

chromosomes of coccids obey quite a different law particularly in respect to orientation and anaphase disjunction, which is parallel. It is, therefore, not unlikely that these chromosomes have a "diffuse" centromere



Fig. 6. Metaphase II, polar view.

similar to that of *Lepidoptera* (FEDELEY¹, SUOMALAINEN²) and *LUZULA* (CASTRO, CAMARA and MALHEIROS³). MENDES⁴ has for the first time reported to have seen the position of primary constriction as a non-staining gap in an heteropteran species, *Dysdercus*, belonging to the

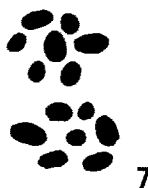


Fig. 7.—Anaphase II.

family Pyrrhocoridae. It is, therefore, not quite improbable to expect a similar centromeric constriction in the chromosomes of other heteropterans as well. An experimental verification similar to that of LACOUR⁵ in *LUZULA* and HUGHES-SCHRADER and RIS⁶ in *Steatococcus* is indeed desirable to support this statement.

I am greatly indebted to Dr. M. L. BHATIA, Head of the Department of Zoology, University of Delhi, for constant encouragement and to Dr. R. I. SAILER, Section of Insect detection and Identification, U. S. Department of Agriculture, Washington, 25, for kindly determining the specimen.

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Résumé

Bagrada picta F. mâle possède 14 chromosomes dont une paire X-Y. Tous les chromosomes sont monocentriques à l'exception de l'Y chez lequel il est impossible de trouver un centromère localisé. Les autres éléments peuvent être, selon leurs dimensions et la position du centromère, rattachés à six types différents.

¹ H. FEDERLEY, Soc. sci. Fenn. 13, 1 (1945).

² E. SUOMALAINEN, Hereditas. 39, 88 (1953).

³ D. CASTRO, A. CAMARA and N. MALHEIROS, Proc. 8th Int. Congr. Genet. Stockholm 548.

⁴ L. O. T. MENDES, Bragantia. 9, 53 (1949).

⁵ L. F. LACOUR, Heredity, Suppl. 6, 77 (1952).

⁶ S. HUGHES-SCHRADER and H. RIS, J. exp. Zool. 87, 429 (1941).

Notes on the Cytological Feature of Male Sterility in the Mule¹

The mule and hinny are noted for their strength and endurance; they are good workers, being active and resistant to sickness. In general, mules and hinnies are

¹ Contribution No. 326 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan. With a financial aid from the Scientific Research Fund of the Ministry of Education.

sterile in both sexes, but female hybrids are said to come on heat and very rarely produce foals to the stallion or the jack-ass. There are no records of fertile male mules or hinnies.

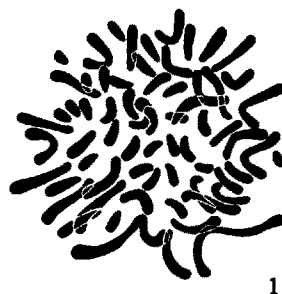


Fig. 1.—Spermatogonial chromosomes of the horse.

The cytological study of the mule has remained incomplete. The chromosomes of the mule have been studied by WODSEDALEK¹ and LEON² who report 51 and 38 chromosomes, respectively. In the light of the results of the chromosome investigations on the horse and ass by MAKINO³, the reinvestigation of the mule chromosomes is desirable, because the work of both WODSEDALEK and LEON is unsatisfactory by present-day standards. The present study became possible through the courtesy of Dr. Y. NISHIKAWA who placed the testicular material of a mule at the author's disposal. The specimen which furnished the material for this study was a male mule, three years of age, produced by crossing of a jack-ass with a mare.

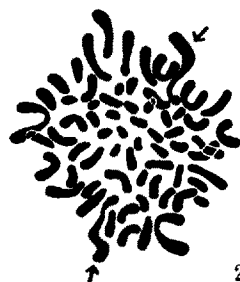


Fig. 2.—Spermatogonial chromosomes of the ass.

