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Chromosomes of *Microtus (Stenocranius) gregalis major* (Ognev, 1923) and Phylogenetic Connections between Sub-arctic Representatives of the Genus *Microtus* Schrank, 1798

[With 1 Fig. and Plates I—II]

A description is given of the karyotype of *Microtus gregalis major* (Ognev, 1923): $2N = 36$, $NF = 54$. The phylogenetic connections between the palearctic and nearctic species: *Microtus middendorffi*, *M. gregalis*, *M. abbreviatus* and *M. miurus*, are discussed on the basis of comparative cytogenetic, morphological and paleontological data. The author puts forward the hypothesis that the evolution of karyotypes in palearctic species took place by means of centric fusions, whereas in the case of nearctic species this occurred through pericentric inversions. The possibility is discussed of the existence in the Pleistocene of three radiation groups which led to the contemporary subarctic species of *Microtidae*.

I. INTRODUCTION

The great variation in the number of chromosomes in different species of the genus *Microtus* is the reason why numerous cytogenetic studies have been undertaken on *Microtidae*. The extreme $2N$ values for 18 species of this genus vary from 17 to 62 (Matthey, 1958). It would not appear that these limits will be greatly widened as the result of research.

Up to the present a description has been given only from North America of the chromosomes of two species considered as belonging to the sub-genus *Stenocranius* Kastschenko, 1901 (Rausch, 1964; Rausch & Rausch, 1968). In order to obtain comparative data which might contribute to a more exact knowledge of the phylogenetic connections between subarctic species of the genus *Microtus* a description has been given in the present study of the karyotype of *Microtus (Stenocranius) gregalis major* (Ognev, 1923), a subspecies inhabiting the Siberian tundra.

Microtus gregalis major, originally described as a separate species (cf. Ellerman & Morrison-Scott, 1951), may be treated in the light of up-to-date research, as a palearctic representative of *M. gregalis* (Schwarz *et al.*, 1960).

II. MATERIAL AND METHOD

The 5 ♂♂ and 2 ♀♀ used for the investigations were taken from the laboratory stock of the Mammals Research Institute at Białowieża. The breeding of this subspecies was begun in 1966, when four pairs were brought from the Lomonosov University in Moscow. The skulls and skins of the animals used for cytogenetic investigations are kept in the collection of the Mammals Research Institute, Polish Academy of Sciences at Białowieża.

Chromosome slides were made from the spleen and bone marrow, and in the case of males additionally from the testes. Intraperitoneal injections of 0.025% colchicine water solution (0.1 ml per 1 g of body mass), was used to arrest mitosis at the metaphase. The animals were usually killed 2 hours after the injection and a cell suspension made in 0.7% sodium citrate solution. After a twenty-minute hypotonic shock the cells were fixed in Carnoy's fixing fluid (3 parts absolute ethanol + 1 part of glacial acetic acid). The slides were made by the »air drying« method after Ford & Woollam (1963).

III. RESULTS

Examination of 274 metaphase plates permitted of establishing the diploid number of chromosomes in *Microtus gregalis major* as: $2N = 36$. This number was confirmed in 210 cases, that is about 77% of all the metaphase plates examined. In 22 plates there were 35 chromosomes in each case, in 15—34, and in 5 cases 37 chromosomes were found.

The karyotype consists of the following pairs of chromosomes: 5 large metacentric, 3 submetacentric, 9 acro- or telocentric, heterochromosomes: X — large metacentric, with ratio of arms 1/1, Y — is a telocentric element (Fig. 1, Plate I—II). The length of chromosome X corresponds to the length of the first pair of metacentric autosomes, while chromosome Y cannot be distinguished from the longest autosomes in the monobrachial series.

The number of arms of chromosomes (NF) in the karyotype of the female was 54, including in this figure the arms of the two metacentric X chromosomes.

IV. DISCUSSION

On the basis of morphological investigations and zoogeographical comparisons Rausch (1964) established that the North American *Microtus miurus* Osgood, 1901 exactly corresponds to the northern group of Eurasian subspecies of *Microtus gregalis*. These studies confirmed the correctness of combining *M. miurus* with *M. gregalis* in one sub-genus. A debatable point here is however whether *Microtus abbreviatus* Miller, 1899 belongs to this same subgenus. Zimmermann (1942) and Rausch (1963) opposed the inclusion of this species in the subgenus *Stenocranius*. It is known from other data that *Microtus middendorffi*

(Poljakov, 1881) is morphologically most closely related to *M. abbreviatus* (Matthey & Zimmermann, 1961).

