

The Occurrence of an Intermediate Didelphid Karyotype in the Short-Tailed Opossum (Genus *Monodelphis*)

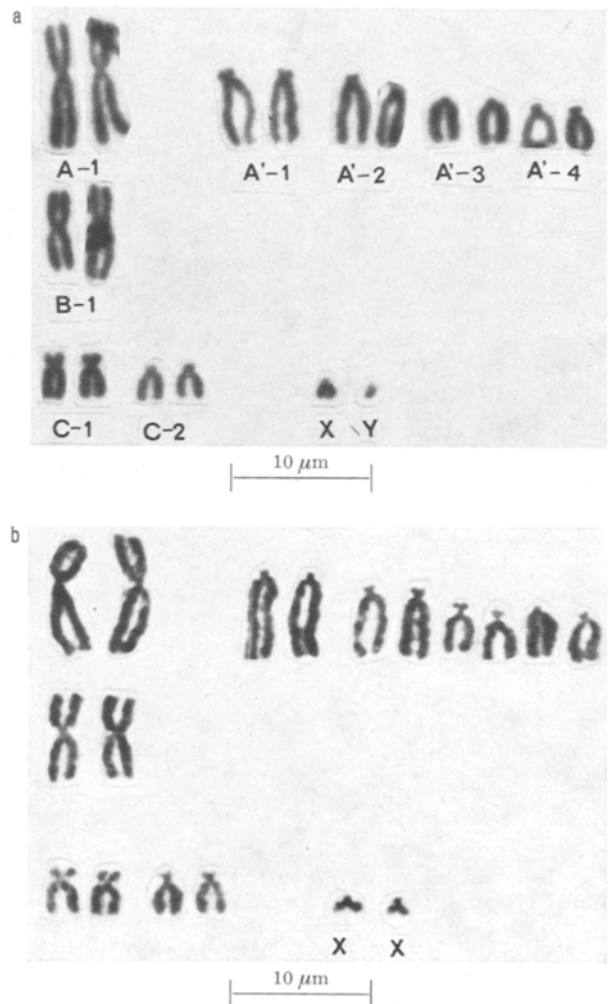
Two different kinds of chromosome complements have been described so far for didelphid marsupials. *Didelphis virginiana* (= *D. marsupialis virginiana* auctorum) is known to have $2n = 22$ chromosomes, comprising 6 pairs of subtelocentric and 4 pairs of telocentric autosomes and a sexual pair formed by a minute submetacentric *X* and an even smaller *Y*¹⁻³. *Didelphis marsupialis*^{4,5}, *Didelphis azarae*⁶ (= *D. paraguayensis*), *Lutreolina crassicaudata*⁷ and *Philander opossum*^{8,9} share a similar $2n = 22$ karyotype slightly differing from that of *D. virginiana* in the fully telocentric nature of all the autosomes and the *X*. Species of *Caluromys*^{9,10} and *Marmosa*^{9,11} share a quite similar karyotype of $2n = 14$ chromosomes composed of 3 pairs of large submetacentrics (Group A), one pair of medium-sized metacentrics (Group B) and 2 pairs of small acrocentrics (Group C), plus an *XY* sexual system essentially like that of *D. virginiana*.

Therefore, and as far as the present information allows us to conclude (only 8 out of a total number of 66 living species of didelphids are known as regards chromosomes), didelphids repeat the distribution of chromosome numbers of Australasian marsupials^{12,13}. In these latter, however, the number of $2n = 16$ was found in 4 out of 56 species studied, but the number of 18 chromosomes, representing the average to the nearest whole number between all the studied Australian forms, has not been reported so far for any marsupial.

The karyotype of 2 species of *Monodelphis* recently investigated by us, show this intermediate number of $2n = 18$ chromosomes. We have studied the chromosomes of *Monodelphis brevicaudata palliolata* (Osgood) from northern Venezuela and *M. dimidiata* (Wagner) from Argentina. Of the former, we investigated 4 males and 1 female from Rancho Grande (Aragua), and 1 female from Kashmera (Zulia), all the 6 specimens being deposited in the collection of mammals of the Institute of Tropical Zoology in Caracas. Only 1 male and 1 female of *M. dimidiata* were studied, both coming from Arroyo Brusquitas, General Pueyrredón, Buenos Aires Province¹⁴. Both bone-marrow and spleen cells were used, following the colchicine-hypotonic pretreatment-air drying sequence described elsewhere^{15,16}. With these 2 species, it was difficult to get good chromosome spreads, the success obtained using different techniques was far less than that achieved for other didelphids. Nevertheless, the karyotypes described below were confirmed in 53 metaphases of *M. dimidiata* and 58 of *M. b. palliolata*.

The Figure shows the karyotypes of a male *M. b. palliolata* and a female *M. dimidiata*. The autosomes have been arranged in 3 groups for a better comparison with the karyotypes of *Caluromys*^{9,10} and *Marmosa*¹¹. The 2 species of *Monodelphis* have quite a similar karyotype composed of a single pair of large submetacentrics (A-1), 1 pair of medium-sized metacentrics (B-1), 2 pairs of medium-sized acrocentrics (A'-1, A'-2), 4 pairs of small acrocentric or subtelocentric autosomes (A'-3, A'-4, C-1, C-2), and a sexual pair composed of a minute telocentric *X* and a dot-like *Y* chromosome. The only appreciable difference between the 2 species lies in the A'-1 pairs: that of *M. dimidiata* is formed by chromosomes with a very minute short arm, whereas those of *M. b. palliolata* have slightly longer short arms and hence a rabbit-ear appearance.

