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Chromosome polymorphism in Polish populations of northern birch mouse *Sicista betulina*[1]

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Abstract. Somatic chromosomes of 17 northern birch mice, *Sicista betulina*, originating from lowland and Tatra Mountain populations were studied. In the whole studied material constant diploid number of chromosomes (2*n* = 32) was found. Polymorphism of a pair of large-sized autosomes was found; acrocentric, subtelocentric and submetacentric chromosomes in homozygous or heterozygous state form the polymorphic pair. Consequently, chromosome arm number (NF) varies within 60 and 62. In this regard Polish populations of northern birch mouse differ from those of more eastward distribution (NF = 63–64).

Key words: variability of karyotype, chromosome evolution

Introduction

Genus *Sicista* is characterised by well-differentiated karyotype. Variability in the number of chromosomes in species belonging to this genus is 2*n* = 16–50 (Table 1). Chromosomes of *Sicista betulina* (Pallas, 1779) were studied in many populations in various parts of species range thus far. The first description of the northern birch mouse's karyotype (2*n* = 32) was based on the material from Białowieża Primeval Forest (Poland) (Walknowska 1960). Karyotypes with 32 chromosomes were also confirmed in various populations of northern birch mouse occurring in European part of former The Union of Soviet Socialist Republics (USSR) as well as in Siberia (Vorontsov & Malygina 1973, Sokolov et al. 1987, Baker et al. 1996). Different chromosome arm numbers (NF) were given; extreme NF or NFa values were 48 (Vorontsov & Malygina 1973) and 64 (Sokolov et al. 1987), respectively. These discrepancies were rather a consequence of a poor quality of chromosome preparations used in karyotype examination and arrangement than the reflection of the real differences in chromosomes morphology.

Chromosome polymorphism was recorded in three *Sicista* species (see Table 1). In *S. subtilis*, three pairs of autosomes have different centromere localization and homo- and heterozygotic morphs occur in populations (Sokolov et al. 1986a). In *S. severtzovi*, several heteromorphic chromosome pairs were described as well as variation in the chromosome number (2*n* = 16–22) was observed (Sokolov et al. 1986a, 1987, Zagorodniuk & Kondratenko 2000). Karyotype of *S. betulina* was for a long time considered to be monomorphic (Walknowska 1960, Vorontsov & Malygina 1973, Sokolov et al. 1987, Baker et al. 1996); although identification of homologous chromosomes made repeated problems. Sokolov et al. (1989) were the first ones that noticed the presence of distinct differences in the localization of centromeres on a pair of large-sized autosomes.

In the present work, we demonstrate karyotype structure and variation in a few Polish populations of *S. betulina*. Three specimens out of the material used in the present work were previously analysed and described by Matan (2007).

[1] *The work is dedicated to the memory of Professor Zdzisław Pucek*

Table 1. *Survey of karyotype variability in the genus* Sicista.

Species/subspecies	Karyotype characteristics*	Remarks	Source
S. severtzovi Ognev, 1935	2n = 16–22; NF = 28–30; X acro (m) Y acro (s)	Originally described as ssp of *S. subtilis*; three unpaired autosomes which differ in size and morphology; number of acroc. autosomes 6–8	1,6,7
S. subtilis sensu lato	2n = 17; NF = 29; X submeta (m)?; Y (d)	This karyotype form probably relate to *S. severtzovi*. One unpaired autosome	2
S. subtilis subtilis Pallas, 1773	2n = 24; NF = 41–44; X acro (s); Y (d)	Three pairs (8, 9, 10) polymorphic (acro and subtelocentric morphs)	1
S. s. sibirica Ognev, 1935	2n = 24; NF = 44 –45; X acro (s); Y (d)	Karyotype similar to *S. subtilis subtilis*; two pair (8, 9) polymorphic acro and subtelo morphs)	1
S. s. vaga Pallas, 1778	2n = 24; NF = 41–42; X acro (m); Y acro (s)	Karyotype similar to *sibirica* and *subtilis*; two pair (8, 9) polymorphic	1
S. s. nordmanni Keyserling & Blasius, 1840	2n = 26; NF = 48; X acro (m); Y (d)	Five large-sized pairs similar to the preceding subspecies with 2n = 24	1
S. kluchorica Sokolov et al., 1980	2n = 24; NF = 44; X acro (m); Y acro (s)		3
S. caucasica Vinogradov, 1925	2n = 32; NF = 48; X acro (m); Y (d)		3
S. tianshanica Salensky, 1903	2n = 32; NF = 54; X acro (m); Y acro (s)		3
S. betulina Pallas, 1779	2n = 32; NF = 63–64; Y (d)	Different size and morphology of X chromosome were reported	see text
S. armenica Sokolov & Baskevich, 1988	2n = 36; NF = 52; X acro (b); Y (d)		4
S. kazbegica Sokolov, Baskevich & Kovalskaya, 1986	2n = 40, 42; NF = 50, 52; X acro (m); Y (d)	The two chromosome types have probably parapatric distribution in Caucasus	5
S. napaea Hollister, 1912	2n = 42; NF = 52; X acro (m); Y acro (s)		3
S. pseudonapaea Strantman, 1949	2n = 44; NF = 52; X acro (m); Y acro (s)		3
S. caudata Thomas, 1907	2n = 50; NF = 50; X acro (l); Y acro (s)		3
S. strandi Formosov, 1931	2n = 44; NF = 52; X acro (l); Y (d)	Originally described as *S. betulina* "B form"	3

