Evolution in ground squirrels

II. Biochemical comparisons in Holarctic populations of *Spermophilus*

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Abstract

A comparison of biochemical similarities among 21 of 23 recognized species in the subgenus *Spermophilus* was made. Thirteen proteins, representing at least 18 loci were studied, and electromorphic variations described. A phenogram and a Wagner network of genetic resemblance are presented, and, together with morphological, chromosomal, and distributional data, used to interpret the systematics of *Spermophilus*. Two lineages diverged in North America, the Nearctic "small-eared" and "big-eared" ground squirrels. Most of the Recent species in these lineages evolved during the late Pleistocene.

Introduction

Ground squirrels (*Spermophilus* = *Citellus*) are widely distributed in and restricted to North America and Eurasia, and probably comprise the most recently evolved of the terrestrial squirrels (Black 1963, 1972). Up to twenty-three species are currently recognized in the subgenus *Spermophilus* (sensu lato): 12 in the Palearctic, and 10 in the Nearctic, with one additional species shared (Gromov et al. 1965; Hall 1981; Lyapunova and Vorontsov 1970; Robinson and Hoffmann 1975). Gromov et al. (1965) subdivided *Spermophilus* into three subgenera, *Spermophilus* (= *Citellus*) (sensu stricto), *Urocitellus*, and *Colobotis*, but others have not recognized this subdivision. The subgenus *Spermophilus* (s. s.) may be further subdivided into informal "groups". Davis (1939) proposed that the Nearctic species could be allocated to a "big-eared" group and a "small-eared" group. Evidence concerning these groups, and a subgeneric classification will be presented below.

Taxa included within the genus *Spermophilus* illustrate the effects of varying periods of geographical isolation by the Bering Strait on evolutionary patterns of cytological, morphological and biochemical characters. Among the long-tailed ground squirrels *(Urocitellus)* is found the only species with a Holarctic distribution, the arctic ground squirrel *(S. parryii)*. This species occupied Beringia during the last glacial period (Würm-Wisconsin) (Repenning et al. 1964). Eastern Siberian and western North American populations were isolated by the Bering Strait about 13,000 years ago as ocean levels rose with glacial retreat (Hopkins 1972). Since then, morphological and biochemical differences have accumulated (Robinson and Hoffmann 1975; Nadler et al. 1976), but gross chromosomal morphology has remained unchanged (Nadler 1966; Lyapunova 1969). The other two species of long-tailed ground squirrels, *S. undulatus* and *S. columbianus*, comprise sister-species (sensu Hennig 1966) in the Palearctic and Nearctic respectively. They also retain apparent chromosomal identity while diverging in morphology and

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biochemistry to a greater degree than *parryii* (Nadler et al. 1975; Robinson and Hoffmann 1975).

The Palearctic subgenus *Colobotis* has no representative in the Nearctic so other Holarctic relationships must be sought within the subgenus *Spermophilus* (s. s.). In an earlier paper we presented detailed comparison of transferrins (Nadler et al. 1974). In this paper, we extend this comparison to other biochemical systems and relate these data to other systematic characters of Holarctic ground squirrels. A revised classification of the subgenus *Spermophilus* in presented, in which we employ the concept of the superspecies, and the notation proposed by Amadon (1966). This is necessary because of the many allo- and parapatric populations in the subgenus, and the occurrence of sporadic hybridization between allospecies (= semispecies) (Lorkovic 1960; Mayr 1969; Petrov and Zivkovic 1977; Reig et al. 1980).

Materials and methods

Specimens analyzed

A total of 1087 specimens were examined. Voucher specimens of most North American specimens reported are deposited in the Museum of Natural History, University of Kansas, and Eurasian specimens are in the collection of the Zoological Institute, Academy of Sciences of the USSR, Leningrad.

Spermophilus townsendii townsendii (Bachman). USA, Washington: Benton County, S. of Yakima River, vic. of Prosser, 27 specimens; Yakima County, Yakima airport, 15 specimens.

S. mollis mollis (Kennicott). Idaho: Cassia County, Burley, 47 specimens.

S. m. idahoensis (Merriam). Idaho: Elmore County, vic. of Mountain Home, 9 specimens.

S. m. nancyae Nadler. Washington: Benton County, N. of Yakima River, vic. of Prosser, 26 specimens; Yakima County, Yakima, 10 specimens.

S. vigilis vigilis (Merriam). Oregon: Malheur County, vic. of Ontario, 16 specimens; vic. of Vale, 39 specimens.

S. v. canus (Merriam). Oregon: Harney County, Narrows, 3 specimens; Hines, 1 specimen.

S. washingtoni (A. H. Howell). Washington: Franklin County, Scootenay Reservoir, 31 specimens.

S. brunneus (A. H. Howell). Idaho: Adams County, 3 mi. S, 0.5 mi. E of Bear Post Office, 8 specimens.

S. beldingi beldingi Merriam. California: Inyo County, Rock Lake, 10 mi. SW of Tom's Place, 13 specimens; Mono County, Rock Creek Lodge, 9–10 mi. SW of Tom's Place, 3 specimens.

S. b. creber (Hall). Idaho: Cassia County, Burley, 2 specimens.

S. b. oregonus (Merriam). Oregon: Grant County, 3.5 mi. S of Mount Vernon, 5 specimens; Harney County, Burns, 3 specimens; Multnomah County, vic. of Portland, 1 specimen.

S. armatus (Kennicott). Montana: Gallatin County, 3.5 mi. S, 1.5 mi. W of Gallatin Gateway, 2 specimens; Madison County, 6.5 mi. S, 1.5 mi. W of Harrison, 19 specimens; 3 mi. W of McAllister, 7 specimens; 4 mi. N, 3 mi. E of McAllister, 9 specimens. Utah: Cache County, vic. of Logan, 5 specimens; Tony Grove Lake, 15 mi. NE of Logan, 10 specimens. Wyoming: Lincoln County, 12 mi. NE of Cokeville, 8 specimens.

S. columbianus columbianus (Ord). Montana: in Granite County, 15 mi. S of Clinton, 1 specimen; Jefferson County, 0.5 mi. E of Whitehall, 2 specimens; in Madison County, 12.5 mi. S, 4 mi. E of Whitehall, 1 specimen; Madison County, 1.5 mi. N, 2 mi. E of Harrison, 83 specimens; 1.5 mi. N of Harrison, 4 specimens; 1.25 mi. S, 1 mi. W of Harrison, 19 specimens; 6.5 mi. S, 1.5 mi. W of Harrison, 1 specimen; Missoula County, 12 mi. E of Potomac, 1 specimen; 5 mi. N of Missoula, 56 specimens. Idaho: Adams County, 1 mi. N of Bear Guard Station, 3 specimens; 5 mi. E of Cuprum, 1 specimen; Idaho County, 4 mi. S, 4 mi. E of Lowell, 1 specimen. Canada, Alberta: vic. of Edmonton, 6 specimens.

S. richardsonii (Sabine). Montana: in Broadwater County, 2 mi. N 0.5 mi. W of Three Forks, 3 specimens; 0.5 mi. N of Jefferson R., 5 mi. SW of Three Forks, 1 specimen; 1.5 mi. N., 2 mi. E of Sappington, 23 specimens; Gallatin County, 5 mi. NW of Bozeman, 32 specimens; W bank, Gallatin R., 7 mi. W of Bozeman, 7 specimens; 3 mi. N of Gallatin Gateway, 3 specimens; 0.5 mi. SW of Three Forks, 3 specimens; 0.5–1 mi. E of Madison R., 15 mi. S. of Three Forks, 5 specimens; S bank, Jefferson R., 0.6 mi. N of Three Forks, 1 specimen; 4.25 mi. S, 2.25 mi. W of Willow Creek, 23 specimens; Jefferson County, 15 mi. SE of Boulder, 1 specimen. Canada, Alberta: vic. of Edmonton, 3 specimens.

S. elegans elegans (Kennicott). Colorado: Gilpin County, 1.75 min. N of Rollingsville, 10 specimens; Eagle County, 6.5 mi. SE of Eagle, 3 specimens; Moffat County, 14 mi. N, 3 mi. W of

Greystone, 1 specimen. Wyoming: Lincoln County, 5 mi. S of Cokeville, 2 specimens; 5 mi. N of Fontenelle, 5 specimens; Sweetwater County, 1.25 mi. NE of Superior, 1 specimen.

S. e. aureus (Davis). Montana: Madison County, 5 mi. E of Cameron, 3 specimens; Wall Creek, 17 mi. S, 1 mi. W of Cameron, 3 specimens; 0.5 mi. S, 2 mi. E of Harrison, 32 specimens; 1.5 mi. S, 3–3.5 mi. E of Harrison, 3 specimens; 3.5 mi. S, 1.5 mi. E of Harrison, 1 specimen; 4.5 mi. S, 6 mi. E of Harrison, 22 specimens; 6.5 mi. S, 1.5 mi. W of Harrison, 8 specimens; 3 mi. N, 2 mi. W of McAllister, 1 specimen; 1 mi. N, 2 mi. E of Norris, 1 specimen; 1.25 mi. N, 0.25 mi. E of Pony, 19 specimens.

S. e. nevadensis (Howell). Nevada: Elko County, 6.5 mi. W of Halleck, 5 specimens.

S. citellus citellus (Linnaeus). USSR, Moldavian ASSR, no exact locality, 2 specimens.

S. suslicus suslicus Guldenstaedt. USSR, Ukraine SSR, Kiev Obl., 75 mi. S of Kiev, 10 specimens; vic. of Odessa, no exact locality, 2 specimens.

