until TLC homogeneous. Over 500 mg of the pure material were synthesized [IR (CCl_4): 1690, 1655, 1600 cm^{-1}; NMR: singlet (1H) $\tau = 1.93$ ppm (N–CH=O), singlets (3H each) $\tau = 6.12$, 6.72, 6.74, 6.77 ppm (4 $-OCH_3$); $m/e = 415$][14]. The synthetic compound **2** was hydrolyzed by heating with methanol-concentrated hydrochloric acid (9:1) at reflux for 24 h. The product **3** was purified by chromatography and crystallization from ether [yield 94%; mp 176°; $m/e = 387$].

Finally, acetylation of the synthetic secondary amine **3** with acetic anhydride-pyridine gave compound **4** in a yield of 95% [mp 188–190° (hexane-ether); IR (CCl_4): 1685, 1650, 1600 cm^{-1}; NMR: singlet (3H) $\tau = 7.90$ ppm ($-\underset{\underset{O}{\|}}{C}-CH_3$); $m/e = 429$].

The totally synthetic racemates **2**, **3** and **4** were proved to be identical with the corresponding optically active 'natural' compounds[2] by TLC in several systems, IR in chloroform and carbon tetrachloride, NMR and mass spectroscopy. Work on the construction of the C–D ring system of delphinine is in progress[15].

Zusammenfassung. Die stereoselektive Totalsynthese eines Delphininabbauproduktes wird beschrieben. Dieses Produkt, das 5 Ringe und 5 Substituenten besitzt, ist von Delphinin aus leicht zugänglich und kann deshalb als Relais-Verbindung für die Totalsynthese dieses Alkaloids dienen.

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T. Kanno, J. Křepinský, Lizzie Poon,
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Natural Products Research Center,
University of New Brunswick,
Fredericton (New Brunswick, Canada), 20 April 1970.

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[15] We wish to thank the National Research Council in Ottawa and the Hoffmann-La Roche Company, Nutley, New Jersey for financial support. We also thank the Merck Co., Mannheim, for a donation of a large amount of crystalline aconitine and S. B. Penick & Company, Orange, New Jersey, for a gift of a large amount of *Delphinium* seeds.

Chromosomes and Some Issues of the Evolution of the Ground Squirrel Genus *Citellus* (Rodentia: Sciuridae)

Chromosome analysis, combined with other methods of systematic zoology, paleontology and zoogeography, can be used to give an integrated analysis of the evolution of taxonomically discrete faunal elements. Such analyses are of special value in studies of the evolution, in relation to space and time, of allied Eurasian and North American forms.

The evolution of the chromosome complements of Nearctic *Citellini* has been thoroughly studied by Nadler et al.[1–10]. The understanding of the evolution of the entire Holarctic genera, however, requires inclusion of the Palearctic species. The present study concerns the chromosomes of 6 species and 20 subspecies and forms of the genus *Citellus*, and a discussion of the results in conjunction with those of Nadler. In the course of the study, we have produced a hypothetical reconstruction of the evolution of the karyotypes of the species studied, with particular reference to the correlation of the range of species with similar and differing chromosome complements.

Materials and methods. The karyotypes of the following forms of *Citellus* have been studied. *C.* (*s. str.*) *relictus relictus* Kaschk., Western Tien Shan, Kuraminsky ridge near Kamtchik pass, 2300 m above sea level, 3 ♂♂, 9 ♀♀. *C. relictus ralli* Kuznetsov, Central Tien Shan, Issyk Kul basin, Tersky Ala Tau ridge, south of the town Prjevalsk, 2100 m, 2 ♂♂. *C. dauricus dauricus* Brandt, Transbaikalia, Chita region, environs of the village Borzia, 6 ♂♂, 5 ♀♀. *C. pygmaeus pallidus* Orlov, Kalmyk A.S.S.R., State farm 'Polinniy', 3 ♂♂, 3 ♀♀. *C. p. pygmaeus* Pall., between Volga and Ural rivers, left bank of Volga, north of Astrakhan, 1 ♂, 2 ♀♀. *C.* (*Colobotis*) *fulvus oxianus* Thom., Central Kyzyl Kum desert, Bukhara region, environs of the village Mubarek, 2 ♂♂. *C. f. orlovi* Ogn., between Volga and Ural rivers, near station Dassang, 2 ♂♂, 1 ♀. *C. f. nigrimontanus* Antipin: Muyun Kum desert, Djambul region, environs of the villages Ak-kol and Oik 1 ♂, 2 ♀♀; plain at the foot of the mountains, north of Transilijski Ala Tau, 74 km west from Alma Ata, 2 ♂♂, 1 ♀; near Alma Ata 1 ♂, 1 ♀. *C. erythrogenys erythrogenys* Brandt: right bank of Ob, Novosibirsk region, environs of the village Toguchin, 2 ♂♂, 2 ♀♀; between Ob and Irtish, between the town Barnaul and the village Kalmanka, 1 ♂; s.w. Altai mountains, right bank of Irtish, environs of the village Predgornoje, 1 ♂, 1 ♀. *C. er. brevicauda* Brandt: Ala-Kul basin, near Uch-Aral, 4 ♂♂, near Ajaguz 1 ♂. *C. er. carruthersi* Thom.: Zaissan basin, Buran 3 ♂♂, 3 ♀♀; near Kokpekty, 5 ♂♂, 5 ♀♀. *C. undulatus stramineus* Obolensky, S. w. Dzungarian Ala Tau, upper course of Karoy, 2400 m, 4 ♂♂, 2 ♀♀. *C. u. eversmanni* Brandt, Altai mountains, Tchujsky highway, 1400 m, 1 ♂, 1 ♀. *C. u. undulatus* Pall., South Transbaikalia, Sayan mountains, Tunkinsky basin, right bank of Irkut, 1 ♂, 1 ♀.

