

Choline acetylase activity of the submaxillary gland of the rat 1 week after duct ligation on one side

No. of glands pooled	Enzyme activity in $\mu\text{g}$ ACh/h/g acetone powder		Enzyme activity in $\mu\text{g}$ ACh/h/whole gland		Gland weight (mg)		Ligatured/contralateral, %
	Ligatured	Contralateral	Ligatured	Contralateral	Ligatured	Contralateral	
7	600	300	10.2	11.5	97 $\pm$ 4.3	169 $\pm$ 9.7	57 $\pm$ 2.8
6	620	260	8.6	9.1	80 $\pm$ 4.2	158 $\pm$ 6.4	51 $\pm$ 3.6

The weight of the submaxillary gland was found to be markedly decreased after duct ligation (Figure), in accordance with previous results<sup>2,5</sup>.

The secretory response to 20  $\mu\text{g}$  adrenalin/kg was about  $1\frac{1}{2}$  drops of saliva from control glands. It was gradually decreased within the first 6 days after the duct was ligatured and later no secretion could be seen. Similarly, the secretory response to 10  $\mu\text{g}$  methacholine/kg was decreased to zero during the first 3 days after the operation, while control glands produced about  $1\frac{1}{2}$  drops. From control glands a maximal flow rate to pilocarpine given repeatedly was reached after a total dose of 1–5 mg pilocarpine/kg; the maximal flow rate was about 4 drops/min. The secretory response to pilocarpine was markedly reduced after duct ligation; usually less than 1 drop of saliva was produced in 10–15 min.

1 week after duct ligation the total activity of choline acetylase was found to be similar in ligatured and control glands. On the other hand, the enzyme concentration was markedly increased, from about 300–600  $\mu\text{g}$  acetylcholine/h/g acetone powder, corresponding to the pronounced decrease in gland weight (Table).

The size of salivary glands is to a large extent dependent on the secretory activity<sup>6</sup>. The atrophy of the rat's submaxillary gland after duct ligation agrees with this suggestion. It seems obvious, however, that not only the secretory inactivity of the ligatured glands is responsible for the atrophy, since the decrease in gland weight is much more marked after duct ligation than after section of the secretory nerves<sup>7</sup>. The ligation atrophy of the rat's submaxillary gland does not seem to be due to changes in the parasympathetic secretory neurones, since the activity of choline acetylase was found to be unchanged after the duct was ligatured. Similarly, the sympathetic neurones of the gland are on the whole unaffected by duct ligation<sup>8</sup>.

A salivary gland after duct ligation has been called a 'resting gland'<sup>5</sup>. The present results indicate that, within the first week after duct ligation, the secretory responses of the rat's submaxillary gland to sialagogue drugs deteriorate markedly; later only very small amounts of secretion can be elicited by intense stimulation with pilocarpine, which evokes a lively flow of saliva from control glands. Thus, the ability of the glandular cells to secrete is almost completely lost after duct ligation, though the tubules, which are supposed to be of great importance for the production of saliva (see OHLIN<sup>3</sup>), remain histologically more or less unaffected<sup>2</sup>.

**Zusammenfassung.** Abbinden des Ausführungsganges der Submaxillarisdrüse der Ratte führt zu ihrer Gewichtsabnahme. Nach i.v. Gaben von Methacholin und Adrenalin verschwindet die Sekretion innerhalb von 4–6 Tagen nach der Operation, während bei Pilocarpin noch eine sehr verminderte Speichelsekretion persistiert. Die spezifische Fähigkeit der sekretorischen Zellen war zusehends verringert. Die Gesamtaktivität der Cholin-Acetylase blieb ähnlich derjenigen der Kontrolldrüsen, was für eine unveränderte Funktion des Parasympathikus spricht.

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22nd September 1966.*

<sup>5</sup> L. C. U. JUNQUEIRA, *Expl Cell Res.* 2, 327 (1951).

<sup>6</sup> H. D. HALL and C. A. SCHNEVER, *Proc. Soc. exp. Biol. Med.* 117, 789 (1964).

<sup>7</sup> P. OHLIN and C. PEREC, *Q. Jl exp. Physiol.* 51, 196 (1966).