** Size of chromosome: (l) large; (m) medium; (s) small; (d) dot-like chromosome.*
Source: 1 – Sokolov et al. 1986a, 2 – Zagorodniuk & Kondratenko 2000, 3 – Sokolov et al. 1987, 4 – Sokolov & Baskevich 1988, 5 – Sokolov et al. 1986b, 6 – Aniskin et al. 2003, 7 – Kovalskaya et al. 2000.

Table 2. *The morphology of polymorphic chromosomes of the pair 2 of* Sicista betulina.

Geographic region	Population	Geographic coordinates	No. of animals	Sex	Chromosome morph (pair No. 2)
Masurian Lake Region	Tylkówko	20º74' E; 53º64' N	4572	f	st/st
			4592	f	st/st
			4607	m	a/a
	Michałki	20º76' E; 53º65' N	4593	m	st/st
	Miluki	20º76' E; 53º67' N	4606	m	st/st
			4608	f	st/st
			4609	f	st/st
	Zgniłocha	20º34' E; 53º33' N	4519	m	a/a
			4520	f	a/st
	Natać	20º34' E; 53º31' N	4652	m	a/a
	Wielka		4653	m	sm/sm
Białowieża Primeval Forest	Białowieża	23º49' E; 52º42' N	4555	m	st/sm
Tatra Mountains	Polana	19º95' E; 49º30' N	4688	m	a/sm
	Biały Potok		4691	m	sm/sm
			4692	m	st/st
			4695	f	st/sm
			4704	f	sm/sm

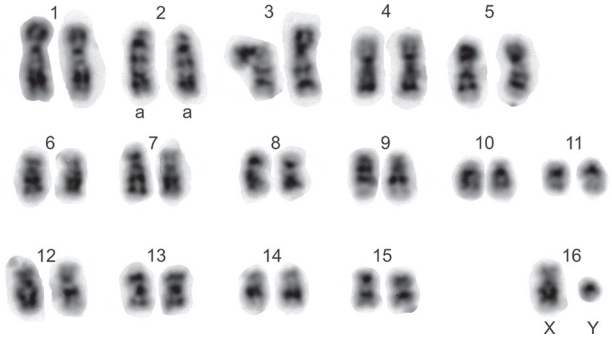

Fig. 1. *The homozygous (a/a) G-banded karyotype.*

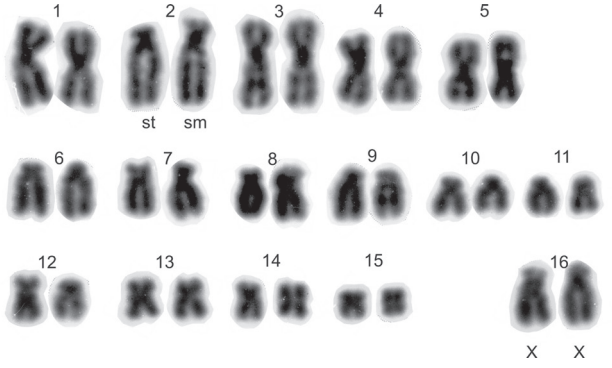

Fig. 2. *The heterozygous (st/sm) karyotype .*

Material and Methods

Material for chromosome studies consisted of 17 individuals originating from six lowland populations and one high mountain population (Table 2). Chromosome preparations were made from spleen and/or bone marrow by using standard method *in vivo*

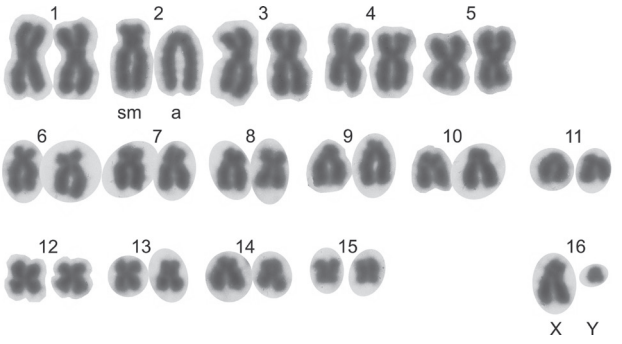

Fig. 3. *The heterozygous (sm/a) karyotype.*

(Fedyk 1980). G-bands were obtained after trypsin digestion of chromosomes and staining with Giemsa buffered solution according to Seabright (1971).