Subspecific distinctions of *Citellus* are given according to Gromov[11] and Vassiljeva[12].

We have caught most of the ground squirrels in the field during the Middle Asian and Siberian expeditions throughout 1965–1968. The cytological preparations were made by the standard methods, mainly from bone marrow cells and more rarely from spleen and corneal epithelial cells. The preparation procedure included colchinization, placement in a hypotonic solution of

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[1] C. F. Nadler and D. A. Sutton, Proc. Soc. exp. Biol. Med. *110*, 36 (1962).

[2] C. F. Nadler, Proc. Soc. exp. Biol. Med. *110*, 785 (1962).

[3] C. F. Nadler, Proc. XVI Intern. Congr. Zool., Washington *4*, 111 (1963).

[4] C. F. Nadler, Am. Midl. Nat. *72*, 2, 298 (1963).

[5] C. F. Nadler, Chromosoma *15*, 289 (1964).

[6] C. F. Nadler, Proc. Soc. exp. Biol. Med. *117*, 486 (1964).

[7] C. F. Nadler, Cytogenetics *4*, 37 (1965).

[8] C. F. Nadler, Sys. Zool. *15*, 199 (1966a).

[9] C. F. Nadler and C. E. Hughes, J. Mammal. *47*, 46 (1966).

[10] C. F. Nadler, J. Mammal. *47*, 579 (1966).

[11] I. M. Gromov, D. I. Bibikov, N. I. Kalabukhov and M. N. Meier, *Fauna SSSR, Mlekopitajuchtchie, Marmotinae* (Publ. House Nauka, Moscow – Leningrad 1965), vol. 3 iss. 2, no. 92, in Russian.

[12] M. V. Vassiljeva, Sbornik Trud. gos. zool. Muz. *10*, 94 (1968), in Russian.

sodium citrate and methyl alcohol glacial acetic acid (3:1) fixation. The slides were stained with orcein, azure eosin and Uhna-blue. The specificity of the procedure of the cytological preparation in hibernating species to which ground squirrels belong was similar to the one used for marmots[13].

Results. According to our data *C.* (*s. str.*) *relictus relictus, C. relictus rallii, C.* (*s. str.*) *dauricus, C.* (*s. str.*) *pygmaeus pallidus, C. p. pygmaeus, C.* (*Colobotis*) *fulvus oxianus, C. f. orlovi, C. f. nigrimontanus, C.* (*Col.*) *erythrogenys erythrogenys, C. e. brevicauda, C. e. carruthersi* are characterized by morphologically similar autosomes, $2n = 36$, $NF = 72$ (Figures 1–5).

Six groups of autosomes are distinguished within the karyotype. Group I includes 2 pairs of large metacentrics. Group II comprises 3 pairs of large submetacentrics. Group III contains 2 pairs of large subtelocentrics; group IV is composed of 3 medium-sized metacentrics; group V consists of 3 pairs of medium-sized submeta- and metacentric autosomes, and group VI has 4 pairs of small submeta- and metacentric autosomes. The sex chromosomes are markerly heteromorphic: the X-chromosome is a large submetacentric similar in size to the autosomes of group II, the Y chromosome is the smalest of the complement; in *C. relictus, C. dauricus, C. pygmaeus* the Y is a metacentric, and in *C. fulvus* and *C. erythrogenys* it is either an acrocentric, or subtelocentric.