Comparing the karyotypes of *Monodelphis* with those of *Caluromys* and *Marmosa*, it is easy to conclude that chromosomes of groups B and C are quite similar in both



Karyotypes from bone marrow cells of: (a) *Monodelphis brevicaudata palliolata* (Osgood), male individual from Rancho Grande, Aragua (Venezuela); (b) *Monodelphis dimidiata* (Wagner), female individual from Buenos Aires Province (Argentina).

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cases, probably comprising the same elements. Pair A-1 of *Monodelphis* agrees in size and arm ratio with the second pair of group A of *Marmosa* and *Caluromys*, whereas the 4 pairs of group A' of *Monodelphis* can be combined to result in the 2 remaining pairs of large submetacentrics of group A of *Marmosa* and *Caluromys*. Therefore, the karyotype of the short-tailed opossums can be derived from the $2n = 14$ karyotype of *Marmosa* and *Caluromys* by the transformation by centromeric fission of 2 pairs of large submetacentrics into 4 pairs of telocentrics. Further pericentric inversions are also to be assumed to explain the short arms in 2 or 3 pairs of the A' group of *Monodelphis*. It could be alleged, however, that the change was in the opposite direction. The $2n = 14$ type should thus be derived from that of *Monodelphis* by the well-known process of centric fusion. *Monodelphis* could hardly be considered, however, as a genus more primitive than either *Marmosa* or the early separated branch of the Microbiotheriinae¹⁷ to which *Caluromys* belongs. It is much more likely that the short-tailed opossums (*Monodelphis* and *Lestodelphis*), which show terrestrial modifications and several advanced characters, are derivatives of the early arboreal marmosoid stock, from which all the didelphid radiation might have taken its origin. By the same assumption, the $2n = 22$ karyotypes shared by a group of large-sized genera of didelphines might be thought of as derived from the $2n = 14$ pattern by the full transformation of 4 pairs of biarmed chromosomes into 8 pairs of telocentric ones.

Resumen. Se han estudiado los cromosomas de dos especies de didelphidos del género *Monodelphis*: *M. brevicaudata palliolata* del norte de Venezuela y *M. dimidiata* de la Prov. de Buenos Aires, Argentina. Ambas especies tienen un cariotipo muy similar, compuesto de 18 cromosomas. Este cariotipo es intermedio entre el que presentan *Caluromys* y *Marmosa* ($2n = 14$) y el de *Didelphis*, *Philander* y *Lutreolina* ($2n = 22$). Se postula la fisión céntrica en dos pares de submetacentricos del primer tipo, para explicar el origen del cariotipo de *Monodelphis*.

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A New Strain of *Aspergillus niger* Producing Citric Acid

A project for the development of superior strains was undertaken in this laboratory with the object of improving the yield of citric acid by taking resort to the technique of induced mutation and strain selection. A strain of *Aspergillus niger* was treated with various mutagenic agents like UV-irradiation, γ -ray irradiation from Co-60 and nitrogen mustard^{1,2}. Fermentations were run in stationary flask cultures at 28–29°C and broth was harvested on the 10th day of incubation. The fermentation medium contained glucose, NH_4NO_3 , KH_2PO_4 and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and the pH was adjusted to 2.0.

The parent strain used in the experiment is *Aspergillus niger* strain 'C' which produced an average of 28.0 mg of citric acid/ml of the fermentation medium. This strain was subjected to the action of mutagens. UV-rays of 2537 Å were used which emitted energy at 100 ergs/mm²/sec. Spores were treated for 8, 12, 16, 24, 28 and 32 min. The doses administered in γ -ray experiment were 10,000 r, 20,000 r and 30,000 r. Nitrogen mustard was used in the strength of 0.1% and 1.0%. However, following each experiment, 500 substrains were grown in fermentation medium to test for their citric acid yielding capacity. Citric acid was estimated colorimetrically by the method of MARIER and BOULET³. Of the substrains studied, the selected mutant was CG110 producing 56.1 mg of citric acid/ml of the medium. This mutant was derived from γ -irradiation experiment, and was next subjected for the second time to UV-irradiation. Surviving 500 substrains were again tested for citric acid production. The best of this population was CGU163 which produced an average of 72.1 mg of citric acid/ml.

Detailed fermentation studies were next made with the strain CGU 163 and it revealed that, under optimum fermentation conditions, the mutant showed itself 4.3 times superior to the original parent strain *A. niger* 'C', as it produced 119.2 mg of citric acid/ml in contrast to 28.0 mg/ml of citric acid produced by the parent.

The final composition of the medium was found to be (g/l) sucrose 150.0; NH_4NO_3 2.3; K_2HPO_4 1.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.25; $\text{Fe}(\text{FeSO}_4 \cdot 7\text{H}_2\text{O})$ 10.0; $\text{Mn}(\text{MnSO}_4 \cdot 4\text{H}_2\text{O})$ 10.0. The development of the second step mutant CGU163 with a considerable increase in the citric acid yield may thus be considered as a partial success in a venture to explore the possibility of evolving outstanding mutations in microorganisms.

Zusammenfassung. Durch Behandlung eines Zitronensäure produzierenden Stammes von *Aspergillus niger* mit γ -Strahlen, N-Lost und UV-Strahlen konnte die Zitronensäureausbeute um ungefähr das Vierfache gesteigert werden.

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