On the second day of illumination there was a very little widening of stomata. Only on the third and particularly fourth day of illumination, the opening reaction of stomata was similar to that of the green leaves. The time required for the maximum widening of stomata in light was 100 min, after which the stomata did not open wider. The closing reaction was faster and stomata narrowed to the initial value within 60 min. As is seen from Figure 1, stomata both of etiolated and green leaves do not close completely in darkness. On both etiolated and green leaves, there were about 28,000 of stomata/cm², and their sizes were similar. The average length of stomata was 19.5  $\mu$ .

Figure 2 shows that the total chlorophyll content in leaves increased linearly within 4 days of greening (5 h of light/day) and reached about 1/4 of concentration in green leaves. The rate of transpiration of etiolated and green leaves is presented in Figure 3. From this Figure it can be read that transpiration of etiolated leaves on the first and second day of greening was by about 3 times less than that of green leaves. On the third day of light period, transpiration raised by about 2 times, and finally on fourth day of greening it was similar to that of green leaves. The data of the Figures 1, 2 and 3 suggest a close relationship between the response of stomata to light, the transpiration and the synthesis of chlorophyll in leaves. The opening reaction of stomata starts to work well when the concentration of chlorophyll in leaves reached a certain level (28.1  $\mu$ g/g fresh weight) below which stomata do not respond to light. The rising in the transpiration rate is correlated in time with the opening reaction of stomata. It is doubtful whether such a response of stomata of onion leaves is due merely to the production of osmotically active substances in guard stomatal cells, because it has been shown<sup>6</sup> that photosynthesis was about 50 times too low to account for the maximum rates of osmoticpressure change observed during the opening.

Recently RASCHKE? has showed that the movements of stomata of maize leaves were a function of light

intensity and were similar either in the atmosphere of air, CO<sub>2</sub>-free air or in pure nitrogen. In other words, the reactions of stomata in light were the same during photosynthetic absorption of CO<sub>2</sub> and after removal of CO<sub>2</sub> from the atmosphere. It is a possibility that, in light in etiolated leaves, the developing mechanism of photophosphorylation in chloroplasts<sup>8</sup> provides the energy, may be involved in the operation of a 'pump' that increases the turgidity of guard cells as postulated by ZELITCH 9. The fact that the stomata of both etiolated and green leaves of onion were similar, suggests that light has no visible effect on the formation and development of stomata, and that they are presumably under genetic control. The observation that the stomata of green and albino mutant barley leaves were similar has been reported by Show 10,11.

Zusammenfassung. In etiolierten Blättern der Zwiebel (Alium cepa L.) kann Licht erst nach Einsetzen der Chlorophyllsynthese die Öffnungsbewegung der Stomata induzieren.

J. Poskuta and J. Tomczyk

Department of Plant Physiology, University of Warszawa (Poland), 18 September 1967.

- <sup>6</sup> M. Show and G. A. MacLachlan, Can. J. Bot. 32, 784 (1954).
- <sup>7</sup> K. RASCHKE, Planta 68, 111 (1966).
- <sup>8</sup> D. von Wettstein, Brookhaven Symp. Biol. 11, 138 (1958).
- <sup>9</sup> I. Zelitch, Biol. Rev. 40, 463 (1965).
- 10 M. Sноw, Can. J. Bot. 36, 575 (1958).
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## The Chromosomes of the Didelphiid Marsupial Marmosa robinsoni Bangs

The murine opposums (Marmosa) are the most diversified neotropical marsupials. Cabrera¹ recognized 37 living species, many of them further split into several subspecies. Such a diversified genus affords interesting material for studies on the causes of species diversification in extremely polytypic genera. The history of Marmosa as a whole has been revised by Tate². Little is known about its evolutionary history, the genus being known in the fossil record only in the Pliocene and Pleistocene of Argentina³.

Chromosome studies provide interesting information for investigating speciation. The available evidence about the chromosomes of Marmosa is almost nil. BIGGERS et al. 4 announced preliminary results in the study of chromosomes of M. mexicana, stating that it has a karyotype very similar to that of  $Caluromys\ derbianus$ , with 2N=14 chromosomes, but they have not yet reported the relevant evidences. It seems useful, therefore, to report the preliminary results reached in the study of M. robinsoni's chromosomes even though they are only based on a few individuals.

Two male and 2 female individuals of *M. robinsoni* have been studied for chromosome analysis. They are the

specimens MBUCV 1-1418, MBUCV 1-1429, MBUCV 1-1423 and MBUCV 1-1424 of the Collection of Mammals of the Institute of Tropical Biology, Central University of Venezuela. The first 2 were captured by the author in Los Llanos Biological Station, near Calabozo, Guárico, Venezuela. The last 2 were caught by C. J. NARANJO in 'hato Acapulco', about 25 km south of La Trinidad de Arauca, Apure, Venezuela. Although the localities are not typical for this species, this is one of the commonest mammals inhabiting the small clusters of trees in the savannas of Guárico and Apure. Series from the above localities closely agree with the description of M. mitis casta given by TATE<sup>2</sup>, which, according to CABRERA<sup>1</sup>, is to be named M. robinsoni robinsoni Bangs, 1898.

<sup>&</sup>lt;sup>1</sup> A. CABRERA, Revta Mus. argent. Cienc. nat. Bernardino Rivadavia, Inst. nac. Invest. Cienc. nat. Zool. 4, 12 (1957).

<sup>&</sup>lt;sup>2</sup> G. H. H. TATE, Bull. Am. Mus. nat. Hist. 66, 1 (1933).