C. (*Urocitellus*) *undulatus stramineus, C. u. eversmanni* and *C. u. undulatus* have a diploid number of $2n = 32$, $NF = 64$ (Figure 6). The karyotypes are classified into 5 groups of autosomes. Group I includes the 4 pairs of large metacentrics. Group II is represented by 2 pairs of submetacentrics somewhat smaller than group I; group III is composed of 2 pairs of medium-sized metacentrics; group IV includes 4 pairs of medium-sized submetacentrics, group V possesses 3 pairs of small submetacentric autosomes. The heteromorphic sex chromosomes are sharply discernible. The submetacentric X is very similar in size to the autosomes of group IV, the Y chromosome is either a small acro- or subtelocentric, it is the smallest of the complement.

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[13] N. N. Vorontsov, E. A. Liapunova and N. G. Zagorujko, Zoologyt. Zh. *48*, 3, 17 (1969), in Russian with English summary.

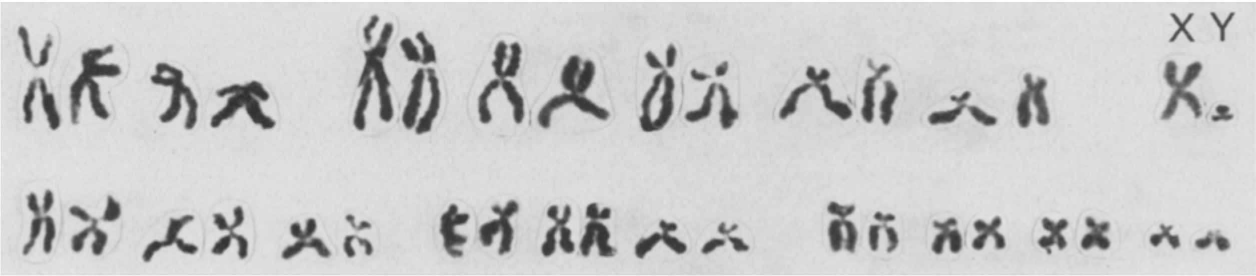

Fig. 1. Karyotype of a male *C.* (*s. str.*) *relictus relictus.*

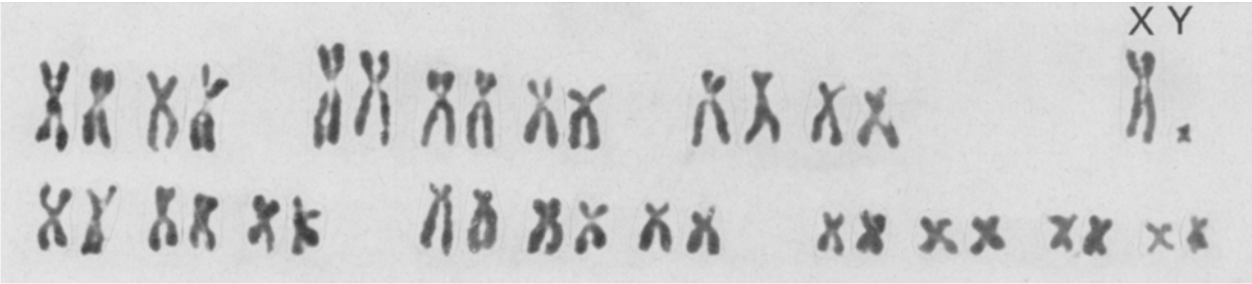

Fig. 2. Karyotype of a male *C.* (*s. str.*) *dauricus dauricus.*

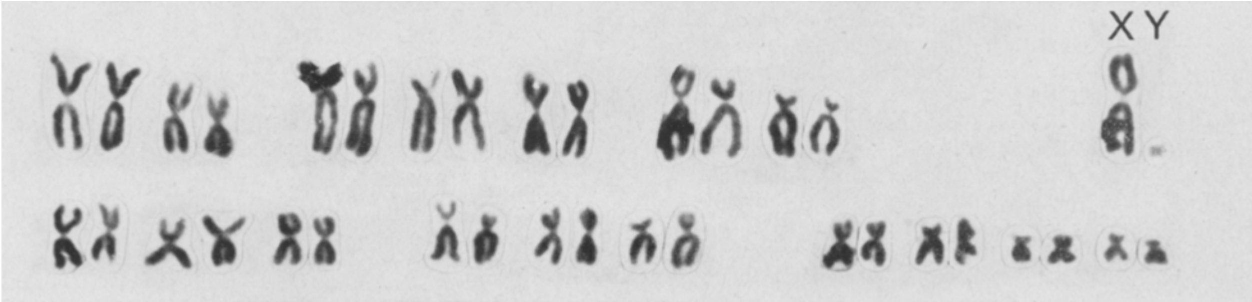

Fig. 3. Karyotype of a male *C.* (*s. str.*) *pygmaeus pygmaeus.*

Discussion. Many authors have expressed the view that *C. undulatus* and *C. parryi* are synonyms for a single species[10, 14–16]. The existence of differences in number and form of the chromosomes of Asiatic *C. undulatus* and American *C. parryi*[10] from Canada, Alaska, and the island Kodjak ($2n = 34$, $NF = 68$) confirms the opinion of GROMOV[11, 17] on the existence of specific differences between *C. undulatus* and *C. parryi*.