We now have karyological data on these four sub-arctic species:

<i>M. miurus</i>	$2N = 54, NF = 72$ — Rausch, 1964; Rausch & Rausch, 1968
<i>M. abbreviatus</i>	$2N = 54, NF = 72$ — Rausch & Rausch, 1968
<i>M. middendorffi</i>	$2N = 50, NF = 54$ — Matthey & Zimmermann, 1961
<i>M. gregalis</i>	$2N = 36, NF = 54$ — Present paper.

When we compare the karyotypes of *M. gregalis* and *M. miurus* — species possessing characters typical of the subgenus *Stenocranius* — we find considerable differences in respect both of the number of chromosomes ($2N$ 36 and 54) and number of arms of the chromosomes (NF 54 and 72). The karyotype of *M. gregalis* is closer to the karyotype of *M. middendorffi*. The number of arms (NF 54) common to both species suggests the possibility of the divergency of these two karyotypes through polymorphism of the Robertson type (association of acrocentric chromosomes with meta- or submetacentric). The karyograms of *M. miurus* and *M. abbreviatus* completely correspond to each other in respect of number of chromosomes, number of arms and the morphology of the various pairs of chromosomes. Finally it must be pointed out that the heterochromosomes of these four species are formed in a different way, and it is only in the case of *M. miurus* and *M. abbreviatus* that the sex chromosomes are identical: X — large metacentric, Y — small telocentric. The relative lengths of these chromosomes are the same in both species. The X-chromosome in *M. gregalis* and *M. middendorffi* is morphologically similar to the X-chromosome in *M. abbreviatus* and *M. miurus*, although the relative lengths of these chromosomes are probably different, whereas the Y-chromosomes in *M. gregalis* and *M. middendorffi* are completely incomparable, since in the first of these species Y is a large telocentric chromosome and in the second Y is distinctly submetacentric.

Several conclusions can be drawn from the above comparisons:

(1) *M. gregalis* is a species cytologically distant from *M. miurus*. In view of the great difference in karyotypes these are most certainly reproductively isolated forms. Differences have also been demonstrated in the structure of the genital organs between these species (Anderson, 1960). It is thus incorrect to combine them into one species (cf. Rausch, 1963; 1964).

(2) The identical character of the karyotypes of *M. miurus* and *M. abbreviatus* argues in favour of including the latter in the subgenus *Stenocranius*, contrary to the opinion expressed by Zimmermann (1942)

and Rausch (1963), who emphasise the morphological differences between these two species. Attempts at cross-breeding *M. abbreviatus* with *M. miurus* (Rausch & Rausch, 1968) were not completely successful, this probably being due more to breeding failures than to the existence of reproductive isolation. The authors found embryos which must be considered as hybrids in one dead female. The final decision as to taxonomic status should, however, be based on results of crossbreeding which leave no room for doubts.

(3) *M. middendorffi* — a species morphologically similar to *M. abbreviatus* — is cytologically more similar to the Euro-Asiatic *M. gregalis*.

(4) The morphological similarity between pairs of the species *M. gregalis* — *M. miurus*, *M. middendorffi* — *M. abbreviatus* and the cytological similarity between *M. miurus* — *M. abbreviatus* and *M. gregalis* — *M. middendorffi* points to the common origin of this whole group of species.

Matthey (1957) showed that in the case of *Microtidae* the transformation of 2 uniarmed chromosomes into 1 biarmed element takes place parallel to progressive speciation. In this author's opinion the primitive number of chromosomes in *Microtidae* was 56, the number of arms of the chromosomes being from 56—58. The evolution of the chromosomes took place by means of centric fusion, and in certain cases other aberrations also played some part.

The number 54 is repeated in the karyotypes of *M. middendorffi*, *M. gregalis*, *M. abbreviatus* and *M. miurus*, as *NF* in the first two species, and as *2N* in the others. It may therefore be assumed that the primitive karyotype for these species was: $2N = 54$, $NF = 54$. The evolution of chromosomes took place in two ways: the karyotypes of Asiatic species evolved by means of the mechanism of centric fusion, and thus retained the original number of arms, while reducing the number of chromosomes. The evolution of the karyotypes of *M. abbreviatus* and *M. miurus*, on the other hand, took place as the result of pericentric inversion, which led to the retention of the original number of chromosomes and increase in the number of arms (Fig. 2).