No karyotype standard of *S. betulina* has been proposed thus far. Authors of previous papers arranged karyotypes based on the size of individual chromosomes (Walknowska 1960, Sokolov et al. 1982, Baker et al. 1996) or they identified chromosome groups depending on the centromere localization (Vorontsov & Malygina 1973, Sokolov et al. 1987, 1989, Baskevich & Okulova 2003). A low quality of chromosome preparations yielding poorly visible localization of the centromere can explain such huge diversity. In the present work, we recommend to use the karyotype of *S. betulina*, published by Sokolov et al. (1989) as a standard.

Results

All the studied individuals had $2n = 32$ chromosomes. Within large chromosomes, pairs 1, 4 and 5 are

metacentric chromosomes; pair 3 is submetacentric, and pair 2 is polymorphic and it is composed of uniarmed (acrocentric) or biarmed chromosomes (Figs. 1–3). Analysis of the arms ratio on biarmed chromosomes demonstrated that there are two distinct variants, which differ by centromere localization. Chromosomes with more proximal localization of centromeres have the ratio 2.13 to 3.20 between the longer and the shorter arm ($r = l/s$); however, the arms ratio on chromosomes with more distal localization of the centromere was between 4.48 and 6.92. These two chromosome variants were classified as submetacentric (sm) and subtelocentric (st), respectively. According to the formal definition (Levan et al. 1964), sm chromosomes have $r \leq 3$, and st chromosomes have $r \geq 3$. Correctness of this differentiation between observed variants is confirmed by the r values (Fig. 4), which do not overlap each other.

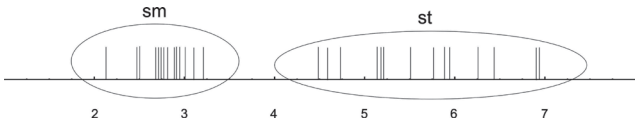

Fig. 4. *The arm length ratio (r = l/s) on biarmed chromosomes of the autosomal pair 2.*

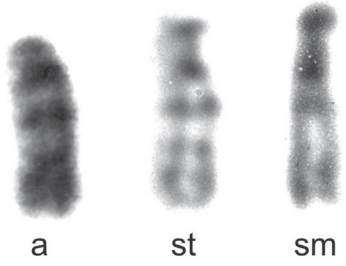

Fig. 5. *G-banding patterns in three variants (a, st, sm) of the polymorphic autosomal pair 2.*

The second size group consists of five pairs (6–10) of submetacentric chromosomes and a pair 11 of acrocentric chromosomes. The third size group consists of four pairs (12–15) of small meta- and submetacentric chromosomes. Small differences in ratio of chromosome arms in various metaphase cells were observed within chromosomes of the second and third group. As the differences were found not only between individuals but also between methaphasal spreads coming from the same individual, this differentiation was not taken into account. A subtelocentric X chromosome is of similar size as the autosomes of pairs 6 and 7. The Y chromosome is the smallest chromosome in the karyotype. The chromosome arm numbers (NF) vary between 60 and 62 depending on the morphology of chromosomes of the pair 2.

The G-banding pattern well characterizes the individual pairs of chromosomes, including the sex chromosomes (Fig. 3). It should be noted that additional bands can occur on short arms of st and sm chromosomes from the pair 2 in comparison with acrocentric chromosomes (Fig. 5).

Discussion

The authors of all previous publications reported unanimously that *S. betulina* has $2n = 32$ chromosomes. The only exception is the Kursk area where the "form B" of *S. betulina* with $2n = 44$ was found (Sokolov et al. 1987). Further studies revealed that the "form B" is a different species, *Sicista strandi* Formosov, 1931 distributed allopatrically in respect of *S. betulina* (Sokolov et al. 1989, Zagorodniuk 2007).