Because of the significant differences in number and forms between the chromosome complements of *C. undulatus* and *C. relictus*, one is forced to disagree with the relatively widely accepted view that these forms are closely related. Their similarity is due rather to the convergent adaptation to mountain habitat. *C. relictus* is more closely related karyologically to *C. pygmaeus* and *C. dauricus*.

The problem of the evolutionary pathways of the chromosomes of the genus *Citellus* was first studied on Nearctic material by NADLER[10]. After analyzing the karyotypes of our Palearctic ground squirrels and studying karyograms of Nearctic ground squirrels[1–10], we think that a different phylogenetic relationship between the subgenera *Citellus-Colobotis-Urocitellus* is possible than that believed by some American authors, who hold that they belong to the single subgenus *Spermophilus* (*s. str.*). Our proposed scheme of the possible karyotypical transformations within the groups mentioned (Figure 7) has been discussed in greater detail in a previous paper[18]. In contrast to NADLER, we draw attention to the differentiation of 2 subgroups of the species group described based on the shape of the *Y* chromosome. This scheme,

Fig. 4. Karyotype of a male *C.* (*Colobotis*) *fulvus nigrimontanus.*

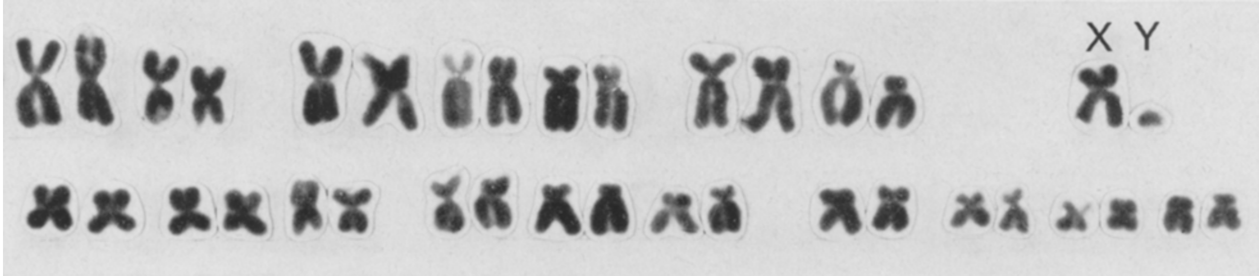

Fig. 5. Karyotype of a male *C.* (*Colobotis*) *erythrogenys brevicauda.*

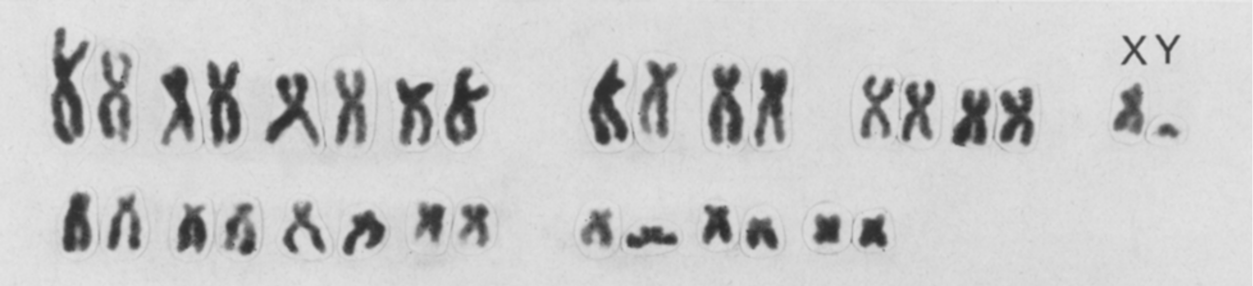

Fig. 6. Karyotype of a male *C.* (*Urocitellus*) *undulatus undulatus.*

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[14] W. G. HEPTNER, Sbornik Trud gos. Zool. Muz. *5*, 184 (1939), in Russian.

[15] S. I. OGNEV, *The Mammals of Russia* (*U.S.S.R.*) *and Adjacent Countries* (Academy of Science Press, Moscow – Leningrad 1947), vol. 5, in Russian.

[16] E. R. HALL and K. R. KELSON, *The Mammals of North America* (Ronald Press, New York 1959), vol. 1.

[17] I. M. GROMOV, *Rodentia in Mlekopitajuchtchie fauni SSSR* (Academy of Science Press, Moscow – Leningrad 1963), vol. 1, in Russian.