The lack of special studies on sex chromosomes makes it impossible to put forward a hypothesis as to the evolution of heterochromosomes in this group of species. It is impossible to establish, on the basis of the cytogenetic data so far available, whether the formation of elements of the neo-XY type took place during differentiation of heterochromosomes, or whether some other aberration played a part here. In any case it would appear likely that changes within the sex chromosomes exert a greater influence on reproductive isolation than any transformation within the autosomes, and thus they could more effectively isolate re-

productively the sympatrically occurring forms. It is also probable that the early differentiation of sex chromosomes formed the basis of the subarctic speciation of species of the genus *Microtus*. The presence of identical heterochromosomes in *M. abbreviatus* and *M. miurus*, on the other hand, is evidence of the relatively late separation of these species. In addition the identical character of autosomes in these species is proof that the evolution of karyotypes in them was completed before attainment of geographical isolation.

In Schwarz's opinion (1963) the subarctic species *M. gregalis* and *M. middendorffi* originate from steppe forms of the «arvalis» type. The lack of reproductive isolation between the southern (*M. gregalis gregalis*) and northern group (*M. gregalis major*) indicates that the originally

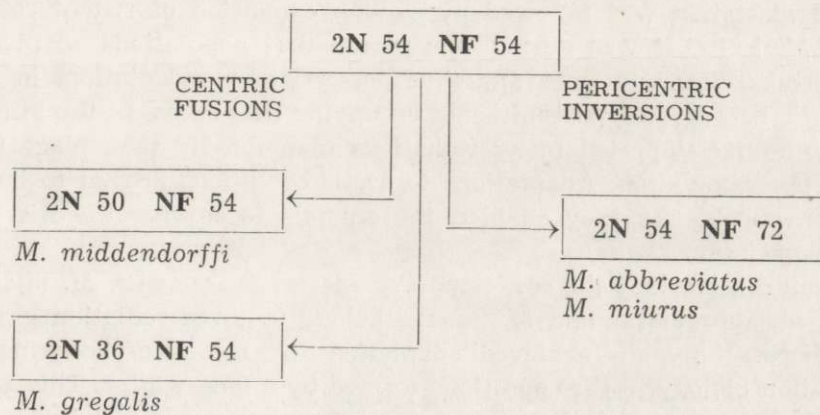


Fig. 2. Diagram of the evolution of the karyotypes of four subarctic species of the genus *Microtus*.

steppe forms spread in a northern direction and occupied the tundra (Schwarz *et al.*, 1960). Populations possessing preadaptation enabling them to live in the subarctic zone must therefore have separated from the primitive population of steppe voles by means of radiation. Schwarz (1963) emphasises that where favourable conditions exist *Microtus gregalis* living in steppes can reproduce before the disappearance of the snow cover. This departure from the reproduction biology might have been of great importance as aromorphosis in the process of spreading into and inhabiting the tundra. At the same time it permitted this species to inhabit the tundra without the necessity for making any great physiological adaptations. *M. middendorffi*, on the other hand, could only occupy the subarctic tundra region after physiological adaptation (Schwarz, 1963).

Paleontological data show that in the late Pleistocene the range of the Euro-Asiatic *M. gregalis* was far greater than it is at present. In Europe (the largest number of discoveries from Würm glaciation period) it was always connected with the faune of the arctic tundra (Kowalski, 1959). In northern Asia *M. gregalis* is known from the Riss and Würm glaciation period. In North America representatives of the Pleistocene *Stenocranius* are known from excavations in the Fairbanks district (Repenning *et al.*, 1964). Although there is only a small amount of data from Alaska on the fauna of the Pleistocene it may be assumed on the basis of Repenning's data, that the range of *M. miurus* at present extends much further to the east than during the Pleistocene. Rausch (1964) also suggested this possibility.

In the light of these data it may be assumed that the retreat of the Mindel glaciation was followed by sudden expansion of rodents of the genus *Microtus*. It was during this period that populations adapted to life in subarctic conditions, and thus possessing preadaptation in this direction, had great opportunities of occupying new areas. In the Mindel-Riss interglacial period many radiations undoubtedly took place from the steppe population. Adaptations to arctic conditions proved to be the most favourable as they enabled the animals to survive the Riss and Würm glaciations.