S. major major Pallas. USSR, Tatarsk ASSR, Ul'yanovsk. Obl., vic. of Cherdakly, 19 specimens.

S. major ungae Martino. USSR, RSFSR, Sverdlovsk. Obl., no exact locality, 4 specimens.

S. dauricus dauricus Brandt. USSR, RSFSR, Chitinisk. Obl., 15 mi. SW of Chindet, 9 specimens.

S. musicus Menetrie. USSR, RSFSR, Karachaevo-Cherkessk. Aut. Obl., no exact locality, 56 specimens.

S. pygmaeus brauneri Martino. USSR, RSFSR, Rostovsk. Obl., vic, of Zernograd, 21 specimens; Rostovsk. Obl., no exact locality, 4 specimens.

S. erythrogenys erythrogenys Brandt. USSR, RSFSR, Novosibirks. Obl., Toguchin, 24 specimens.

S. fulvus nigrimontanus Antipin. USSR, Kazakh SSR, Dzhambilsk. Obl., vic. of Otar, 2 specimens; in Kazakh SSR, 28 mi. NE of Frunze, 6 specimens.

S. relictus relictus Kashkarov. USSR, Uzbek SSR, Tashkentsk. Obl., Kuraminsk. Mts., Angren Plateau, 23 specimens.

S. relictus ralli Kuznetsov. USSR, Kirgiz SSR, Issyk-Kul'sk. Obl., vic. of Przheval'sk, 2 specimens.

S. undulatus undulatus Pallas. USSR, RSFSR, Irkutsk. Obl., vic. of Angarsk, 4 specimens.

S. u. eversmanni Brandt. USSR, RSFSR, Altaisk. Krai, Gorno-Altaisk. Aut. Obl., 1.5 mi. N of Shebalino, 1 specimen.

S. u. jacutensis Brandt. USSR, RSFSR, Yakutsk. ASSR, 52 mi. N of Yakutsk, W bank, Lena R., 5 specimens.

S. u. menzbieri Ognev. USSR, RSFSR, Amursk. Obl., 15 mi. S of Belogorsk, 1 specimen.

S. parryii parryii Richardson. Canada, Manitoba: 5 mi. N of Seal River, W shore, Hudson Bay, 12 specimens; Northwest Territories: Thelon R., 22 mi. NW of Baker Lake, 17 specimens; 32 mi. NW of Baker Lake, 29 specimens; Thelon R. at E end of Schultz Lake, 1 specimen. USA, Alaska: East Fork, Chandalar R., Arctic Village, 2 specimens; 25 mi. N of Arctic Village, 22 specimens 30 mi. N of Arctic Village, 5 specimens.

S. p. ablusus Osgood. Alaska: Alaska Peninsula, Cold Bay, 8 specimens; 13 mi. N of Egegik, 3 specimens; Alaska Range, Otto Lake, 2 specimens; Tikchik Lake, 6 specimens.

S. p. plesius Osgood. Alaska: Wrangel Mts., no exact locality, 6 specimens.

S. p. leucostictus Brandt. USSR, RSFSR, Khabarovsk. Krai, Chukotsk. Nats. Okr., Pevek, 14 specimens; Magadansk. Obl., vic. Atka, 19 specimens; 125 mi. NNW of Magadan, 1 specimen; Yakutsk. ASSR, Cherskii, 10 specimens; E bank, Kolyma R., 15 mi. below Sredne Kolymsk, 10 specimens.

Laboratory methods

Blood was drawn from live animals into heparinized syringes, then centrifuged at ambient temperatures in the field or in a refrigerated centrifuge in the laboratory to separate plasma and red cells. Red cells were washed twice in normal saline and following centrifugation were hemolyzed with an equal volume of distilled water and 0.1 M of CCl_4. The hemolysate was shaken vigorously, centrifuged and the supernatant stored at −20° to −60 °C. Multiple small samples of both plasma and red cell hemolysates were stored in separate vials for multiple electrophoretic analyses, thus avoiding thawing and refreezing. Livers and kidneys removed in the laboratory were minced in distilled water and ground in a glass homogenizer on ice; after centrifugation (10,000 rpm at 0–4 °C) for 30 min, the supernatant was lyophillized and stored at −20 °C until use. Tissues collected in the field were placed directly into plastic vials and stored in a liquid nitrogen freezer until return to the laboratory where they were thawed, homogenized, and lyophillized as described above. At the time of electrophoresis whole plasma and red cell hemolysates were thawed and analyzed in undiluted form; 10 mg of lyophillized tissue was reconstituted with 0.1 ml of distilled water. Samples were absorbed on 5 × 9 mm pieces of Whatman #3 filter paper prior to insertion in the gel.

Starch-gel electrophoresis on horizontal or vertical apparatus in a cold room was used for the analysis of all proteins; size of the horizontal tray was 6 × 14 × 0.9 cm and that of the vertical 18 × 13 × 0.9 cm. Each gel could be cut into three slices which permitted the examination of several proteins separated simultaneously during a sample run. Reference samples of known electrophoretic mobilities were included in each run and isozymes of each polymorphic protein were arbitrarily designated by numbers or lettes according to their time of discovery, rather than their relative mobilities.

Serum transferrin (Tf-one locus) was analysed by horizontal starch-gel electrophoresis on 14 % Connaught starch according to the methods of Poulik (1957) and Poulik and Smithies (1958); these data were reported previously (Nadler et al. 1974). Serum albumin (ALB-2 loci?) were examined on the half of the gel used to separate transferrins after staining with Buffalo Black. Hemoglobin (Hgb-two loci), serum leucine aminopeptidase (LAP-one locus), red cell aldolase (ALD-one locus), red cell alpha glycerophosphate dehydrogenase (αGPD-one locus), and red cell lactic dehydrogenase (LDH-two loci) were run on a 14 % electrostarch gel made with pH 8.1 gel buffer (0.05 M Tris, 0.0018 M EDTA disodium salt, 0.0075 M boric acid) and pH 8.1 electrode buffer (0.3 M boric acid, 0.0625 M NAH) and stained according to the techniques of Shaw and Prasad (1970). Glucose-6-phosphate dyhydrogenase (G6PD-one locus), 6-phosphogluconate dehydrogenase (6PGD-one locus), red cell and liver malate dehydrogenase (MDH-two loci), liver and kidney isocitric dehydrogenase (ICD-two loci), and liver glutamate oxalate transaminase (GOT-two loci) were analyzed on 12,5 % electrostarch utilizing the buffers of Engel et al. (1970) and the stains of Shaw and Prasad (1970). Red cell phosphoglucomutase (PGM-two loci) was analyzed in the vertical apparatus utilizing 14 % electrostarch, the buffer system of Koen (1971) and the stain of Shaw and Prasad (1970).

Computation methods

Heterozygosity (h), average heterozygosity ($\overline{H}$), and its variance (V_H) were all calculated following Nei (1975).

For systematic analysis, electromorphs at each locus for each taxon were coded for presence (1) or absence (0) and arranged in two-dimensional arrays for analysis on a Honeywell 66/60 Computer using FORTRAN IV programs. Two methods for determining relationships were employed, the association coefficient (S) of Jaccard (Sneath and Sokal 1973), and a rootless Wagner network (Farris 1970; Sneath and Sokal 1973). The association coefficient is given by $S = a/(a + u)$, where a and u are the number of respective matched (1 : 1) and mismatched (0 : 1 or 1 : 0) electromorphs, respectively. S values were arranged in a similarity matrix and using the NT-SYS program package at The University of Kansas Computation Center, we performed an unweighted pair group cluster analysis (UPGMA, Sokal and Sneath 1963; Sneath and Sokal 1973) from which a phenogram was derived. We chose Jaccard's measure of relationship rather than other measures that have been proposed (Selander 1970; Hedrick 1971; Nei 1972; Rogers 1972) because (1) preliminary analysis showed it was highly correlated with the four measures we tested (see also Hedrick 1975); (2) sample sizes for many of the populations considered were small and variable, and Jaccard's S is less sensitive to sample size differences; and (3) it was more appropriate for systematics in that presence of electromorphs is more important than relative frequency (Avise 1974).

Results

Protein mobility

Ten of the 13 variable proteins (representing at least 11 loci) in Holarctic *Spermophilus* are listed in the table together with electromorphs observed at the various loci. Figure 1 illustrates their relative mobilities. Interspecific variation was observed in ALD, LAP, Hgb and ICD_1, with different electromorphs fixed in different species. Polymorphism within populations was observed in Tf, Alb, PGM_1, PGM_2, G6PD, 6GPD, and MDH_1. Seven additional loci were monomorphic in all species examined, and are not listed (see also Serov et al. 1974).

Monomorphic loci

Two lactate dehydrogenase loci, LDH_1 and LDH_2, were monomorphic in all North American and Eurasian species, as was the malate dehydrogenase found in red cell hemolysates, (MDH_{rbc}) (cf. Serov et al. 1974). A second locus, MDH_1 of liver, was polymorphic (see below) (cf. Sukernik 1975). Isocitrate dehydrogenase (ICD_2) from kidney was invariant, whereas the liver enzyme (ICD_1) displayed three electromorphs (see below). GOT exhibited a fast fraction migrating toward the anode and a slow fraction migrating cathodally from the origin, each considered to be under the control of a separate locus. These two fraction appeared with identical mobilities in all Holarctic taxa, as did the single α GPD fraction observed.

