237 pigmy-mice studies in his previous papers, the author brings new data concerning the cytogenetics of 25 Leggada. One first set of 12 mice from Nigeria belongs to the same polymorphic series containing the *musculoides* from the Ivory Coast and the Central African Republic.

A small sample from Ippy (Central African Republic), although the individuals are morphologically all alike and referable to *musculoides*, shows an amazing diversity: ♂/259. $2N = 28$, *N.F.* = 36, sex chromosomes of the translocated type (*TR*). We meet here a new robertsonian combination in the polymorphic series, 34/33/32/31 . . . 28/ . . . 22/ . . . 19/18.

♀/260. $2N = 32$, *N.F.* = 36, sex chromosomes *TR*. Both big biarmed autosomes are metacentric – submetacentric with a centromeric index of about 0.25 by the typical *musculoides* having the same diploid number.

♂/261. $2N = 19$, *N.F.* = 36, sex chromosomes X/Y_1Y_2. The first occurrence of such sex chromosomes by the Leggada. By the *musculoides* from Rhodesia we were dealing with the type X_1X_2/Y.

♀/262. $2N = 18$, *N.F.* = 36, sex chromosomes X/X. Very likely the female of ♂/261.

In the complex *minutoides-musculoides*, we meet many different caryotypes and it is obvious that the chromosomal mutations play here the part of a primary factor of isolation, previous to the gene mutations which later will allow the taxonomic determination of the species actually *'in statu nascendi'*.

R. MATTHEY

Institut de Biologie animale et de Zoologie,
Université de Lausanne,
CH-1005 Lausanne (Suisse), le 20 octobre 1969.

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Differential RNA-Synthesis of Eu- and Heterochromatin in *Microtus agrestis*

Many observations indicate that heterochromatin is genetically inactive or less active than euchromatin (for review see [1]). Condensed chromatin of mammalian lymphocytes was shown to synthesize RNA at a lower rate than loosely packed chromatin[2,3]. Autoradiographic studies of H^3-uridine incorporation in living cells of a mouse strain[4], and of female human tissues in vitro[5] indicated also a low rate of RNA synthesis in heterochromatic regions. However, these objects have the disadvantage of not well defined or very small heterochromatic regions limiting the interpretation of autoradiographic preparations.

A very favorable mammal in which to study heterochromatin is the field vole (*Microtus agrestis*) which has very large heterochromatic sex chromosomes, forming distinct chromocentres in certain somatic interphase nuclei in both sexes[6].

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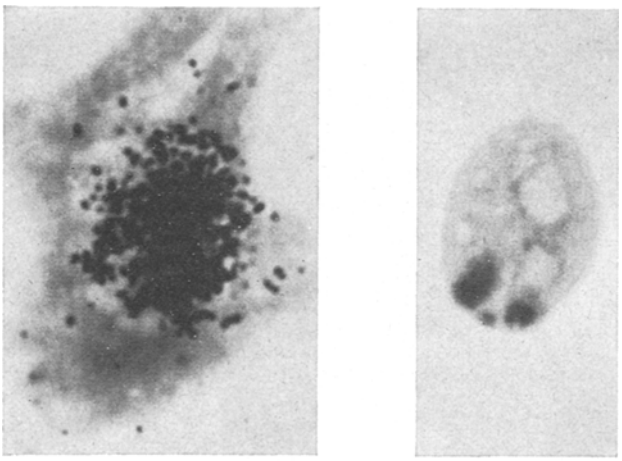
Epithelial cell cultures were set up from trypsinized kidney tissues of female and male *Microtus agrestis* (McCoy's medium 5a with 15% fetal calf serum). The cells were grown on coverglasses and incubated with tritiated uridine (50 µC/ml medium; specific activity 24.9 and 28.3 µC/m*M*) for 3, 10 and 30 min before fixation in 95% ethyl alcohol. They were autoradiographed (Kodak AR 10 stripping film), stained with pararosanilin-methylgreen, and photographed. After removal of the grains they were Feulgen stained and again photographed. Some of the preparations were treated with RNAse (1 mg/ml for 1 h at 37 °C) as controls.

Most of the cell nuclei of kidney epithelial cultures show 2 distinct chromocentres. Sometimes the 2 are fused together resulting in a single large one. In the remaining interphase nuclei the sex chromosomes form either loose structures or are not conspicuous, with the exception of the 'true' sex chromatin part of the *X* chromosomes in female cells[7,8]. If chromocentres are present, they show no or only few grains after labelling with H^3-uridine, whereas the euchromatic regions and the nucleoli are clearly labelled (Figure). No difference in this respect can be observed in preparations which were treated with H^3-uridine for 3, 10 and 30 min. Similar experiments on other cell types, e.g. suspensions of surviving cells from brain and lung tissues, showed also no or only little H^3-uridine incorporation into heterochromatin compared to euchromatin.

Of particular interest are the cells without distinct chromocentres in interphase except a small heterochromatic body, corresponding to sex chromatin in female cells. These cells show a lack of H^3-uridine incorporation in circumscribed nuclear regions which are presumably occupied by the sex chromosomes. This would indicate that heterochromatic chromosomal regions are genetically inactive also in a less or possibly non-condensed state.



a b

Kidney epithelial cell of *Microtus agrestis* in vitro. Absence of grains in heterochromatic regions after H^3-uridine labelling (incubation for 10 min, 40 days exposure). × 2000. (a) Autoradiography, pararosanilin-methylgreen. Chromocentres are stained with methylgreen and appear faint in green filtered photography. (b) After removal of grains and Feulgen staining.

Zusammenfassung. Autoradiographische Untersuchungen nach H^3-Uridineinbau von Nierenepithelzellkulturen der Erdmaus (*Microtus agrestis*) zeigen, dass die Chromozentren nicht oder viel schwächer markiert sind als Euchromatin und Nukleolen. Auch in Zellen ohne sichtbare Chromozentren finden sich Aussparungen im H^3-Uridin-Markierungsbild, die vermutlich den heterochromatischen Geschlechtschromosomen entsprechen. Heterochromatische Chromosomenabschnitte sind demnach genetisch inaktiv oder weniger aktiv, wobei es keine Rolle zu spielen scheint, ob sie in der Interphase zu mikroskopisch sichtbaren Chromozentren kondensiert sind oder nicht.

M. SIEGER, F. PERA[9] and H. G. SCHWARZACHER[9]

Anatomisches Institut, Lehrstuhl II, der Justus Liebig-Universität, D-63 Giessen (Germany), 18 Juli 1969.

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A Hypothesis Concerning the Use of Colchicine as a Polyploidy Inducer[1]

It has been demonstrated in a great variety of plant and animal species that colchicine has the ability to arrest the development of the spindle during mitosis[2], and thereby causes the accumulation of cells at metaphase. In woody plants, however, colchicine may fail to produce its mutagenic effects[3]. This has been explained by the lower rate of division of the cells of the woody plants[4]. Taking this into consideration, ZATYKÓ and SIMON[4] have succeeded in increasing the percentage of myxoploids treating black-currant hybrids with gibberellic acid – a hormone which stimulates cellular division[5] – along with colchicine. ZATYKÓ and SIMON have concluded that the higher number of myxoploid cells obtained is partly the result of the activation of cytokinesis caused by the gibberellin. On this basis, it is suggested that any substance physiologically related to the gibberellin may equally intensify the polyploidy activity of colchicine. The experiments described here seem to show that a low dose of colchicine may fulfill the hormonal requirements which have been outlined.

[1] Acknowledgments. This work has been supported by a research grant from the National Research Council of Canada.
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