<sup>&</sup>lt;sup>3</sup> O. A. Reig, Acta geol. lilloana 2, 255 (1958).

<sup>&</sup>lt;sup>4</sup> J. D. BIGGERS, H. I. FRITZ, W. C. D. HARE and R. A. McFeely, Science 148, 1602 (1965).

Chromosome preparations were made from tissue of the bone marrow, following the techniques describedby Nadler and Bolk<sup>5</sup>. Seventy cells have been observed and 5 karyotypes have been constructed on the basis of enlarged photographs obtained with a Leitz Orthoplan photomicroscope.

The diploid chromosome set of *M. robinsoni* (Figures 1 and 2) comprises 14 chromosomes and it is strikingly similar to that of *C. derbianus* recently described by different authors <sup>6-8</sup>. As in this species, the autosomes clearly fall into 3 groups: 3 pairs of large homologues with

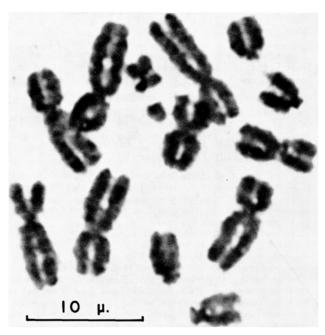


Fig. 1. Mitotic metaphase from a cell of the bone marrow of M. robinsoni Bangs. Acetic orcein stain.  $\times$  2250. Individual No. MBUCV 1-1423.

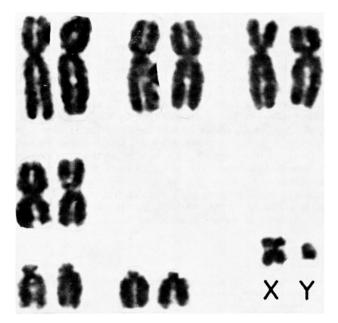


Fig. 2. Karyotype of the male M. robinsoni Bangs, constructed on the basis of the same cell of Figure 1.  $\times$  2 250.

a centromere median or submedian in position, 1 pair of medium sized chromosomes with a clearly median centromere, and 2 pairs of subterminal smaller chromosomes. The sexual pair is formed by a small X of the m type, and an even smaller Y fully telocentric (T). The absolute size of the chromosomes is large, the largest autosomes of the first pair measuring about 11  $\mu$ . Although rather similar in shape and size, the pairs of the first group are easily distinguishable by relative length and arm ratio, as it also the case in C. derbinus. Small differences among M. robinsoni and C. derbianus are apparently shown in the relative length and centromeric position of the autosomes of this first group, but this must be confirmed by measurements in a large number of cells of both species. One clear-cut difference lies in the X chromosome, which is metacentric in M. robinsoni and which has a terminal centromere in C. derbianus.

In any case, the difference between the karyotypes of M. robinsoni and C. derbianus is only in minor details, and these do not affect the challenge of finding a strong similarity in chromosome morphology between members of 2 genera that are far appart in morphology and evolutionary history 4,10-12. As the author has advocated elsewhere 10, Caluromys and their relatives Dromiciops, Caluromysiops and Glironia are members of the subfamily Microbiotheriinae, whereas Marmosa belongs to the subfamily Didelphiinae. The separation of these 2 subfamilies seems to have been very old, as Clemens 13 has described one genus from the late Cretaceous (Glasbius, from the Lance formation of Wyoming) which, in the author's opinion, clearly falls within the microbiotherines. Additional studies in other species of Marmosa and the different genera of living microbiotheres, are certainly required; the field promises to afford interesting material to investigate the role played by chromosome changes in the evolution of such a typically bradytelic group as the didelphiid marsupials.

Résumé. L'étude des chromosomes somatiques du marsupial sudaméricain Marmosa robinsoni Bangs, démontre que l'équipement chromosomique diploïde de cette espèce de la subfamille Didelphiinae est composé de 14 chromosomes. Trois paires d'autosomes sont grands et submétacentriques; 1 paire est formée par de chromosomes moyens, métacentriques, et 2 paires sont acrocentriques et plus petites. Le complexe sexuel est du type XY, formé par un X métacentrique et un Y télocentrique, tous les 2 étant les chromosomes les plus petits du complément. La similitude de ce caryotype avec celui du genre Caluromys, appartenant à la subfamille Microbiotherinae, est remarquable.

O. A. Reig

Instituto de Zoología Tropical, Universidad Central de Venezuela, Caracas (Venezuela), 21 August 1967.

- <sup>5</sup> C. F. NADLER and M. H. Bolk, Chromosoma 13, 1 (1962).
- <sup>6</sup> J. LEGATOR, C. JACOBSON, J. PERRY and D. DOLIMPIO, Life Sci. 5, 397 (1966).
- <sup>7</sup> G. H. Mickey, Mammal. Chromosome Newsl. 8, 41 (1967).
- <sup>8</sup> A. K. Sinha, Mammal. Chromosome Newsl. 8, 54 (1967).
- 9 A. Levan, K. Fregda and A. Sandberg, Hereditas 52, 201 (1964).
- <sup>10</sup> O. A. Reig, Investnes zool. chil. 2, 121 (1955).
- 11 J. P. Hill and E. A. Fraser, Proc. zool. Soc. Lond. 189 (1925).
- <sup>12</sup> J. D. BIGGERS, in Comparative Biology of Reproduction in Mammals (Ed. I. W. Rowlands; Academic Press, London and New York 1966), p. 251.
- <sup>13</sup> W. A. CLEMENS, Univ. Calif. Publs geol. Sci. 62, 24 (1966).