Before dealing with the account of the mule, it seems necessary to present some descriptions on the cytological features of the horse and ass, based on the results of the present author's former studies. The number of chromosomes of the horse was reported to be 66 in diploid (MAKINO⁴). The diploid complement shows at least 12 V-shaped chromosomes, each being characterized by submedian centromeres with dissimilar arms (Fig. 1). They are very prominent in appearance in contrast to the others with terminal centromeres.

The author's observations (MAKINO⁵) revealed that in general morphology the chromosomes of the ass were very similar to those of the horse though not entirely identical. The diploid complement of the ass consists of 66 chromosomes which reduce in meiosis into 33 bivalents. There are, as in the horse, at least 12 elements

¹ J. E. WODSEDALEK, Biol. Bull. 30, 1-56 (1916).

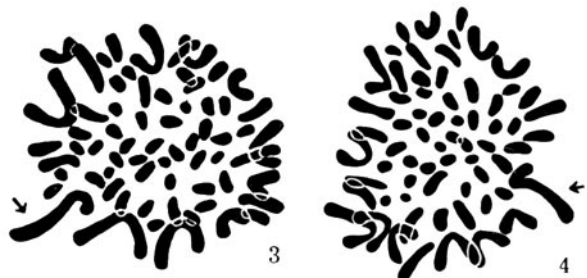
² M. VAN LEON, Ann. Gembloux 1938, 44.

³ S. MAKINO, Cytologia 13, 26-38 (1943); Zool. Mag. (Tokyo) 56, 8-15 (1944); Cytologia 16, 288 (1952).

⁴ S. MAKINO, Cytologia 13, 26 (1943); Zool. Mag. (Tokyo) 56, 8 (1944).

⁵ S. MAKINO, Zool. Mag. (Tokyo) 56, 8 (1944); Cytologia 16, 288 (1952).

characterized by submedian centromeres in the complex (Fig. 2). But closer comparison reveals the constant occurrence of a pair of considerably large-sized J-shaped chromosomes with subterminal centromeres in the ass (indicated by arrows in Fig. 2). Since such a pair does not exist in the complex of the horse, it readily serves to differentiate cytologically the ass from the horse.



Figs. 3–4.—Spermatogonial chromosomes of the mule. $\times 4000$.

The chromosomes of the mule were studied in the spermatogonial division, since the sections of the mule testis showed no cells in process of meiotic division. The number of chromosomes was found to be the same in the mule and its parent animals, horse and ass, namely 66 in diploid (Figs. 3 and 4). In general morphology, the chromosomes of the mule, horse and ass were found also to be very similar but not entirely identical. The diploid set of the mule contains also at least 12 chromosomes which are remarkable for having submedian centromeres. The most noticeable feature of the mule chromosomes, however, is the occurrence of a J-shaped element of considerably large-size, single in number (indicated by an arrow in Figs. 3–4). It is evident that this J-shaped element originated from the ass. From the cytological point of view, therefore, this J-shaped chromosome serves as a striking criterion for distinguishing the chromosome pattern of the mule from that of the parent species.

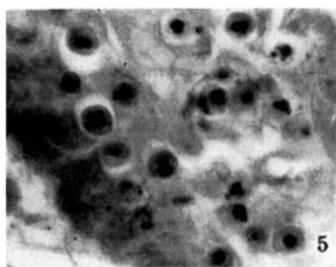


Fig. 5.—Showing pycnotic degeneration of spermatogonial cells in the mule testis. $\times 500$.

So far as the testicular material here under study is concerned, the spermatogonial division proceeds regularly in the mule testes, but the spermatogenesis does not proceed beyond an early meiotic prophase. The germ-cells degenerate without forming the metaphase spindle of the first meiotic division. It seems probable that there are also many which undergo degeneration in the spermatogonial stage without going through meiosis (Fig. 5). Thus the meiotic division is entirely absent in the mule testes and no spermatozoa are formed. The seminiferous tubules of the testes show a remarkable decrease in diameter and contain a very small number of germ-cells (Fig. 6). In the majority of tubules, only one or two layers of such cells line their inner wall, and most of the cells found there are spermatogonia. The tubular lumina are empty, or sometimes contain a mass of de-

generating cell debris. In striking contrast to the shrinkage of the seminiferous tubules, the interstitial tissue shows a remarkable development between the widely separated tubules. The structural difference of the testicular tissue is very striking between the mule and a horse of similar age (three years old), as seen in Figures 6 and 7.