Table

Polymorphic electromorphs of Holarctic *Spermophilus*

Group/Taxon	2n	ICD_1	ALD	LAP	Hgb	Tf	ALB	MDH_1	6PGD	PGM_1	PGM_2	G6PD	$\bar{H}$	V_H	N
Nearctic "small-eared"													X100		
S. (t.) townsendi	36	1	1–2	1	A–B	1, 3	C–2	A–1	2, 4	A	A, B, C	B	7.2	.157	42
S. (t.) vigilis	46	2	1–2	1	A–B	1	C–2	A–0, A–1	4	A, B	A, B, C	B	3.5	.085	59
S. (t.) mollis	38	2	1–2	1	B–0	1, 3, 17	C–2	A–1	4	A	A, B	C	4.3	.094	47
S. (t.) m. idahoensis	38	2	1–2	1	A–B	1	C–2	A–1	4	A	A, B	B	2.8	.075	9
S. (t.) m.? nancyae	38	2	1–2	1	A–B	3	C–2	A–1	2	A	A, B	B	2.8	.075	36
Nearctic "big-eared"															
S. (a.) armatus	34	1	1–2	1	A–0	18	B–1	A–0, A–1	3	A	A, B	A, B	8.2	.194	62
S. (a.) beldingi	30	1	1–2	1	A–0	3	B–1	A–1	3	A	A, B	A	2.8	.075	27
S. (r.) richardsonii	36	1	1–2	1	A–0	2, 4	C–3	A–0, A–1	3	A	A, B, C	A	5.0	.091	105
S. (r.) elegans	34	1	1–2	1	A–0	4	C–0, C–3	A–0, A–1	3	A	A, B	A	4.4	.086	120
S. washingtoni	36	1	1–2	1	A–B	3	B–0	A–1	5	A	A, B, C	A	3.2	.098	31
Long-tailed															
S. columbianus	32	1	1–2	1	A–0	1, 3, 4, 7	C–0, C–3	A–0, A–1	1, 3	A, B	A, B, C	A	10.4	.197	179
S. undulatus ssp.	32	1	1–2	1	A–0	10	C–2	A–1	3	A	A, B	A, B, D	6.0	.183	5
S. u. menzbieri, jacutensis	32	1	1–2	1	A–0	11	A–1	A–1	3	A	A, B	A, B	0.0	–	6
S. parryii, Siberia	34	1	1–2	1	A–0	6, 7	C–0	A–1	3	A	A, B	A, B	6.2	.139	54
S. parryii, N. Amer.	34	1	1–2	1	A–0	5, 6, 7	C–0	A–1	3	A	A, B, C	B	4.9	.116	113
"Intermediate"															
S. relictus	36	3	1–2	2	E–F	12	B–0	A–1	3	A	A, B	B	1.9	.038	25
S. dauricus	36	2	2–3	2	A–C	4, 7	B–0	A–1	3	A	A, B	A	0.8	.007	9
S.brunneus	38	2	1–2	2	A–0	5	B–0	A–1	4	A	A, B	A	2.7	.073	8
Palearctic "small-eared"															
S. suslicus	36	2	2–3	2	C–D	2, 4	B–0	A–1	3	A	A, B	B	2.4	.057	12
S. citellus	40	2	2–3	2	B–0	2	B–0	A–1	3	A	A, B	B	0.0	–	2
S. musicus	36	2	2–3	2	B–0	4	B–0	A–1	3	A	A, B	B	0.0	–	56
S. pygmaeus	36	2	2–3	2	B–0	15, 16	B–0	A–1	3	A	A, B	B	1.5	.023	25
Palearctic "desert-steppe"															
S. erythrogenys	36	2	2–3	2	B–0	6	B–0	A–1	3	A	A, B	A	0.0	–	24
S. major	36	2	2–3	2	B–0	2, 5	B–0	A–1	3	A	A, B	A	3.5	.123	23
S. fulvus	36	2	2–3	2	B–0	12, 13	B–0	A–1	3	A	A, B	A	1.8	.035	8

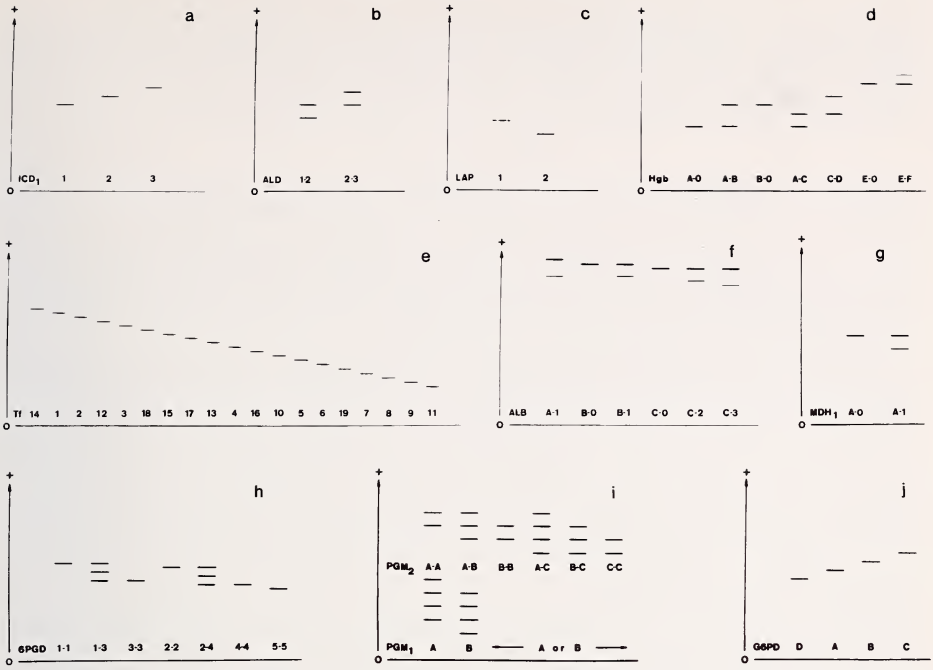


Fig. 1. Relative mobilities of isozymes in the subgenus *Spermophilus.* a Isocitrate dehydrogenase; b Aldolase; c Leucine aminopeptidase; d Hemoglobin; e Transferrin; f Albumin; g Malate dehydrogenase; h 6-phospogluconate dehydrogenase; i Phosphoglucomutase; j Glucose-6-phosphate dehydrogenase

Interspecifically variable loci

Isocitrate dehydrogenase. ICH_1 in liver displayed three electromorphs, each controlled by a single fraction (Fig. 1a).

Adolase. Two different, double-banded patterns, of unknown genetic control, were observed (Fig. 1b).

Leucine aminopeptidase. Two LAP electromorphs, 1 and 2, characterized the species studied and they occurred monomorphicaly as single, presumably homozygous fractions in all taxa (Fig. 1c).

Hemoglobin. Electrophoretic analysis of hemoglobin revealed species patterns containing either one or two fractions. Where two fractions were found (*S. townsendii* group except *S. mollis; S. suslicus, S. dauricus; S. relictus*), intrapopulational polymorphism was absent (Fig. 1d). The lack of polymorphism indicates that the double fractions are not heterozygotes resulting from segregation of two alleles at a single locus and instead suggested that two loci were operative in the production of double-banded hemoglobin pattern. Subsequent studies, to be published in detail elsewhere, indicate presence of a β-chain gene duplication. For the purpose of the present taxonomic analysis we have scored hemoglobin according to phenotypic pattern with fractions designated by letters A through F depending on electrophoretic mobility; single fractions were designated A–O, B–O and E–O and double fractions A–B, A–C, C–D and E–F (Fig. 1d).

Both Palearctic and Nearctic species exhibited two hemoglobins (Table) which suggest that the gene duplication responsible for the phenomenon occurred at an early time in the evolutionary history of these ground squirrels or that the duplication occurred independently in the two regions. In the case of *S. suslicus* (Hgb C–D) and *S. relictus* (Hgb E–F)

both loci have undergone mutational divergence from the Hgb A–B pattern seen in Nearctic species. Furthermore, the Hbg B–O pattern of one species of the *townsendii* group, *S. mollis*, indicates that the locus responsible for Hgb A production was deleted during evolution of that group.

Polymorphic loci

Transferrin. Nineteen alleles plus two additional variants of undetermined relative mobility (TfA and TfB of *S. erythrogenys*) have now been identified (Fig. 1e). *S. parryii* populations exhibited six alleles (Tf 5, 6, 7, 8, 9, 19), the greatest number of alleles observed at any locus or in any species (Nadler and Hoffmann 1977). Transferrin polymorphism was generally associated with those species examined most extensively both in numbers and distribution (Nearctic *S. parryii, S. columbianus*).

Albumin. Albumin patterns appeared electrophoretically as single or double fractions (Fig. 1f). In species exhibiting double fractions, specimens were never observed with single fast or slow fractions which would be anticipated if the double fractions represented the heterozygous expression of two alleles. We therefore scored albumins on the basis of their patterns of phenotypes, the faster fractions designated by letters and the slower fractions by a numerical designation or, if absent, a zero. Two loci probably account for the two observed fractions in some species. Only *S. columbianus*, characterized predominantly by Alb C–0, displayed intraspecific variation with Alb C–3 appearing in two populations at Harrison and Missoula, Montana.