[18] N. N. VORONTSOV and E. A. LIAPUNOVA, Bull. Moscow Soc. of Naturalists, Biological Series *75*, 3 (1970), in Russian with English summary.

while not illustrating the phylogenetic relationship of the species whose cytology was studied, but suggesting only a way of possible karyotypical transformation, can be used to investigate this relationship. A final scheme of the phylogeny of chromosomes can only be drawn up when the karyotypes of *C. major, C. xanthoprymnus, C. citellus, C. suslicus*[19] and Asiatic *C. parryi*[20] and some unknown American species of *Citellus* are investigated.

There is an interesting relationship between the ranges of Palearctic *Citellus*. Only 2 largest species of the plains ground squirrels *C. fulvus* and *C. major* are sympatric with one of the small species *C. pygmaeus*. The range of *C. undulatus* in some areas overlaps with that of *C. erythrogenys* (foothills of Altai) and *C. dauricus* (Transbaikalia). The semisympatric species *C. fulvus* and *C. pygmaeus* differ very considerably in body size and color and are also characterized by slight differences in the morphology of the *Y* chromosomes. The mountain species *C. undulatus*, which is semisympatric with *C. erythrogenys* and *C. dauricus*, is characterized by allobiotopy, while differing considerably both in karyotypes and in the 'usual' morphological characteristics. Thus, it seems that the differences in the size, shape and the morphology of the chromosomes secure a totally reliable reproductive isolation between all the semisympatric species of the Eurasian ground squirrels. It is characteristic of most of the Eurasian species to have allopatric ranges. That allopatry is widespread among Palearctic ground squirrels as compared to the wide sympatric occurrence of the American ground squirrels is evidence of Palearctic *Citellus* being relatively more recent than the Nearctic species.

The data of chromosomal analysis indicate that in the Palearctic within the karyologically studied species 3 phylogenetically disjunct groups should be distinguished: (I) *parryi*; (II) *undulatus*; (IIIa) *dauricus, pygmaeus, relictus*; (IIIb) *erythrogenys, fulvus*.

It is of interest to compare the suggested scheme of the phylogeny of the karyotypes with the actual geographical distribution area of some of the American *Citellus* species. Of the recent species studied of the American ground squirrels, *C. vigilis* ($2n = 46$) has the most primitive chromosome complement and is restricted to a very narrow range of an obviously relict character. *C. washingtoni* is isolated allopatrically from the latter species and has the diploid chromosome number of $2n = 36$. The karyotype of *C. washingtoni* can be derived directly from the ancestral karyotype: the fusion of 20 autosomes into 4 metacentric and 6 submetacentric chromosomes through the fusion of centromeres (Robertsonian rearrangements) gives rise to the karyotype of *C. washingtoni* with 16 metacentric, 16 submetacentric and 2 acrocentric autosomes.

C. townsendii which is sympatric with *C. washingtoni* has $2n = 36$. The karyotype of *C. townsendii*, however, could have arisen independently only from the ancestral karyotype of *C. vigilis* due to Robertsonian fusion of 20 acrocentric chromosomes into 4 metacentrics and 6 submetacentrics and also in consequence of a single pericentric inversion that has led to the transformation of