No polymorphism of autosomes has been detected in the early studies of *S. betulina*. In addition, previous authors achieved only very poor quality chromosomal preparations. Therefore, not only the arrangement of chromosomal pairs but also the determination of the X chromosome was wrong in many publications. Application of the outdated "squash" method in pioneering work gave preparations with poor morphology of chromosomes (Walknowska 1960). Usage of the air-drying preparation method significantly improved the results. However, Vorontsov & Malygina (1973) did not provide a detailed morphology of *S. betulina* chromosomes as well. Some of chromosomes were described as "small, near acrocentric elements", and as a consequence, the number of autosomal arms (NFa = 48) was underestimated. Poor quality of chromosome preparations also did not enable the correct determination of the X chromosome (Vorontsov & Malygina 1973). Sokolov et al. (1982) obtained better quality chromosome preparations from northern birch mice collected near Moscow, but they also arranged the karyotype incorrectly. A subtelocentric chromosome from the heteromorphic pair (pair 2 at present work) was determined as the X chromosome and only biarmed chromosomes were described in the karyotype ($2n = 32$, NF = 64). In a comprehensive study on the material collected from Moscow, Kurgan, Novosibirsk, Kemerowo, Tomsk and Krasnojarsk regions and from the former Buryat ASSR, Sokolov et al. (1987) gave the same characteristics ($2n = 32$, NF = 64) and again incorrectly determined the sex chromosomes. Chromosomes of pair 4 (according

to the nomenclature used in the present paper) were determined as the X chromosomes, however, there is no mention of polymorphism, though the heteromorphic pair (sm/st) of large autosomes is clearly visible in the published karyotype. Sokolov et al. (1989) realized that pair 2 was polymorphic and they re-analyzed material from previous publications (28 individuals from 13 populations from the European part of the former USSR and Siberia). The authors described two chromosome variants (sm and st) of the pair 2. The sm chromosomes were more common and they were found in homozygous state (sm/sm) in 15 individuals originating mostly from the European part of species range. The st chromosomes occur mostly in Siberia, where they are rare and were found in heterozygous state (sm/st) in 13 individuals. These data confirm our observations of geographic differentiation of polymorphic morph frequency; st chromosomes occur more frequently on the lowlands in Poland, whereas sm chromosomes prevail in the mountain population. In our material, the frequency of the acrocentric chromosome variant is 0.23 in the whole sample (Table 2). It is surprising that this variant was not identified in the earlier paper, as an acrocentric chromosome in heterozygous state is apparent in the complement of individuals from the former Buryat ASSR (see Sokolov et al. 1989 – Fig. 2).

The karyotype of *S. betulina* reported by Sokolov et al. (1989) was used as a standard in the present study. However, it must be emphasized that Sokolov et al. (1989) determined the X chromosome as subtelocentric, with its size similar to the smallest autosomes (pairs 13–15), whereas in the Polish populations (excluding of Walknowska 1960), X chromosome is reported as submetacentric of comparable size to pairs 6 and 7. The number of chromosome arms also differs. All the autosomes are considered biarmed in Sokolov et al. (1989), whereas pair 11 is evaluated as acrocentric in Polish populations. Baker et al. (1996) described at Chernobyl, a region close to the eastern border of Poland, a karyotype, which is the most similar to our findings. Pair 2 is heteromorphic (sm/st) and one of the autosomal pairs is acrocentric. The centromere position on the X chromosome is not clear but the relative size of this chromosome is comparable with X chromosome from our material. Baskevich & Okulova (2003) on the basis of the G-banding spreads, determined X chromosome of similar size in the material from Moscow and Riazian environs. However, it should be mentioned that G-banding karyotype presented in their work is not similar to that presented in this paper (Fig. 1).

G-banding pattern (Fig. 5) suggests that shorter arms of st and sm chromosomes of pair 2 originated by chromatin addition rather than pericentric inversion. The presence of large heterochromatin blocks (C bands) in the centromeric region of the autosomal pair 2 (Baskevich & Okulova 2003) supports this hypothesis. It is surprising that the G-banding pattern of northern birch mice from Moscow region (Baskewich & Okulova 2003) is totally incomparable with that presented in our study (Fig. 3).

Polymorphism similar to *S. betulina* was recorded also in *S. subtilis*. Variants a, st and sm of three autosomal pairs were found in three subspecies with $2n = 24$ (*S. subtilis subtilis*, *S. s. vaga*, and *S. s. sibirica*). It was suggested that this polymorphism resulted from pericentric inversions. The fourth subspecies (*S. s. nordmanni*) differs from the above-mentioned with its chromosome number, which is $2n = 26$ (Sokolov et al. 1986a, Baskevich et al. 2005). Generally, based on the obtained results, it could be hypothesised that there is possible polymorphism of more than one pair of chromosomes that exists in *S. betulina.*

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