Malate dehydrogenase. The locus for this enzyme in liver exhibited two patterns of undetermined genetic control: pattern A–0 consisted of a single fraction whereas pattern A–1 was comprised of fraction A and a second, more slowly migrating fraction (Fig. 1g).

6-Phosphogluconate dehydrogenase. Both red cell hemolysates and liver homogenates yielded single fractions with identical mobilities in the case of homozygotes and triple fractions in the case of heterozygotes (Fig. 1h); five electromorphs were observed.

Polymorphism occurred in one large population of *S. columbianus* from Missoula, Montana (6PGD-1 and -3), but not in the other populations from Montana. A similar polymorphism involving 6PGD-2 and -4 occurred in *S. t. townsendii* from the Yakima Airport colony, Yakima County, Washington. All populations of other species were monomorphic for a singel electromorph.

Phosphoglucomutase. The multiband PGM pattern was considered to be controlled by at least two loci, the faster constellation of fractions assignable to PGM_2 and the slower group to PGM_1 (Fig. 1i). In PGM_2, ground squirrels followed the pattern of genetic regulation described in humans where each allele controlled two electrophoretic fractions (Hopkinson and Harris 1969); thus, homozygotes displayed two fractions whereas heterozygotes exhibited either four fractions, or three, when one fraction controlled by each allele had similar mobilities. Three electromorph designated A, B and C were observed at the PGM_2 locus (Fig. 1i).

The slower fractions were all assigned to PGM_1 in the present study; two patterns of unknown genetic control designated PGM_1 A and B were observed (Fig. 1i). Attempts to explain intrapopulation of variations by postulating control of two fractions by each allele, as was possible with PGM_2, were not successful, and a third homozygous locus with its resultant two fractions added to those of PGM_1 may exist. Nevertheless, PGM_1 was scored as either PGM_1 phenotype A or B for the taxonomic purposes of this study.

At the PGM_1 locus two pattern, A and B, were recorded in *S. columbianus* and *S. vigilis* whereas the other Nearctic taxa and Palearctic *S. parryii* and *S. undulatus* were monomorphic for the A pattern. Sukernik (1975) reported both A and B from *S. major*. PGM gene frequency data for certain extensively analyzed species including *S. parryii, S. columbianus, S. richardsonii, S. elegans,* and *S. armatus* will be published elsewhere.

Glucose 6-phosphate dehydrogenase. Four electromorphs, G6PD A, B, C, and D, appeared in *Spermophilus,* each reflecting a sex-linked allele controlling a single fraction (Vorontsov et al. 1980), and with identical electrophoretic mobility in both red cell hemolysates and liver (Fig. 1j). Although both G6PD-A and -B occurred in the same population of *S. armatus*, heterozygotes with double-banded patterns were not identified, even in females.

Discussion

Biochemical comparisons and systematic relationships

Biochemical similarity. A phenogram based on the association coefficient of Jaccard (proportion of electromorphs in common) between pairs of species and subspecies is displayed in Figure 2a. The major division separates most Palearctic from most Nearctic species. Among Palearctic *Spermophilus, S. relictus* is quite isolated; Nearctic *S. brunneus* and *S. dauricus* are also isolated, but form a small cluster of their own. The remainder from two clusters, *S. erythrogenys, S. major* and *S. fulvus* in one ("subgenus *Colobotis*"), and the remaining species in the other (subgenus *Spermophilus* [s. s.]).

Among Nearctic *Spermophilus,* the *townsendii* complex is quite isolated from the other species. Almost as distant is *S. washingtoni,* usually considered on morphological grounds as close to *S. townsendii* (Howell 1938). The remaining Nearctic "big-eared" and Holarctic "long-tailed" ground squirrels do not separate along conventional lines, as proposed in the subgeneric classification of Gromov et al. (1965). The closest relationships are shown by Siberian and North American populations of *S. parryii,* only recently separated by the Bering Strait (Nadler et al. 1973b; Nadler and Hoffmann 1977), and the sibling species *S. richardsonii* and *S. elegans,* whose divergence is probably also postglacial in age (Nadler et al. 1971; Neuner 1975).

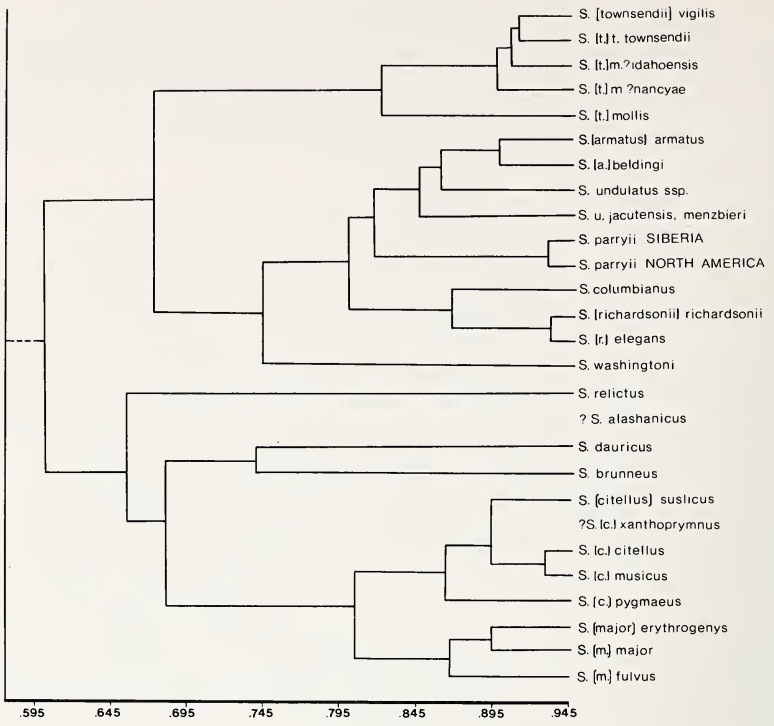
The above description also fits the results of the unrooted Wagner network, the only difference being the somewhat greater distance between the two *S. parryii* groups (Fig. 2b).

Systematic relationships. Other available evidence-morphological and chromosomal as well as biochemical-should be considered in evaluating systematic relationships within the Holarctic ground squirrels. We make summary comparisons here, since a detailed phylogeny of the subgenus of *Spermophilus* is not yet feasible.

Spermophilus in the Old'World exhibit somewhat less diversity in chromosome number and form, and in external and cranial morphology, than do New World ground squirrels of this subgenus (s. l.). The majority belong to a group morphologically similar, allopatrically distributed taxa sharing the same gross chromosomal morphology (2n = 36–42) (Fig. 3). *S. xanthoprymnus* is sometimes considered conspecific with *S. citellus* on the basis of external morphology (Corbet 1978; Flint et al. 1965) but the two differ in chromosome number (2n = 42 and 40, respectively). *S. suslicus* is morphologically distinctive with its white dorsal spotting, but is known to hybridize with *S. pygmaeus* (Denisov 1963; Denisov and Smirnova 1976) with which it shares a chromosome number of 2n = 36, in the zone of potential contact (Fig. 3a). *S. musicus* consists of several isolated, montane populations (Fig. 3a) that have often been considered conspecific with *S. pygmaeus* (Corbet 1978; Flint et al. 1965). Biochemically *S. musicus* is very close to *S. citellus* but shares 2n = 36 with *S. suslicus* and *S. pygmaeus,* and resembles the latter in external and cranial morphology (Gromov et al. 1965).

S. pygmaeus is slightly more divergent biochemically, and morphologically appears to link the Palearctic small-eared ground squirrels with the larger, more specialized "desert-steppe" ground squirrels some-times referred to the subgenus *Colobotis* (Gromov et al. 1965). This latter group includes three taxa with largely allopatric ranges. *S. fulvus* to the south shares a zone of potential contact with *S. major* to the northwest and *S. erythrogenys* to the northeast (Fig. 3b); the ranges of the former pair of species overlap in the steppe

a



b

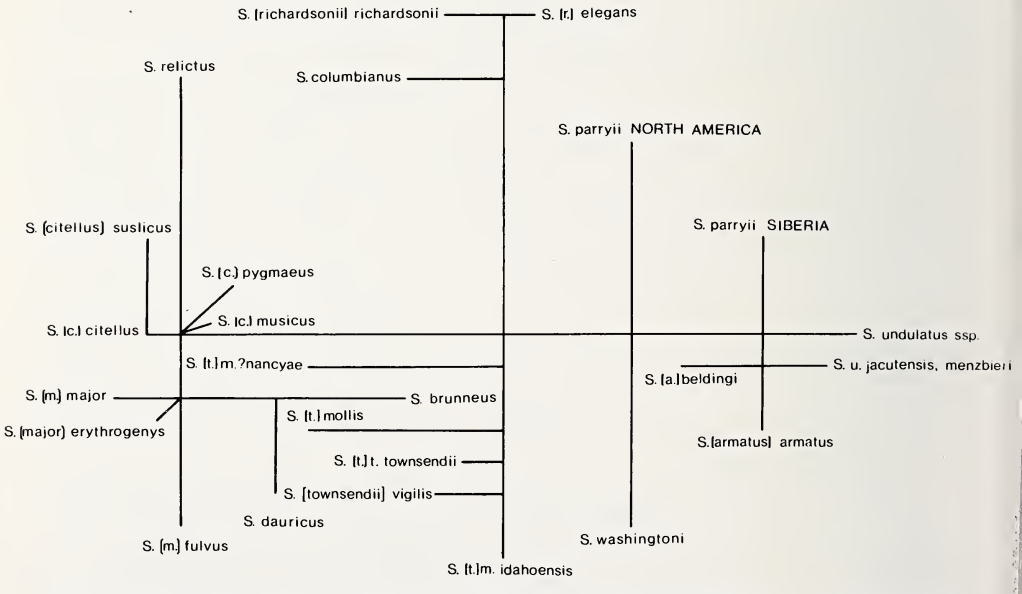


Fig. 2. Dendrograms based on biochemical similarity. a: Jaccard's Coefficient; b: rootless Wagner network