It would seem that the contemporary species *M. gregalis*, *M. middendorffi*, *M. abbreviatus* and *M. miurus* belong to three radiation groups:

I. *Microtus gregalis* achieved adaptations by means of adapting reproduction biology to the conditions formed by a long winter. This group expanded to and occupied a large area in Asia and Europe and was characterized by rapid evolution rate, proof of which is the relatively considerable degree of subspecies differentiation. The karyotype of this species, evolving by means of the mechanism of centric fusion, achieved a high degree of complication.

II. *Microtus middendorffi* forms a separate group, the speciation of which proceeded in the direction of physiological adaptations. It is a conservative group from the morphological and karyological aspects. The karyotype of *M. middendorffi* is most similar to the primitive karyotype in this group of *Microtidae* and also evolved by means of centric fusion. Physiological adaptations created the possibility of making better use of the habitat, and it therefore settled evenly over its range, contrary to *M. gregalis*, which is characterized by mosaic-like distribution over its range (Schwarz, 1963).

III. *Microtus abbreviatus* and *M. miurus* belong to a common radiation group. After reaching and occupying the platform connecting North America with Asia, this group was geographically isolated from the

Asian population and from that time on evolved independently. During the period when the platform lost its connection with the continents the rate of speciation probably increased, due certainly to deteriorating conditions. The evolution of the karyotype must have been completed by the time a new connection was formed between the continents of Asia and America, since the karyotypes of *M. abbreviatus* and *M. miurus* are identical and during the subsequent period these species were completely isolated, this state of affairs having continued up to the present.

M. abbreviatus (the endemic form) adapts itself to specific island conditions, while *M. miurus* occupied the subarctic areas of the American tundra during the postglacial period (R a u s c h, 1963, 1964). This latter species adapted itself to a wide variety of habitats and achieved a fairly great degree of subspecies differentiation.

Morphological comparisons suggest that there are two radiation groups. On the basis of morphological characters only it is possible to allocate the species *M. gregalis* and *M. miurus* (species possessing characters typical of the subgenus *Stenocranius*) to the first group, while the second group will consist of *M. middendorffi* and *M. abbreviatus*, similarly to Matthey & Zimmermann (1961) who proposed, on the basis of comparisons of the morphological characters, the creation for these species of the »middendorffi« group. If we assume that morphological connections between pairs of these species reflect phylogenetic connections, we should have to accept the existence of two such completely separate radiation groups. This is refuted by karyological data, since it would be necessary to accept that after achieving total geographical isolation, the chromosomes of *M. abbreviatus* and *M. miurus* from two different initial forms evolved independently of each other, in exactly the same way, finally leading to the formation of identical formulae, which would appear to be completely improbable. It is surely more correct to accept the existence of three radiation groups originating directly from one Mendelian population of voles inhabiting the Euro-Asiatic steppes. The morphological similarity between *M. miurus* and *M. gregalis* must therefore be explained by convergence. The two species occupy similar ecological niches, and *M. miurus* thus achieved the same idioadaptations during the postglacial period which *M. gregalis* must have acquired during the Mindel-Riss interglacial period.

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CHROMOSOMY *MICROTUS (STENOCRANIUS) GREGALIS MAJOR* (OGNEV, 1923) I ZWIĄZKI FILOGENETYCZNE MIĘDZY SUBARKTYCZNYMI PRZEDSTAWICIELAMI RODZAJU *MICROTUS* SCHRANK, 1798