<sup>8</sup> N.-E. ANDÉN, K.-A. NORBERG and L. OLSON, *Acta physiol. scand.* 66, 501 (1966).

## Chromosomes of Some Squirrels (Mammalia – Sciuridae) From the Genera *Sciurus* and *Glaucomys*

The family Sciuridae contains the subfamilies Sciurinae and Petauristinae<sup>1</sup>. North American Sciurinae include the tribes Sciurini (genus *Sciurus*, *Microsciurus*, *Syntheosciurus*, *Guerlinquetus*), *Tamiasciurini* (genus *Tamiasciurus*) and *Marmotini* (genus *Marmota*, *Spermophilus*, *Ammospermophilus*, *Cynomys*, *Eutamias*, *Tamias*)<sup>2</sup>. Among the Petauristinae, only the genus *Glaucomys* is found in North America. Another classification included *Tamiasciurus* within the tribe Sciurini and raised *Tamias* and *Eutamias* to tribal rank, *Tamiini*<sup>3</sup>.

Chromosome analysis has been applied successfully to taxonomic study of many genera of Sciuridae including *Spermophilus*<sup>4</sup>, *Ammospermophilus*<sup>5</sup>, *Tamias* and *Eutamias*<sup>6</sup>, *Marmota*<sup>7</sup>, *Cynomys*<sup>8</sup> and *Tamiasciurus*<sup>9,10</sup>. Because considerable interspecific and intraspecific variation was present in most taxa, chromosomes were of greater systematic value at these rather than suprageneric levels.

The present investigation describes chromosomes from 4 species of *Sciurus* and the 2 species which comprise *Glaucomys*, thereby completing a karyological survey of North American Sciuridae.

The following specimens were studied: *Sciurus carolinensis carolinensis* GMELIN, Florida, Lake County, 1 male;

*Sciurus carolinensis pennsylvanicus* ORD, Illinois, Cook County, Winnetka, 3 females and 2 males; *Sciurus niger rufiventer* E. GEOFFROY ST. HILAIRE, Illinois, Henry County, Kewanee, 1 male and 2 females; *Sciurus griseus griseus* ORD, California, Butte County, Chico, 1 male; *Sciurus aberti* Woodh., unknown locality, karyotype from a male kindly provided by Dr. T. C. Hsu; *Glaucomys sabrinus lascivus* BANGS, California, Butte County, Paradise, 1 male; *Glaucomys volans*, unknown locality, 1 female.

Chromosomes were analyzed from bone marrow using a Colcemide, hypotonic citrate acetic orcein squash method<sup>10</sup>. The fundamental number (FN)<sup>11</sup> was computed by counting the number of autosomal arms.

*Sciurus carolinensis carolinensis* and *S. c. pennsylvanicus* have a diploid number ( $2n$ ) of 40 and similar karyotypes that contain 14 metacentric and 24 submetacentric autosomes, a submetacentric  $X$  and an acrocentric  $Y$  chromosome (Figure 1). FN, 76.

The  $2n$  of *S. niger rufiventer* is 40 and the karyotype exhibits 14 metacentric and 24 submetacentric autosomes, a submetacentric or nearly metacentric  $X$  and an acrocentric  $Y$  chromosome (Figure 2). FN, 76.

The single karyotype from *S. aberti* contains 40 chromosomes including 14 metacentric and 24 submetacentric autosomes. A large unpaired metacentric and an unpaired acrocentric probably represent the  $X$  and  $Y$  chromosomes respectively (Figure 3). FN, 76. The karyotypes of *S. carolinensis*, *S. niger* and *S. aberti* are indistinguishable,

although study of a larger series may reveal differentiating features.