Fig. 6.—Sterile seminiferous tubules of the mule testis. $\times 80$.

It was thus shown that the degeneration of male germ-cells of the mule which directly leads to sterility took place during an early stage of the meiotic prophase and no spermatozoa were produced. The mule from which the present material was derived, showed normal sex drive and mated with mares, but was completely sterile.

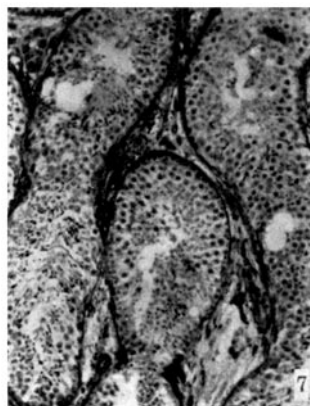


Fig. 7. Seminiferous tubules of the horse testis. $\times 80$.

The cytological phenomena of sterility here observed in the male mule show general similarity to those of hybrid sterility in the cross between *Drosophila melanogaster* and *D. simulans*. These two species possess metaphase chromosomes which appear quite identical each other, whereas the hybrids between them are completely sterile. Neither spermatogenesis nor oogenesis advance beyond spermatogonia and oogonia in the hybrid gonads (KERRIS¹). Sturtevant² showed that the arrangement of genes was different between these two species of *Drosophila*. It was assumed that structural

¹ J. KERRIS, J. Exp. Zool. 66, 477 (1933); Amer. Nat. 70, 81 (1936).

² A. H. STURTEVANT, Genetics 5, 488 (1920); 6, 179 (1921); Carnegie Inst. Publ. 399, 1 (1929).

differences in gene sequence between the parental chromosomes may be the cause of failure of pairing at meiosis in hybrids. But it is known that in *Drosophila*, failure of pairing is not due to mere mechanical impediments. Probably, failure of synapsis in hybrids must be due to a combination of mechanical and physiological impediments. It should be considered, as pointed out by WHITE¹, that each of the parental species probably contains within its chromosomes a large number of genes which control many physiological processes essential to fertile reproduction. The fertile offspring may be produced when all these genes are sufficiently identical in the two parents.

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Zusammenfassung

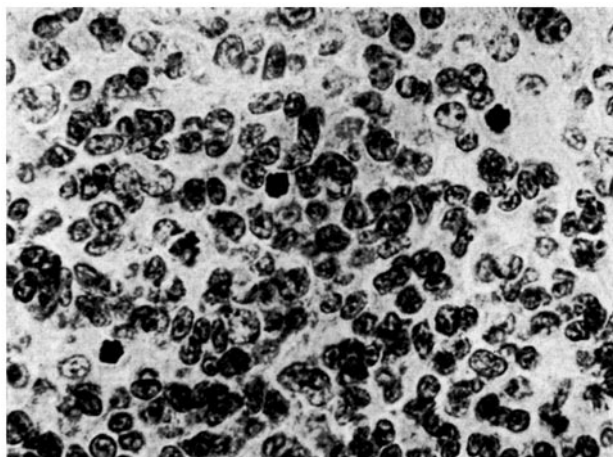
Untersuchungen der Spermatogenese beim Pferd und Esel haben die diploide Chromosomenzahl 66 und die haploide Zahl 33 eindeutig ergeben. Die gleiche Zahl 66 kann beim Maulesel festgesetzt werden. Der Hoden des Maulesels enthält keine Spermatozytenteilung, trotzdem Spermatogonienteilung vorhanden ist. Unfruchtbarkeit beim Maulesel wegen Mangels beider Reifeteilungen hat sich mit Sicherheit feststellen lassen.