Streszczenie

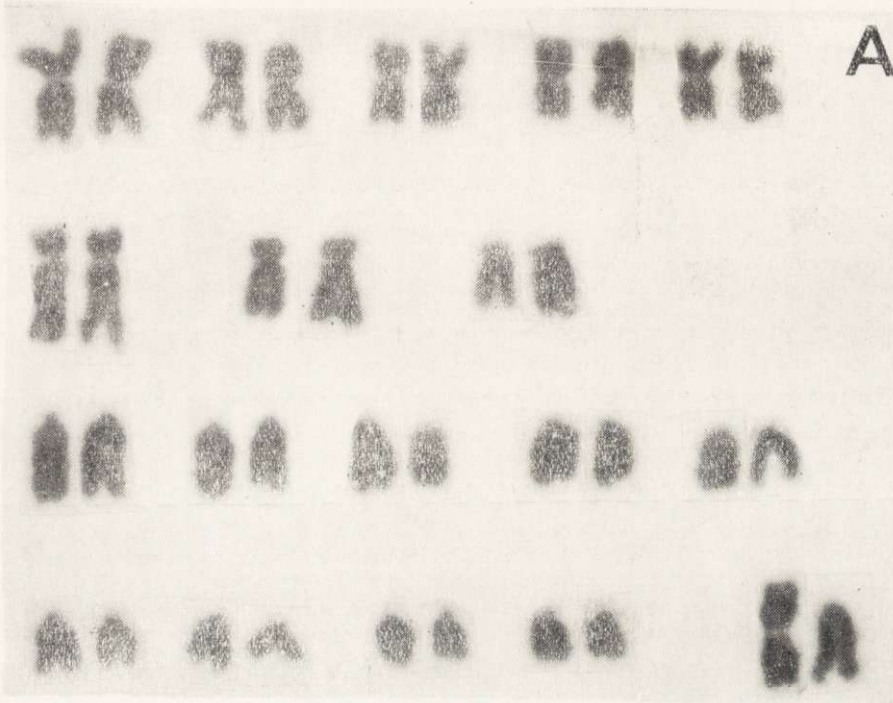
Opisano kariotyp *Microtus gregalis major* (Ognev, 1923). Liczba chromosomów w komórkach somatycznych $2N = 36$, liczba ramion chromosomów $NF = 54$ (Ryc. 1). Ustalono, że *M. gregalis* posiada różny kariotyp od nearktycznych przedstawicieli podrodzaju *Stenocranius*. W oparciu o dane porównawcze wysunięto hipotezę o wspólnym pochodzeniu subarktycznych gatunków: *M. gregalis*, *M. middendorffi*, *M. abbreviatus* i *M. miurus*. Ewolucja kariotypów gatunków palearktycznych i nearktycznych poszła odmiennymi drogami (Ryc. 2).

Na podstawie danych porównawczych z cytogenetyki, morfologii i paleontologii przedyskutowano możliwość istnienia w plejstocenie trzech grup radiacyjnych *Microtidae*. *M. middendorffi* i *M. gregalis* tworzą dwie zbliżone kariologicznie grupy radiacyjne, różniące się tempem ewolucyjnych zmian, co powoduje morfologiczne zróżnicowanie ich mimo sympatrycznego zasięgu. *M. abbreviatus* i *M. miurus* połączono we wspólną grupę, różnice morfologiczne między tymi gatunkami tłumaczono izolacją geograficzną i ścisłą adaptacją do różnych warunków środowiskowych.

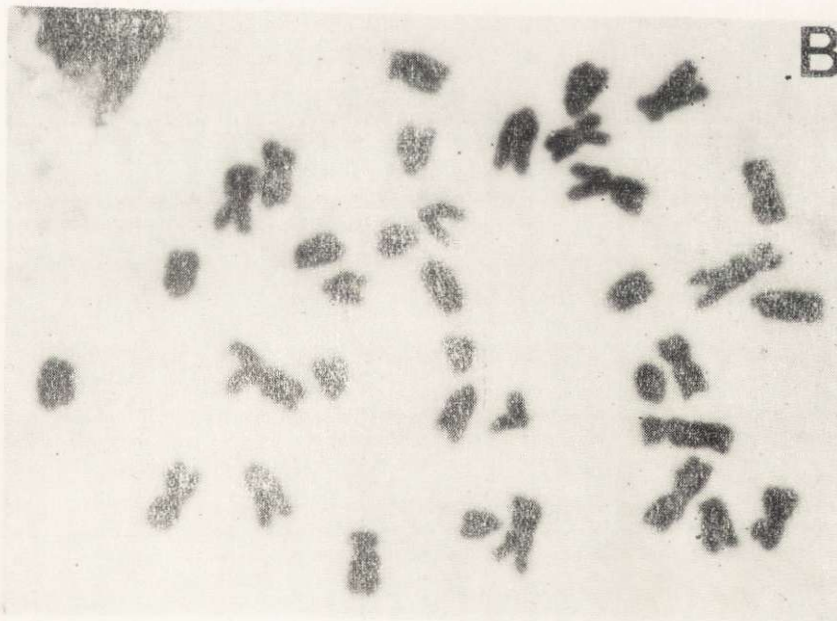
EXPLANATION OF PLATES

Plate I & II

Fig. 1. *Microtus gregalis major*. Metaphase chromosomes plate. A — male karyotype, B — male metaphase plate, C — female karyotype, D — female metaphase plate.