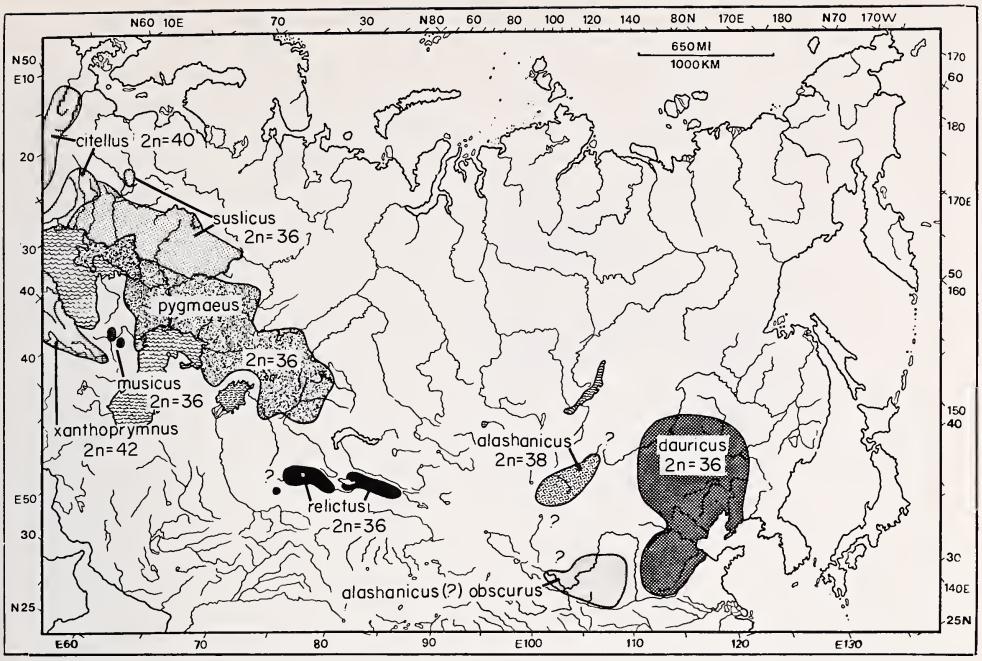


Fig. 3a

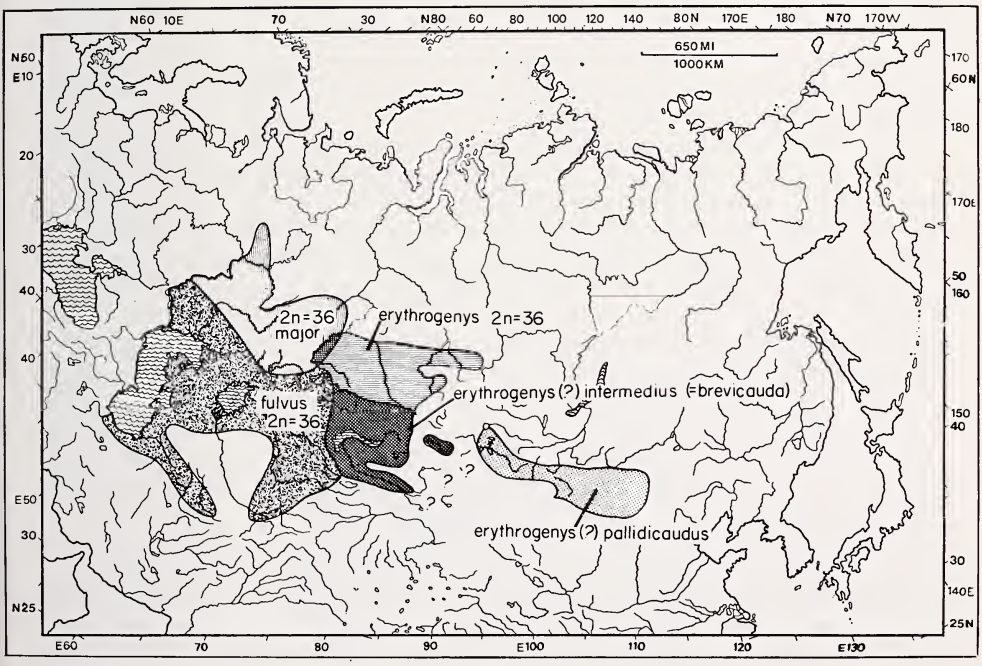


Fig. 3b

Fig. 3. Range of the superspecies *S. [citellus]*, including *S. [c.] citellus, S. [c.] xanthoprymnus, S. [c.] suslicus, S. [c.] pygmaeus*, and *S. [c.] musicus*, and the *dauricus* species group including *S. dauricus, S. alashanicus*, and *S. relictus* (a); the superspecies *S. [major]*, including *S. [m.] erythrogenys, S. [m.] fulvus*, and *S. [m.] major* (b)

zone of central Kazakhstan, while the latter two taxa are reported to overlap in distribution between the Tobòl and Ishim rivers southeast of Tyumen', also in Kazakhstan (Fig. 3b). Within these zones of sympatry, hybridization is known (Denisov 1963; Ognev 1947). The range of *S. pygmaeus* overlaps those of two semidesert-steppe taxa (*fulvus, major*; Fig. 3a, b) but though they too share 2n = 36 chromosomes, hybridization between them and *S. pygmaeus* in the zone of sympatry is rare (Bazhanov 1944, 1945; Denisov 1964).

Thus, both of these groups of allopatric Palearctic ground squirrels may best be regarded as superspecies, listed, in Amadon's (1966) notation, as *S. [citellus]* and *S. [major]*. The low level of morphological differentiation, close biochemical resemblance, and identical chromosome morphology argue against conferring subgeneric recognition to the desert-steppe ground squirrels; accordingly we do not recognize the subgenus *Colobotis*.

Of the remaining three taxa of Palearctic *Spermophilus, S. dauricus* (Fig. 3a) is morphologically similar to the *S. [citellus]* superspecies, and was considered conspecific with *S. citellus* by Ellerman and Morrison-Scott (1951); it also shares the common chromosome number, 2n = 36. Detailed morphological study of *S. dauricus* by Gromov et al. (1965) subsequently supported its specific distinction while confirming its close morphological to *S. citellus*. However, *S. dauricus* shares a sufficient number of electromorphs in common with Nearctic *S. brunneus* that it clusters with it, rather than its Palearctic congeners, although at a low level. *S. alashanicus* is a poorly known species with a montane distribution (Fig. 3a), sometimes considered conspecific with *dauricus* (Allen 1940; Ellerman and Morrison-Scott 1951), and sharing certain morphological characters with that species and with *S. relictus* (Gromov et al. 1965). Its biochemical characteristics are unknown, but it possesses 2n = 38 (Orlov and Davaa 1975), a karyotpe otherwise not seen in Palearctic ground squirrels, but fairly common in Nearctic taxa, including *S. brunneus* (Nadler et al. 1973a). *S. relictus* (2n = 36), also with a montane distribution (Fig. 3a), although clustering biochemically with the Palearctic species, does so at a very low level; morphologically it bears some resemblance to the Nearctic species *S. richardsonii* (2n = 36) (Gromov et al. 1965).

The Nearctic *townsendii* group proper forms a cluster of its own on both biochemical and morphological evidence (Fig. 2). However, chromosomal differentiation is considerable. No chromosomally intermediate individuals have been described among *townsendii* (2n = 36), *mollis* (2n = 38), and *vigilis* (2n = 46), and judging from other species within the genus, a difference of this magnitude is likely to indicate a specific difference (Lyapunova and Vorontsov 1970). We consider these allopatric taxa as species or semispecies belonging to a single superspecies, *S. [townsendi]* (Fig. 4a). Although Howell (1938) in the most recent revision of North American ground squirrels affiliated *S. brunneus* with *S. washingtoni* and the *townsendi* group of "small-eared" squirrels, Davis (1939) noted that *brunneus* shares many morphological characters with the "big-eared" squirrels. As noted above, *S. brunneus* is also distinct biochemically, and its restricted range (Fig. 4a) indicates a relict distribution. *S. washingtoni* may also be a relict species (Fig. 4a). Its morphology places it with the small-eared group (Howell 1938), but biochemically it clusters with the big-eared group, albeit at a fairly low level.