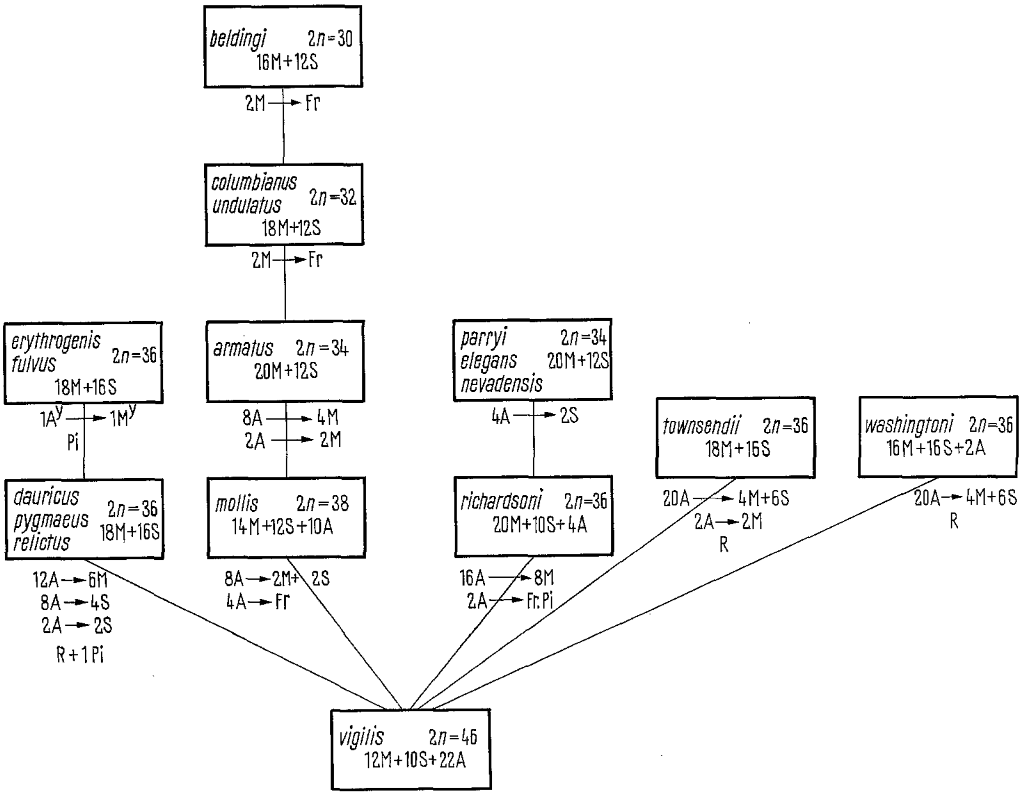

Fig. 7. Theoretical karyotype transformation in the group *Citellus* (*s. str.*), *Colobotis*, *Urocitellus*. R, Robertsonian centric fusion; Pi, pericentric inversion; Fr, fragmentation with translocation of the fragments to the other chromosomes; A, acrocentric; M, metacentric; S, submetacentric autosomes.

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[19] N. N. Vorontsov and E. A. Liapunova, in *The Mammals* (*Evolution, Karyology, Taxonomy, Fauna*) (Ed. N. N. Vorontsov; Novosibirsk 1969), p. 41, in Russian.

[20] E. A. Liapunova, in *The Mammals* (*Evolution, Karyology, Taxonomy, Fauna*) (Ed. N. N. Vorontsov; Novosibirsk 1969), p. 53, in Russian.

a pair of acrocentric chromosomes into a meta- or submetacentric one.

C. richardsoni is isolated allopatrically from *C. vigilis*. The karyotype of *C. richardsoni* can be derived also from that of *C. vigilis*: the transformation of 16 acrocentrics into 8 metacentrics through Robertsonian rearrangements in addition to the fragmentation of a pair of acrocentric chromosomes with the subsequent translocation of the fragments to the other autosomes gives rise to the karyotype of the species *C. richardsoni* ($2n = 36$) which is at present widely spread and fluorishing.

The karyotype of *C. elegans*, *C. nevadensis* and *C. parryi* can be derived directly from the karyotype of *C. richardsoni* through the Robertsonian fusion of 4 acrocentric chromosomes into 2 submetacentrics. All the 3 species are characterized morphologically by identical karyotypes with 20 metacentric and 2 submetacentric autosomes ($2n = 34$). The first two species are morphologically extremely similar to *C. richardsoni* and hitherto have been considered only as subspecies of the latter. *C. nevadensis* is an allopatric isolate inhabiting Nevada. The range of *C. elegans* includes Wyoming and Colorado and in the north its range borders with the range of *C. richardsoni*. In America the range of *C. parryi* extends from northern British Columbia through the Continental areas of Keewatin, Mackenzie, Yukon and Alaska and includes St. Laurence and the adjacent islands. Of the ground squirrels having more southern ranges only 2 species, *C. colombianus* and *C. richardsoni*, have penetrated northern most (to the southwestern regions of Canada). It should be noted that *C. parryi* is similar karyologically to *C. richardsoni* only. All the species considered above (*C. washingtoni*, *C. townsendii*, *C. richardsoni*, *C. elegans*, *C. nevadensis*) are characterized by a small Y chromosome. The karyotype of *C. mollis* can be derived from *C. vigilis*: Robertsonian fusion of 8 acrocentrics into 4 meta and submetacentric chromosomes as well as the fragmentation of 2 pairs of acrocentric chromosomes followed by the translocation of the fragments to the other chromosomes have been responsible for the rise of this line. In the north-east the range of *C. mollis* borders with that of *C. armatus*. *C. armatus* has derived its karyotype from *C. mollis*: 8 acrocentrics underwent Robertsonian fusion and formed 4 metacentric chromosomes, 2 acrocentric chromosomes through pericentric inversion have been transformed into 2 metacentrics. *C. armatus* has a $2n$ of 34 and its karyotype possesses 20 metacentric autosomes.