*S. griseus*,  $2n$  40, has 14 metacentric and 24 submetacentric autosomes and 2 unpaired submetacentric sex chromosomes of slightly unequal size and centromere location (Figure 4). FN, 76. *S. griseus* differs from the

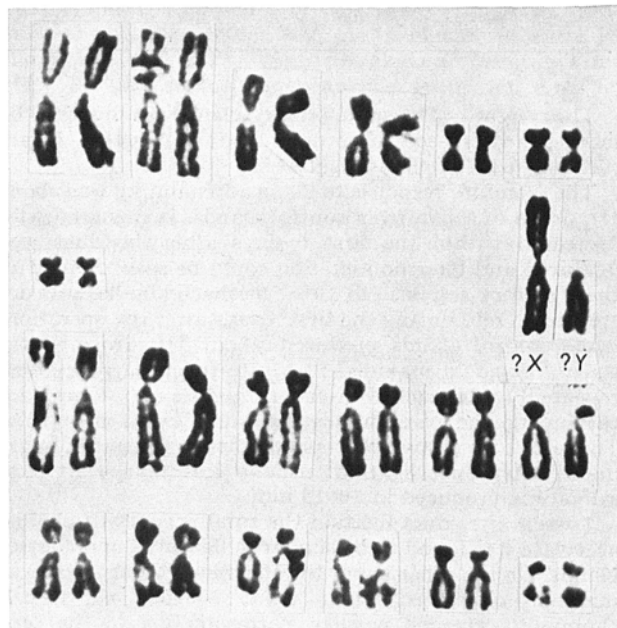


Fig. 3. Karyotype of a male *S. aberti* (by courtesy of Dr. T. C. Hsu).

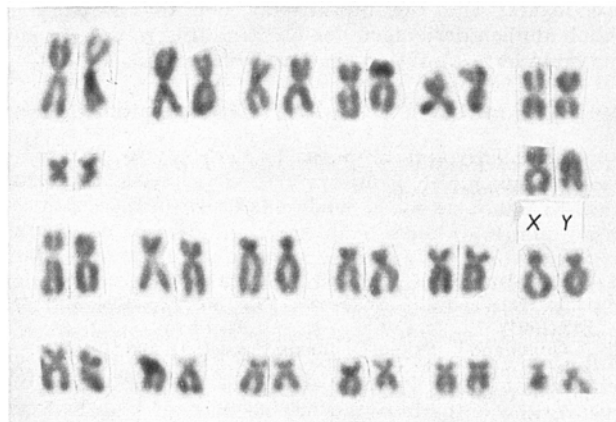


Fig. 1. Karyotype of a male *S. carolinensis carolinensis*.  $\times 2000$ .

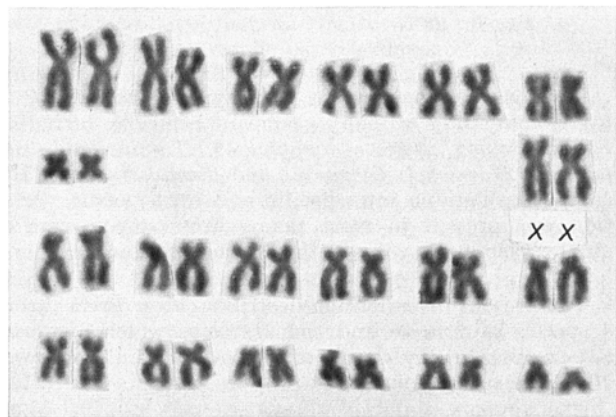


Fig. 2. Karyotype of a female *S. niger rufiventer*.  $\times 2000$ .

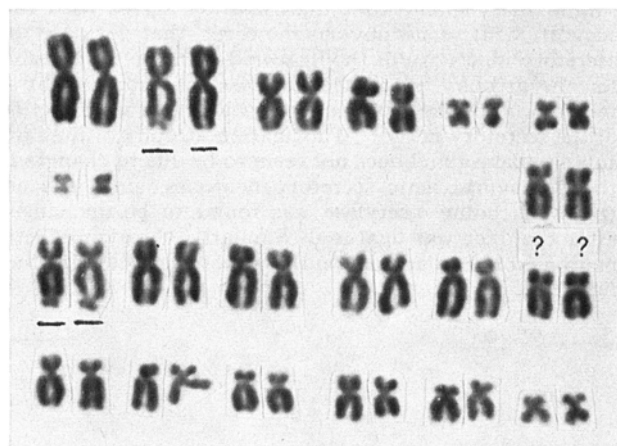


Fig. 4. Karyotype of a male *S. griseus griseus*.  $\times 2000$ . Chromosome pairs with secondary constrictions are underlined. Question marks identify the sex chromosomes.