The remaining Nearctic big-eared ground squirrels include species placed by some authors in two subgenera *Spermophilus* (s. s.) and the long-tailed ground squirrels, *Urocitellus*. The first includes *S. beldingi* (2n = 30), *S. armatus* (2n = 34), *S. elegans* (2n = 34), and *S. richardsoni* (2n = 36). Within this chromosomally diverse group, sympatry and ecological niche segregation is considerable (Durrant and Hansen 1954). However, the pairs exhibiting parapatric distributions *(armatus-beldingi; elegans-richardsoni)* (Fig. 4b, c) are also most similar to one another biochemically (Fig. 2) and morphologically (Robinson and Hoffmann 1975). While hybridization has been confirmed between *elegans* and *richardsonii*, it is very limited (Nadler et al. 1971), and between *armatus* and *beldingi*, it has only been inferred (Davis 1939; but see Hansen 1956). No

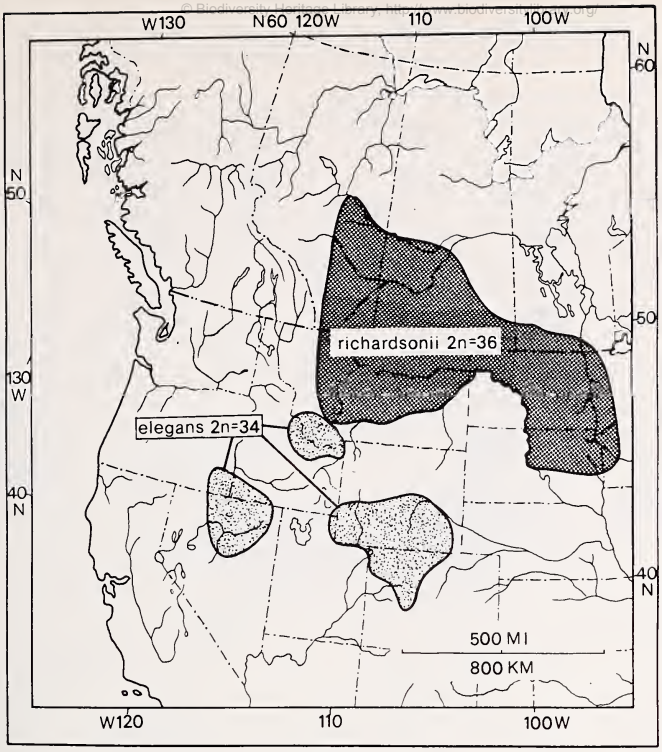

W130
N60
120W
110
100W
N
60
N
50
50
130
W
40
N
40
N
richardsonii 2n=36
elegans 2n=34
500 MI
800 KM
W120
110
100W

Fig. 4a

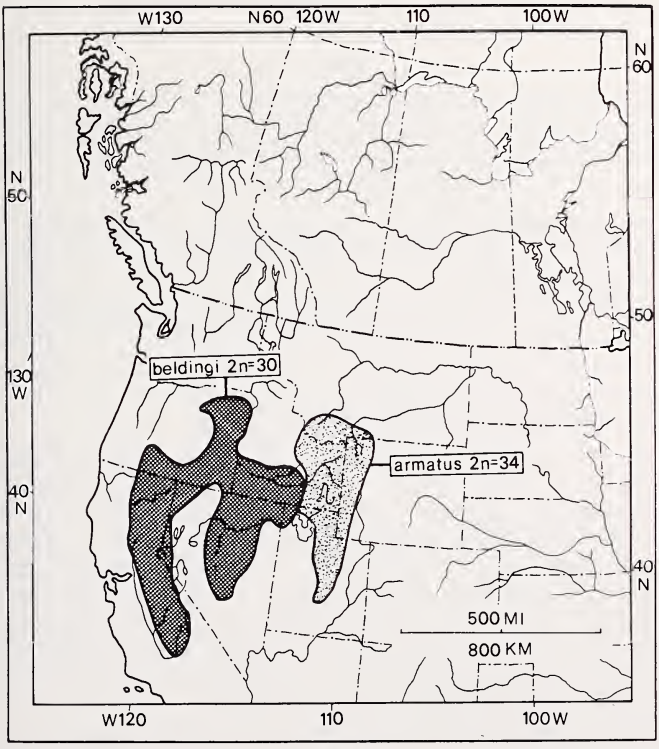

W130
N60
120W
110
100W
N
60
N
50
50
130
W
40
N
40
N
beldingi 2n=30
armatus 2n=34
500 MI
800 KM
W120
110
100W

Fig. 4b

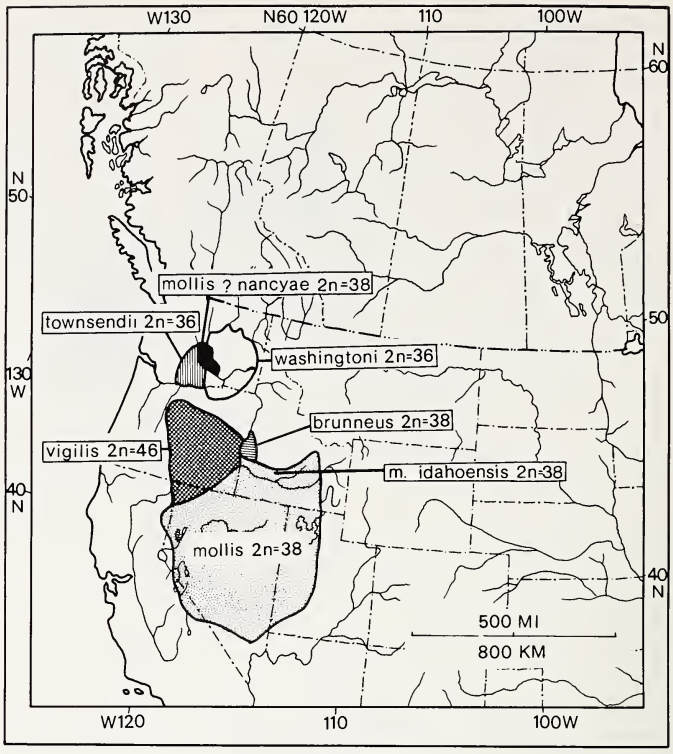


Fig. 4c

Fig. 4a–c. Range of the *townsendi* superspecies including *S. [t.] townsendii, S. [t.] mollis,* and *S. [t.] vigilis,* with the range of the relict species *S. brunneus* and *S. washingtoni* also shown (a); the superspecies *S. [armatus],* including *S. [a.] armatus* and *S. [a.] beldingi* (b); and *S. [richardsonii],* including *S. [r.] elegans* and *S. [r.] richardsonii* (c)

hybrids between *elegans* and *beldingi* or *armatus* are known, although they are sympatric in some places. Thus, each pair may best be regarded as comprising a distinct superspecies, *S. [armatus]* and *S. [richardsoni].*

The last Nearctic species placed by Davis in the big-eared group was *S. columbianus.* However, this taxon, together with *S. parryii* and *S. undulatus,* is best regarded as forming the group of long-tailed ground squirrels sometimes placed in a distinct subgenus, *Urocitellus.* Together they have allopatric ranges from central Siberia to northwestern North America, centering on the Beringian region (Fig. 5).

Of the three extant species, *S. undulatus* is the most variable, and has the widest distribution (Fig. 5). All subspecies of *S. undulatus* have a 2n = 32 karyotype which is indistinguishable in gross morphology from that of North American *S. columbianus,* and Gromov et al. (1965) regarded the two species as derived from the same lineage, suggesting that the sister species may have diverged as early as the Pliocene (Vorontsov and Lyapunova 1976). Nadler et al. (1975) suggested, however, that the isolation between Siberian and North American populations probably dates from the end of the Mindel-Kansas glacial period. In any event, the level of biochemical similarity between *S. undulatus* and *S. columbianus* is quite low, and parallels the pattern of cranial divergence (Robinson and Hoffmann 1975).

The only of taxon ground squirrels with a Holarctic distribution at the species level is *S. parryii* (Fig. 5). Its morphological resemblance to *S. undulatus* is attested to by the fact that

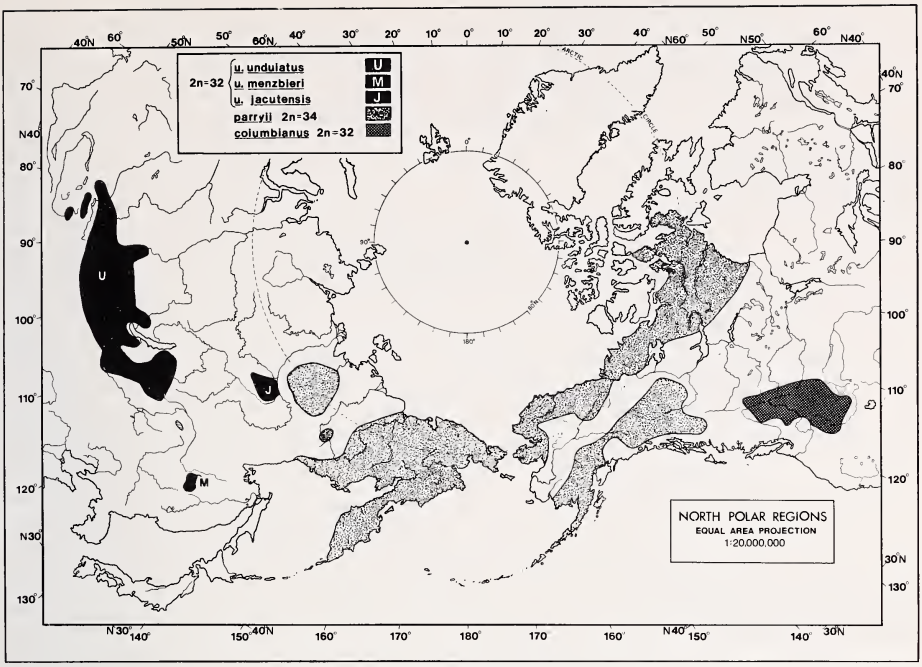


Fig. 5. Range of the long-tailed species group, including *S. undulatus, S. parryii,* and *S. columbianus*

they were considered conspecific (Heptner 1941; Ognev 1947; Rausch 1953; Hall and Kelson 1959) until Gromov et al. (1963) separated them on morphological grounds. Their specific distinctness was later supported by chromosomal evidence (Vorontsov and Lyapunova 1969). Biochemically, the Siberian and North American populations of *S. parryii* have a very high coefficient of similarity, not surprising in view of their very recent (13,000 yr. B. P.) post-glacial isolation (Nadler et al. 1975). However, as in the case of *S. columbianus* and *S. undulatus, S. parryii* shows rather low biochemical resemblance to the other two long-tailed ground squirrels, and this is also true of cranial morphology (Robinson and Hoffman 1975). In fact, while *S. parryii* and *S. undulatus* cluster with *S. (armatus), S. columbianus* clusters with *S. (richardsoni).*