We have published elsewhere the detailed discussion of the causes of the morphological identity of the karyotypes of the Palearctic *C. undulatus* and the Nearctic *C. columbianus*[18,21]. The karyotypes of *C. columbianus* and *C. undulatus* are derived from the karyotype of *C. armatus*. It may be assumed that one of the pairs of the metacentric chromosomes of *C. armatus* underwent fragmentation with the subsequent translocation of the fragments to the other chromosomes. Similar chromosomal rearrangements occurred in the line evolving from *C. columbianus* and *C. beldingi*. Of the karyologically studied ground squirrels, the latter species distinguished by its small chromosomal number ($2n = 30$).

The karyotypes of the Palearctic *C. dauricus*, *C. pygmaeus* *C. relictus* can probably be derived also from the primitive karyotype similar to that of *C. vigilis*. The transformation of 12 acrocentric chromosomes into 6 metacentric chromosomes due to Robertsonian fusion, and 8 acrocentric chromosomes through Robertsonian fusion into 4 submetacentric chromosomes in addition to a pericentric inversion, which has transformed a pair of acrocentric chromosomes into a pair of submetacentric chromosomes; such is the possible evolutionary pathway of the karyotype of the small species of the Palearctic ground squirrels from their ancestral chromosome complement of the type *C. vigilis*. These transformations possibly did not occur synchronously and there existed some intermediates with transitional karyotypes between these forms. It is less likely that the karyotypes of the Eurasian ground squirrels of the group *dauricus-pygmaeus-relictus* have evolved from a karyotype similar to that of *C. mollis*. If such were the mode of emergence of its karyotype, it should have undergone the fusion of 4 acrocentrics and 2 metacentrics, and also in consequence of 3 pericentric inversions have been subjected to the transformation of 6 acrocentrics into 6 meta- and submetacentrics.

Finally, the karyotypes of *C. erythrogenys* and *C. fulvus* are derived from the karyotype of the groups *dauricus-pygmaeus-relictus* as the result of a single rearrangement, the pericentric inversion of a small acrocentric into a small metacentric Y chromosome.

The establishment of the morphological similarity between the chromosome complements of *C. columbianus* and *C. undulatus* as well as the fact that there is no similarity between the karyotypes of these species and those of *C. parryi* suggests the following evolutionary pathway of some of the Holarctic species of ground squirrels. *C. armatus* ($2n = 34$) has evolved independently from *C. mollis* ($2n = 38$) in the northeastern areas of its range. In the northwestern range of the latter *C. columbianus* ($2n = 32$) or its ancestor with a similar karyotype has evolved independently. By the close of the Pliocene or the beginning of the Eopleistocene *C. columbianus* has migrated across the Bering land bridge to Palearctic where fossil records of *C. undulatus* date from the Middle Eopleistocene. In East Asia *C. undulatus* has survived through the Samarov (≈ Kansas) glaciation period. *C. undulatus* has become extinct over the territory from the right bank of Lena to Alaska and Canada, probably, during the Kazantsev (≈ Sangamon) interglaciation period when the xerophyte flora of the tundra-steppe receded. During the xerophyte stage of the Early Wisconsin glaciation, when the tundra-steppe approached the prairies due to the recession of the forested areas, *C. richardsoni* migrated to the north, and *C. parryi* originated from it. The significant differences in the chromosome complements of these species prompts us to suggest that a small number of ground squirrels were involved in the migration. Rearrangements occurred in the karyotypes of the populations of the founder stocks and after that the species dispersed over the territory of northern Canada and Alaska. The spread of ground squirrels over northeastern Asia across the Bering land bridge dates from the Zyrjan (≈ Salmon, ≈ Early Wisconsin) glaciation period because during the Sartan (≈ Late Wisconsin) glaciation period *C. parryi* already inhabited Alaska and northeastern Asia. Spreading westwards over Asia, *C. parryi* occupied the mountainous-steppe regions of northeastern Asia from Chukotka to the right bank of Lena, the region from which *C. undulatus* had disappeared earlier. Similar habitats have resulted in the convergent similarity between *C. parryi* and *C. undulatus*. This similarity has confused many taxonomists. A more detailed history of the ranges of *C. columbianus*, *C. undulatus*, *C. richardsoni*, and *C. parryi* is given in another paper which includes paleontologic and paleo-

[21] N. N. VORONTSOV and E. A. LIAPUNOVA, Dokl Akdad. Nauk SSSR *187*, 1, 207 (1969), in Russian.

geographic evidence of the hypothesis we have put forward[21].

Leaving open the question of the actual date of the penetration of the ancestors of the subgenera *Citellus* (*s. str.*) and *Colobotis* into the Palearctic, we must accept that the North American longtailed ground squirrels have migrated to Asia across the Bering land bridge during at least two disjunct periods[22].