¹ M. J. D. WHITE, *Animal Cytology and Evolution*, 2nd ed. (Cambridge Univ. Press, 1954).

De l'existence d'une multiplication de noyaux dans le cerveau du rat après la naissance

Au cours de recherches antérieures¹, il a été démontré que l'acide désoxypentosenucléique (A.D.N.) du cerveau continue à s'accroître après la naissance chez certaines espèces animales telles que le rat, le chien, le chat, le lapin ainsi que chez l'homme. Etant donné que la quantité moyenne d'A.D.N. des noyaux ne subit pas dans ces conditions de variations sensibles, il convenait de déduire de l'accroissement de l'A.D.N. une augmentation du nombre des noyaux, voire des cellules. Cette notion est évidemment en contradiction avec les données classiques selon lesquelles la multiplication cellulaire se termine à la naissance. Il nous a donc paru intéressant de chercher dans une étude histologique la confirmation de la multiplication nucléaire post-natale, au niveau du cerveau. Par la même occasion, il était intéressant de définir la nature des cellules qui continuent à se multiplier. Compte tenu de l'accroissement de l'A.D.N. qui est, chez le rat, de l'ordre de 11,4% par jour jusqu'au 15^e jour, on pouvait prévoir que le taux des cellules en mitoses doit être très faible, surtout si ces mitoses ne sont pas de longue durée. Aussi n'avons-nous pas été étonnés de l'impossibilité de mettre en évidence directement des mitoses au niveau du cerveau de rat dans les premiers 15 jours après la naissance. Nous avons, de ce fait, procédé à l'injection de colchicine avant le prélèvement et l'examen des coupes de cerveau.

Nos essais ont porté sur 8 rats dont l'âge variait de 4 à 8 jours, qui ont reçu 0,1 mg de colchicine pour 100 g de poids frais en injection sous-cutanée, puis 0,2 mg



Microphotographie à l'immersion d'une zone profonde de l'hémisphère. On remarque facilement l'existence de 4 mitoses typiques (sur une diagonale allant du coin inférieur gauche au coin supérieur droit) d'éléments de nature gliale. (Rats âgés de 8 jours.) (Hémaroxyline au fer seule; grossissement $\times 650$.)

après un intervalle de 3 h. Les animaux ont été sacrifiés 2 h après la 2^e injection. Les cerveaux prélevés ont été fixés au liquide de BOUIN et inclus à la paraffine. Les coupes ont été colorées aux techniques usuelles: hémalum-éosine; hémaroxyline au fer seule; trichrome de MASSON; hémaroxyline phospho-tungstique de MALLORY et examinées à l'immersion. Les résultats de nos essais ont été parfaitement concluants. Nous avons pu mettre en évidence des mitoses au niveau de la névroglie comme en témoigne la microphotographie ci-contre.

Nous pensons avec HERLANT¹ qu'il n'est pas possible de déterminer exactement un taux de mitoses après injection de colchicine, mais cela n'a pas été notre but, le problème quantitatif étant résolu par les dosages d'A.D.N.

Il nous paraît également utile d'insister sur l'intérêt des études quantitatives de l'acide désoxyribonucléique au cours de la croissance, études qui permettent de mettre en évidence la présence d'une multiplication de noyaux et d'en évaluer l'intensité, là où l'histologie classique peut être défailante.

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Summary

The increase of the central nervous system desoxyribonucleic acid of the rat after birth has led to the recognition of a multiplication of the brain cells nuclei during the period after birth². Histological studies confirm the conclusions of the biochemical investigations and allow localization of nuclear multiplications in the neuroglia.

¹ M. HERLANT, *Ann. Endoc.* 10, 313 (1949).

² P. MANDEL et R. BIETH, *C. r. Acad. Sci.* 235, 485 (1952).

¹ P. MANDEL et R. BIETH, *C. r. Acad. Sci.* 235, 485 (1952).