A revised classification of the subgenus *Spermophilus*

Genus *Spermophilus*
Subgenus *Spermophilus*

Nearctic "small-eared" group

Spermophilus [towsendii] townsendi
[t.] vigilis
[t.] mollis (incl. *idahoensis* and *nancyae*?)
Spermophilus washingtoni

Nearctic "big-eared" group

Spermophilus [armatus] armatus
[a.] beldingi

Spermophilus [richardsonii] richardsonii
[r.] elegans
Spermophilus brunneus incertae sedis

Intermediate; *dauricus* group

Spermophilus dauricus
Spermophilus alashanicus
Spermophilus relictus

Palearctic "small-eared" group

Spermophilus [citellus] citellus
[c.] xanthoprymnus
[c.] suslicus
[c.] musicus
[c.] pygmaeus
Spermophilus [major] major
[m.] fulvus
[m.] erythrogenys

Long-tailed group (= subgenus *Urocitellus*?)

Spermophilus undulatus
Spermophilus columbianus
Spermophilus parryii

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Zusammenfassung

Evolution der Erdhörnchen II. Biochemische Vergleiche holarktischer Populationen von Spermophilus

Biochemische Ähnlichkeiten innerhalb 21 von 23 anerkannten Arten der Untergattung *Spermophilus* wurden verglichen. Dreizehn Proteine, wenigstens 18 Loci vertretend, wurden untersucht und die elektromorphen Variationen beschrieben. Phänogramm und Wagner Netzwerk der genetischen Ähnlichkeiten sind dargestellt und zusammen mit morphologischen Daten, mit Daten ihrer Chromosomen und Verbreitung benutzt, um die Systematik des holarktischen *Spermophilus* zu interpretieren. Zwei Entwicklungslinien spalten in Nordamerika voneinander ab, die nearktischen „klein-ohrigen" und „groß-ohrigen" Erdhörnchen. Die meisten der rezenten Arten in diesen Entwicklungslinien haben sich im späten Pleistozän entwickelt.

References

Allen, G. M. (1940): The mammals of China and Mongolia. Natural history of Central Asia. Vol. **11**, 2. New York: Amer. Mus. Nat. Hist.
Amadon, D. (1966): The superspecies concept. Syst. Zool. **15**, 245–249.

Avise, J. C. (1974): Systematic value of electrophoretic data. Syst. Zool. **23**, 465–481.
Ayala, F. J.; Tracey, M. L.; Hedgecock, D.; Richmond, R. C. (1975): Genetic differentiation during the speciation process in *Drosophila*. Evolution **28** (for 1974), 576–592.
Bazhanov, V. S. (1944): Gibridy suslikov. Dokl. Akad. Nauk SSSR **42**, 321–322.
Bazhanov, B. S. (1945): K sistematike dvukh vidov suslikov iz Kazakhskoi SSR. Vest. Kazakh. Fil., Akad. Nauk SSSR **5**, 37–39.
Black, C. C. (1963): A review of the North American Tertiary Sciuridae. Bull. Mus. Comp. Zool. (Harvard Univ.) **130**, 109–248.
Black, C. C. (1972): Holarctic evolution and dispersal of squirrels (Rodentia: Sciuridae). In: Evolutionary biology Vol. **6**, 10. Ed. by T. Dobzhansky, M. K. Hecht, and W. C. Steere. Appleton-Century-Crofts, New York. Pp. 305–322.
Corbet, G. B. (1978): The mammals of the Palaearctic region: a taxonomic review. London: Brit. Mus. Nat. Hist. and London and Ithaca, N. Y.: Cornell Univ. Press.
Davis, W. B. (1939): The recent mammals of Idaho. Caldwell, Idaho: Caxton Printers.
Denisov, V. P. (1963): O gibridizatsii vidov roda *Citellus* Oken. Zool. Zhur. **42**, 1887–1889.
Denisov, V. P. (1964): Rasprostranenie malova (*Citellus pygmaeus* Pallas) i rizhatovo (*Citellus major* Pallas) suslikov v Zavol'zhe. Nauch. Dokl. Vysshe Shkol., Biol. Nauki **2**, 49–54.
Denisov, V. P.; Smirnova, N. I. (1976): Immunological relationships of sousliks genus *Citellus* in the Povolgje region. Acta Theriol. **21**, 267–278.
Durrant, S. D.; Hansen, R. M. (1954): Distribution patterns and phylogeny of some western ground squirrels. Syst. Zool. **3**, 82–85.
Ellerman, J. R.; Morrison-Scott, T. C. S. (1951): Checklist of Palaearctic and Indian mammals 1758–1946. London: Brit. Mus. Nat. Hist.
Engel, W.; Bender, K.; Kadir, S.; Op't Hos, J.; Wolk, U. (1970): Zur Genetik der 6-Phosphogluconate-dehydrogenase (EC:1.1.1.44) bei Säugern. Humangenetik **10**, 151–157.
Farris, J. S. (1970): Methods for computing Wagner trees. Syst. Zool. **19**, 83–92.
Flint, V. E.; Chugunov, Yu. D.; Smirin V. M. (1965): Mlekoptitayushchie SSSR. "Misl'", Moscow.
Gromov, I. M.; Gureev, A. A.; Novikov, G. A.; Sokolov, I. I.; Strelkov, P. P.; Chapskii, K. K. (1963): Mlekopitayushchie fauni SSSR. Vol. **2**, Moscow-Leningrad: Acad. Sci.
Gromov, I. M.; Bibikov, D. I.; Kalabukhov, N. I.; Meier, M. N. (1965): Fauna SSSR. Mlekopitayushchie. Vol. **3**, 2. Nazemnye Belich'i (Marmotinae). Moscow-Leningrad: Nauka.
Hall, E. R. (1981): Mammals of North America. Vol. **1**, New York: J. Wiley.
Hall, E. R.; Kelson, K. R. (1959): The mammals of North America. Vol. 1, New York: Ronald Press.
Hansen, R. M. (1956): Remarks on reported hybrid ground squirrels, *Citellus*. J. Mammalogy **37**, 550–552.
Hedrick, P. W. (1971): A new approach to measuring genetic similarity. Evolution **25**, 276–280.
— (1975): Genetic similarity and distance: comments and comparisons. Evolution 29, 362–366.
Hennig, W. (1966): Phylogenetic systematics. Urbana: Univ. Illinois Press.
Heptner, V. G. (1941): Taxonomic and zoogeographical interrelations of some Asiatic and North-American ground-squirrels. Zoogeographica **4**, 21–27.
Hopkinson, D. A.; Harris, H. (1969): Red cell acid phosphatase, phosphoglucomutase and adenylate kinase. In: Biochemical methods in red cell genetics. Ed. by G. Yunis. New York: Academic Press.
Hopkins, D. M. (1972): The paleogeography and climatic history of Beringia during late Cenozoic time. Inter-Nord, Paris, **12**, 121–150.
Howell, A. H. (1938): Revision of the North American ground squirrels, with a classification of the North American Sciuridae. North American Fauna **56**, 256 pp.
Koen, A. L. (1971): Use of blood clots as enzyme source in gel electrophoresis. Biochemical Genetics **5**, 449–450.
Lorković, Z. (1962): Wesen, Anwendungsbereich und Nomenclatur des Taxon Semispecies. XI Intern. Kongr. Entom., Wien, 1960, **3**, 325–328.
Lyapunova, E. A. (1969): Opisanie khromosomnovo nabora i podtverzhdenie vidovoi samostoyatel'nosti *Citellus parryi* (Marmotinae, Sciuridae, Rodentia). In: Mlekopitayushchie. Ed. by N. N. Vorontsov. Novosibirsk: Acad. Sci. USSR, Pp. 53–54.
Lyapunova, E. A.; Vorontsov, N. N. (1970): Chromosomes and some issues of the evolution of the ground squirrel genus *Citellus* (Rodentia: Sciuridae). Experientia **26**, 1033–1038.
Mayr, E. (1969): Principles of systematic zoology. New York: McGraw-Hill.
Nadler, C. F. (1966): Chromosomes and systematics of American ground squirrels of the subgenus *Spermophilus*. J. Mammalogy **47**, 579–596.
Nadler, C. F.; Hoffmann, R. S. (1977): Patterns of evolution and migration in the arctic ground squirrel, *Spermophilus parryii* (Richardson). Can. J. Zool. **55**, 748–758.
Nadler, C. F.; Hoffmann, R. S.; Greer, K. (1971): Chromosomal divergence during evolution of ground squirrel populations (Rodentia: *Spermophilus*). Syst. Zool. **20**, 298–305.
Nadler, C. F.; Hoffmann, R. S.; Vorontsov, N. N.; Sukernik, R. I. (1976): Evolyutsionnye

svyazi nekotorykh Beringiiskikh mlekopitayushchikh. Pp. 325–336. In: Beringia in Cenozoic. Ed. by V. L. Kontrimavichus. Vladivostock: Acad. Sci.