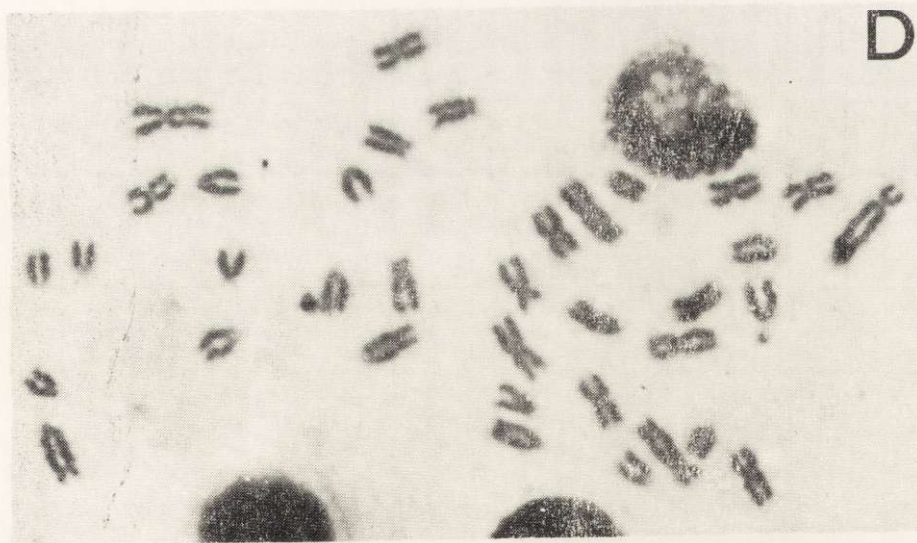
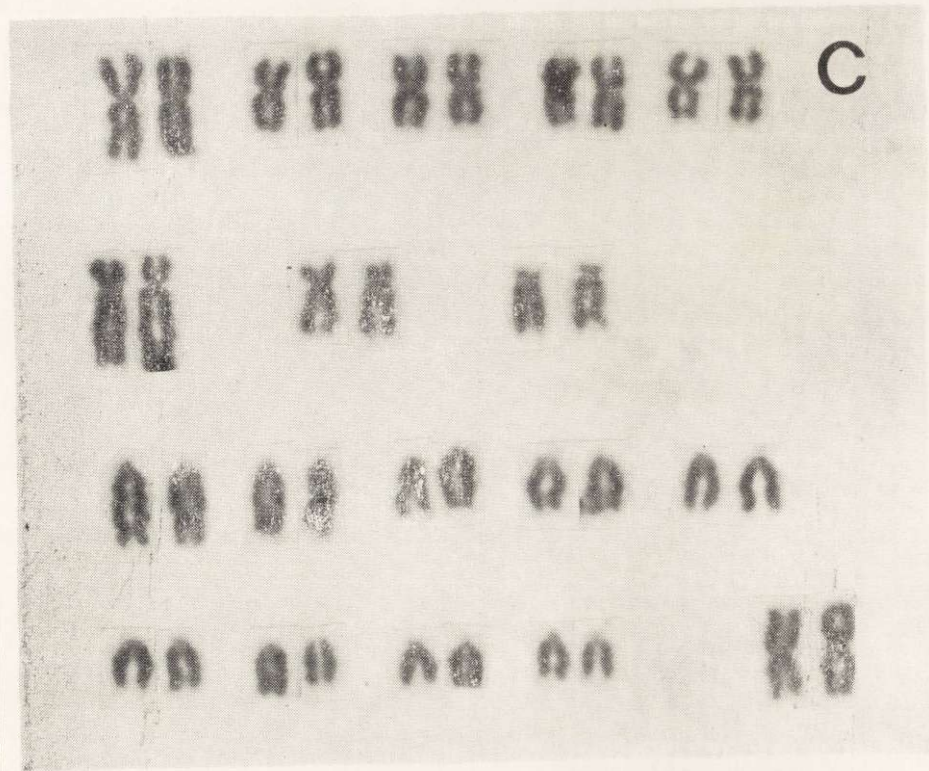
A



B

S. Fedyk

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