<sup>1</sup> G. G. SIMPSON, Bull. Am. Mus. nat. Hist. 85, 1 (1945).

<sup>2</sup> J. C. MOORE, Bull. Am. Mus. nat. Hist. 118, 155 (1959).

<sup>3</sup> C. C. BLACK, Bull. Mus. comp. Zool. Harv. 130, 109 (1963).

<sup>4</sup> C. F. NADLER, Syst. Zool. 15, 199 (1966); J. Mammal., in press.

<sup>5</sup> C. F. NADLER and D. A. SUTTON, Proc. Soc. exp. Biol. Med. 170, 36 (1962).

<sup>6</sup> C. F. NADLER, Am. Midl. Nat. 72, 298 (1964).

<sup>7</sup> R. L. RAUSCH and V. R. RAUSCH, Chromosoma 16, 618 (1965).

<sup>8</sup> C. F. NADLER and K. E. HARRIS, Experientia 23, 41 (1967).

<sup>9</sup> T. C. HSU, Mammal. Chromosome Newsl. 19, 22 (1966).

<sup>10</sup> C. F. NADLER and M. H. BLOCK, Chromosoma 13, 1 (1962).

<sup>11</sup> R. MATTHEY, Experientia 1, 50, 78 (1945).

other 3 species of *Sciurus* by the absence of an acrocentric Y and the consistent presence of secondary constrictions in the second largest pair of metacentric and largest pair of submetacentric autosomes. These unique features are considered tentative species characters for *S. griseus* until additional populations are sampled.

A male *Glaucomys sabrinus lascivus*,  $2n$  48, has 12 metacentric, 16 submetacentric and 18 acrocentric autosomes, an unpaired submetacentric and a minute chromosome (Figure 5); the latter 2 chromosomes are probably X and Y respectively. FN, 74.

The female *G. volans*,  $2n$  48, has a karyotype containing 12 metacentric, 16 submetacentric and 20 acrocentric chromosomes. The X chromosomes were not identified. *G. volans* has chromosomes nearly similar to those of *G. sabrinus*; they appear to differ only in the centromere position of the sex chromosomes or a pair of autosomes.

Chromosomes from species of *Sciurus* and *Glaucomys* exhibit an intrageneric homogeneity comparable to that of *Eutamias* and *Tamias* ( $2n$  38)<sup>6</sup>, *Ammospermophilus* ( $2n$  32)<sup>5</sup> and *Tamiasciurus* ( $2n$  46)<sup>9</sup>. Because their chromosomes are numerically or morphologically distinctive and share no recognizable relationship to other genera they may be considered valid generic characters. In contrast, other genera of Sciuridae are chromosomally diverse, particularly *Spermophilus* ( $2n$  30–46)<sup>4</sup> and *Marmota* ( $2n$  36–42)<sup>7</sup>; here chromosomes are less definitive indicators of generic status even though cytologically heterogeneous species within a genus are interrelated by rearrangements such as Robertsonian centric fusions.

The availability of chromosome data from species belonging to 4 of 6 subgenera warrants a reappraisal of the infrageneric classification of *Sciurus*<sup>12</sup>. MOORE has already proposed, based on cranial and baculum similarities, that the subgenus *Neosciurus* (*S. carolinensis*) is invalid and should be included within the subgenus *Sciurus*<sup>13</sup>. It was also suggested that the subgenus *Parasciurus* (*S. niger*) be dropped and its species included within *Sciurus* because only one character of possible subgeneric significance distinguished *S. niger* from *S. carolinensis* (*Neosciurus*)<sup>13</sup>. Although we have not examined chromosomes from *S. vulgaris*, type species of the subgenus *Sciurus*, our data indicate a close relationship between *S. niger* and *S. carolinensis* and therefore confirm MOORE's revision. Our limited data indicate that the subgenus *Otosciurus* may also be referable to *Sciurus* because the chromosomes of *S. aberti* are similar to those of *S. niger*

and *S. carolinensis*. Only *S. griseus* of the monotypic subgenus *Hesperosciurus* has a karyotype sufficiently different to support its subgeneric rank; however, the specialized baculum of *S. griseus* is the strongest evidence for subgeneric recognition.