Выводы *Citellus* (*s. str.*) *relictus*, *C. dauricus C. pygmaeus* обладают идентичными кариотипами ($2n = 36$, $NF = 72$). Группа *C.* (*Colobotis*) *erythrogenys* и *C. fulvus* отличается от этих видов лишь незначительными различиями в форме y-хромосомы. Кариотип *C.* (*Urocitellus*) *undulatus* ($2n = 32$, $NF = 64$) существенио отличается от всех описанных выше видов но идентичен кариотипу *C. columbianus*. Обсуждаются причины кариологической гомогенности большинства современных азиатских *Citellus*, характеризующихся аллопатрическим распространением, в сравнении с неарктическими *Citellus*, характеризующимися широким распространением симпатрии и резкими различиями в строении хромосом. Сопоставляются цитологические, зоогеографические и палеонтологические данные по эволюции сусликов.

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Novosibirsk 90 (U.S.S.R.), 26 February 1970.

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[22] We thank Prof. N. I. Kalabukhov, Prof. N. V. Nekipelov and Dr. V. K. Shepeleva for some living specimens, Dr. S. I. Rajabli for the preparation of the chromosomes of *C. dauricus*, *C. pygmaeus* and *C. f. orlovi*; Y. Mirokhanov, E. Ivanitskaja and E. Zholnerovskaja for their technical assistance.

PRO EXPERIMENTIS

A Nylon Reograd Rotor Embodying New Principles

Anderson et al.[1] introduced the principle of the reorienting gradient (reograd) rotor. This rotor was subsequently superceded by the continuous flow and the large scale gradient rotors (as manufactured by Beckman Instruments Incorporated). A detailed review on the application of zonal centrifugation may be found in the Spinco DS326 brochure entitled 'Large-scale Zonal Rotors for use in the Beckman Preparative Ultracentrifuges'.

The reograd rotor was reinvestigated in this laboratory and it was found that after the introduction of a few new features it became a valuable addition to the family of rotors of modern preparative ultracentrifuges.

The Nylon Reograd Rotor. Nylon was selected as a suitable material for the construction of this prototype rotor because of its favourable tensile strength-density ratio, its chemical inertness and the ease with which it may be machined on a lathe.

The rotor described here was machined from a cylindrical block of nylon 21 × 10 cm. The final product has a diameter near the base of 20.5 cm and 16 cm at the top. The cylindrical cavity which is 12.3 cm wide and 5 cm deep with the base sloping towards the centre at an angle of 3° from the horizontal. The rotor is fitted with a duraluminum lid, and a base of the same material designed

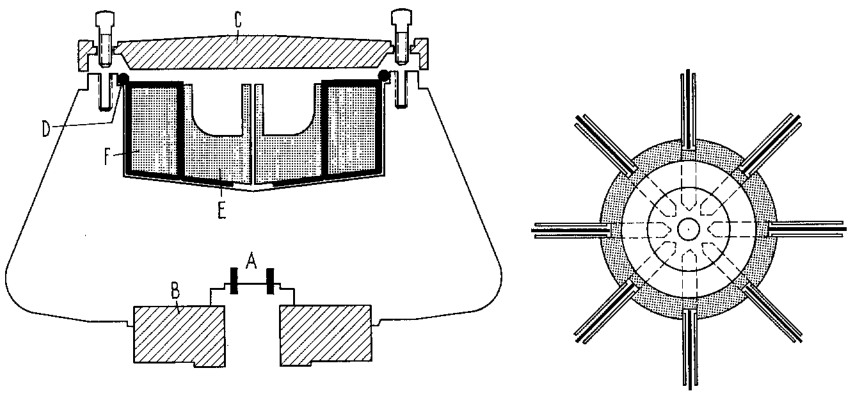

Fig. 1. Left: A cross-sectional drawing of the nylon zonal rotor. A) The main body of the rotor with the cylindrical central cavity. The base of the cylinder slopes towards the centre at 3.0°. B) The base, made of duraluminum, is screwed on to A with 4 steel screws. 2 brass pins protrude from the main body. The base and pins enable it to be driven on the Spinco Model L ultracentrifuge. C) The lid, made of duraluminum is screwed on to the rotor by means of 6 aluminium screws. D) An O ring fits exactly into the rim of the cylinder. When the lid is screwed down a water-tight seal is formed. E) The central core composed of a perspex cylinder into which a cup-like cavity is cut holding an inner column through which a capilliary, 1 mm in diameter, is drilled through its entire length. F) The central core is attached to 8 perspex septa with O rings wrapped over recesses cut into their peripheries. 8 strips of rubber are glued on to the base of the central core to raise it approximately 0.8 mm from the lower surface of the cylinder. Right: A plan view of the central core showing the O rings wrapped around each of the 8 septa, and the rubber strips radiating from the centre. The capillary down the centre rod is also indicated.