Nadler, C. F.; Lyapunova, E. A.; Hoffmann, R. S.; Vorontsov, N. N.; Malygina, N. A. (1975): Chromosomal evolution in Holarctic ground squirrels *(Spermophilus).* 1. Giemsa-band homologies in *Spermophilus columbianus* and *S. undulatus.* Z. Säugetierkunde **40**, 1–7.

Nadler, C. F.; Sukernik, R. I.; Hoffmann, R. S.; Vorontsov, N. N.; Nadler, C. F. Jr.; Fomichova, I. I. (1974): Evolution in ground squirrels. I. Transferrins in Holarctic populations of *Spermophilus.* Comp. Biochem. Physiol. **47a**, 663–681.

Nadler, C. F.; Turner, L. W.; Hoffmann, R. S.; Deutsch, L. (1973a): Chromosomes and Giemsa-bands of the Idaho spotted ground squirrel, *Spermophilus brunneus* (Howell). Experientia **29**, 893–894.

Nadler, C. F.; Vorontsov, N. N.; Hoffmann, R. S.; Fomichova, I. I.; Nadler, C. F. Jr. (1973b): Zoogeography of transferrins in arctic and long-tailed ground squirrel populations. Comp. Biochem. Physiol. **44b**, 33–40.

Nei, M. (1972): Genetic distance between populations. Amer. Nat. **106**, 283–292.

— (1975): Molecular population genetics and evolution. Frontiers of biology. Vol. **40**, Amsterdam: North-Holland.

Neuner, M. (1975): Evolution and distribution of the *Spermophilus richardsonii* complex of ground squirrels in the middle and late Pleistocene: a multivariate analysis. Unpubl. M.A. thesis, Univ. of Kansas, Lawrence.

Ognev, S. I. (1947): Zveri SSSR i prilezhashchikh stran. Vol. 5. Gryzuny. Moscow-Leningrad: Akad. Nauk.

Orlov, V. N.; Davaa, N. (1975): O sistematicheskom polozhenii Alashanskovo suslika *Citellus alashanicus* Buch. (Sciuridae, Rodentia). In: Sistematika i tsitogenetika mlekopitayushchikh. Ed. by V. N. Orlov. Moscow: Nauka. Pp. 8–9.

Petrov, B.; Zivković, S. (1977): Some questions of the actual systematics in the light of use of karyological methods. Biosistematika (Beograd) **3**, 13–22.

Poulik, M. D. (1957): Starch-gel electrophoresis in a discontinuous system of buffers. Nature **180**, 1477–1479.

Poulik, M. D.; Smithies, O. (1958): Comparison and combination of the starch-gel and filter-paper electrophoretic methods applied to human sera: two-dimensional electrophoresis. Biochem. J. **68**, 636–643.

Rausch, R. (L.) (1953): On the status of some arctic mammals. Arctic **6**, 91–148.

Reig, O. A.; Aquilera, M.; Barros, M. A.; Useche, M. (1980): Chromosomal speciation in a Rassenkreis of Venezuelan spiny rats (genus *Proechimys,* Rodentia, Echimyidae). In: Animal genetics and evolutions. Ed. by N. N. Vorontsov and J. M. Van Brink. The Hague: Junk. Pp. 291–312.

Repenning, C. A.; Hopkins, D. M.; Rubin, M. (1964): Tundra rodents in a late Pleistocene fauna from the Tofty placer district, central Alaska. Arctic **17**, 177–197.

Robinson, J. W.; Hoffmann, R. S. (1975): Geographical and interspecific cranial variation in big-eared ground squirrels *(Spermophilus):* a multivariate study. Syst. Zool. **24**, 79–88.

Rogers, J. S. (1972): Measures of genetic similarity and genetic distance. Studies in Genetics VII (Univ. Texas Publ. 7213), 145–153.

Selander, R. K. (1970): Bichemical polymorphism in populations of house mouse and old field mouse. In: Variation in mammalian populations. Ed by R. J. Berry and H. N. Southern. New York: Acad. Press.

Serov, O. L.; Vorontsov, N. N.; Frisman, L. V. (1974): A comparison of enzyme and other protein spectors in some species of *Citellus* (Rodentia). Trans. lst Instern. Theriol. Congr. Moscow, 1974, **2**, 145.

Shaw, C. R.; Prasad, R. (1970): Starch-gel electrophoresis of enzymes – a compilation of recipes. Biochem. Genetics **4**, 297–320.

Sneath, P. H. A.; Sokal, R. R. (1973): Numerical taxonomy. San Francisco: W. H. Freeman.

Sokal, R. R.; Sneath, P. H. A. (1963): Principles of numerical taxonomy. San Francisco: W. H. Freeman.

Sukernik, R. I. (1975): Geneticheskie vzaimootnosheniya mezhdu vosem'yu vidami suslikov *(Citellus)* po resul'tatami elektroforeza belkov i izofermentov krovi. Izv. Akad. Nauk SSSR, Ser. Biol. **5**, 713–718.

Vorontsov, N. N.; Lyapunova, E. A. (1969): Stroenie khromosom *Citellus undulatus* i istoriya stanovleniya arealov *C. undulatus* i *C. parryi.* Dokl. Akad. Nauk SSSR **187**, 207–210.

— — (1976): Genetica i problemy transberingiiskikh svyazei Golarkticheskikh mlekopitayushchikh. In: Beringia in Cenozoic. Ed. by V. L. Kontrimavichus. Vladivastok: Acad. Sci. Pp. 337–353.

Vorontsov, N. N.; Frisman, L.V.; Lyapunova, E. A.; Mezhova, O. N.; Serdyuk, V. A.; Fomichova, I. I. (1980): The effect of isolation on the morphological and genetical divergence of populations. In: Animal genetics and evolution. Ed. by N. N. Vorontsov and J. M. Van Brink. The Hague: Junk. Pp. 339–359.

Vorontsov, N. N.; Lyapunova, E. A.; Borissov, Yu. M.; Dovgal, V. E. (1980): Variability of sex chromosomes in mammals. In: Animal genetics and evolution. Ed. by N .N Vorontsov and J. M. Van Brink. The Hague: Junk. Pp. 361–372.

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Zyklische Bestandswechsel (Gradationen) bei der Feldmaus *(Microtus arvalis)*, festgestellt durch Analyse von Eulen-Gewöllen

Von E. Bethge

Eingang des Ms. 29. 12. 1981

Abstract

Cyclic population changes of the common vole (Microtus arvalis) studied by analysis of owl pellets

Studied over 13 years (1968/69 until 1980/81) the pellets of longeared owls, which regularly winter in a park at the edge of Würzburg. The pellets contained the remains of 51 682 small vertebrates. The numbers of the main prey animals common vole *(Microtus arvalis)* and longtailed fieldmouse *(Apodemus sylvaticus)* and of the captured birds fluctuated regularly with peaks of 3 year intervals, except for one 2 year interval. The yearly portion of the common vole and the longtailed fieldmouse together was rather constantly 82–95 %. Therefore there was a reciprocal relationship of the occurrence of these two species. It was concluded that these fluctuations reflect the real fluctuations of the population density of the common vole. The 3 year cycle of the longtailed fieldmouse seemed to be dependent upon the course of the cycle of the common vole.

Einleitung

Jedes Jahr erscheinen in Deutschland Waldohreulen, um hier den Winter zu verbringen. Die Ankunftszeiten dieser wohl aus dem Norden und Osten einfliegenden Eulen liegen in Norddeutschland 2–3 Wochen früher als in Unterfranken. Frühester Zeitpunkt war nach meinen Beobachtungen in Hamburg der 20. August, in Würzburg der 10. September. Die Verweildauer beträgt 5–6 Monate.

Untersuchungsgebiet und Methodik

Seit dem Winter 1968/69 wurden an verschiedenen Stellen um Würzburg, seit 1974/75 vor allem in einem Park am Stadtrand von Würzburg, Ansammlungen von Waldohreulen regelmäßig beobachtet (Bethge 1975). Dieser auf der „Sieboldshöhe" gelegene Park ist etwa 8 ha groß und erhält jeden Herbst Besuch von 14 bis 40 Eulen. Unter den Ruhebäumen, 20 m hohen Schwarzkiefern (es sind fast immer die gleichen Bäume besetzt) wurden regelmäßig Gewölle aufgesammelt, um ihren Inhalt auszuwerten. So entstand in 13 Wintern zwischen 1968/69 und 1980/81 eine Gesamt-Beuteliste von 51 682 Wirbeltieren in etwa 30 000 Gewöllen. Käfer und andere Wirbellose spielen bei der Winternahrung keine Rolle.

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