The chromosomal similarity of *Glaucomys sabrinus* and *G. volans* argues for a closer relationship than is suggested by their markedly different bacula<sup>14</sup>; other lines of evidence are needed to clarify this taxonomic problem.

Although application of chromosomes to suprageneric classification is limited by inability to ascertain the cytologic rearrangements responsible for karyotype divergence, certain problems merit comment. First, there is no evidence for a chromosomal relationship between *Tamiasciurus* ( $2n$ , 46, FN ?80–88)<sup>9</sup> and species of *Sciurus* ( $2n$  40, FN 76); this, in addition to cranial and genital tract data, supports the tribal classification of *Tamiasciurus*<sup>3</sup>. Second, BLACK<sup>8</sup> questioned the taxonomic status of *Glaucomys* and several other genera of the Petauristinae by observing that, except for the gliding membrane, they share many similarities with *Sciurus*. His suggestion that *Glaucomys* evolved from ancestral stock similar to that of the Sciurinae is strengthened by the presence of nearly identical FNs (74 vs 76) in *Glaucomys* and *Sciurus*; their karyotypic differences may originate from several centric fusions.

Secondary constrictions are uncommon in chromosomes of the Sciuridae. In addition to *S. griseus*, they are seen in lung cultures of *Tamiasciurus hudsonicus* and *T. douglasii*<sup>9</sup> and in lung but not marrow cultures of *S. niger*<sup>15</sup>. Hsu<sup>9</sup> reported in *Tamiasciurus* 'that the number of chromosomes with secondary constrictions varied from cell to cell and the presumed homologous pairs rarely showed constrictions on both elements'. In contrast, our data indicate constrictions are present in all cells examined and in both members of a chromosome pair. Further study of this phenomenon in other species of *Sciurus* is needed and may provide a valuable means for more exact identification of individual chromosomes and a method for tracing pathways of interspecific karyotype evolution<sup>16</sup>.

*Zusammenfassung.* Die Analyse der mitotischen Chromosomen bei *Sciurus carolinensis*, *S. niger* und *S. aberti* ergibt die diploide Zahl 40 und gleichartige Karyotypen. *S. griseus*,  $2n$  40, hat einzige Y Chromosomen und sekundäre Konstruktionen in 4 Autosomen. *Glaucomys sabrinus* und *G. volans*,  $2n$  48, zeigten kleinere Unterschiede der Karyotypen. Taxonomische Bezeichnungen von *Sciurus*, *Glaucomys* und *Tamiasciurus* werden erörtert.

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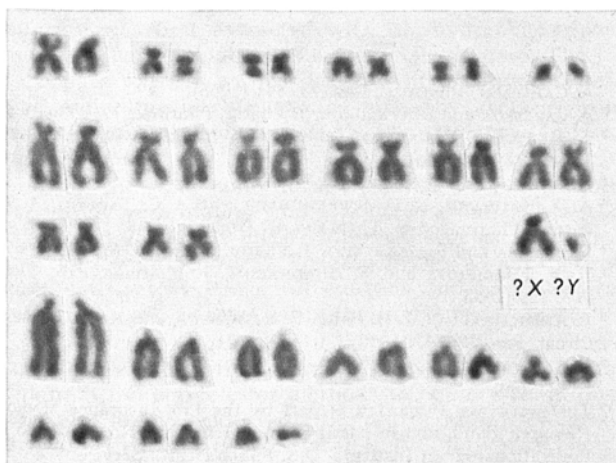


Fig. 5. Karyotype of a male *Glaucomys sabrinus lascivus*.  $\times 2000$ .

<sup>12</sup> A. H. HOWELL, N. Am. Fauna 56, 1 (1938).

<sup>13</sup> J. C. MOORE, Southeastern Assoc. Game and Fish Comm. 13th Ann. Conf. (1959), p. 356.

<sup>14</sup> W. H. BURT, Misc. Publs Mus. Zool. Univ. Mich. 173, 1 (1960).

<sup>15</sup> T. C. HSU, Personal communication.

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