CYTOGENETIC ANALYSIS OF A HYBRID ZONE BETWEEN THE MOSCOW AND NEROOSA RACES OF THE COMMON SHREW (*SOREX ARANEUS*) DIFFERING BY A SINGLE WART-LIKE CHROMOSOME REARRANGEMENT

© *S. V. Pavlova*

A. N. Severtsov Institute of Ecology and Evolution RAS, Moscow; e-mail: swpavlova@mail.ru

Three karyotypic variants were revealed in the Moscow—Neroosa chromosomal hybrid zone by comparative cytogenetic analysis: homozygotes of the pure parental races and complex heterozygotes (F_1 hybrids). As expected, a ring-of-four configuration (RIV) was observed in diakinesis spreads of hybrids. No disturbances in the structure of the RIV were found. Distribution of telomeric repeats and rDNA on the chromosomes of an individual of the Neroosa race were studied using dual-colour FISH.

K e y w o r d s: karyotype, hybrid zone, meiosis, FISH, *Sorex araneus.*

As a result of high karyotypic variability, at least 72 parapatric chromosomal races have been described in the common shrew (*Sorex araneus* L., 1758), a small insectivorous mammal (White et al., 2010). These races are distinguished by metacentrics deriving from 12 originally acrocentric autosomes by Robertsonian (Rb) centric fusions and whole-arm reciprocal translocations (WARTs). Three pairs of biarmed autosomes (*af*, *bc*, *tu*) and the sex chromosomes (XX in females and XY_1Y_2 in males) are invariant in all chromosomal races, while the other autosomal arms (from *g* to *r*) may occur as acrocentrics and/or metacentrics.

The chromosomal races of *S. araneus* make contact and hybridise, forming hybrid zones (Searle, Wójcik, 1998). Chromosomal heterozygotes may be expected to have low fertility due to meiotic abnormalities, and hybrid zones are excellent places to examine such individuals and to assess the reproductive isolation between the chromosomal races.

In this paper the results of cytogenetic studies carried out in the Moscow—Neroosa hybrid zone, recently discovered in the European part of Russia (Moscow Region, Ozery city, N 56°56′ E 38°31′), are presented. These chromosomal races differ by two pairs of race-specific metacentrics *gm-no* and *go-nm*, respectively, such that a ring-of-four configuration (RIV) is expected at meiosis I of hybrids. It may be anticipated that such a complex meiotic configuration would disrupt the meiotic process. This is examined here, together with cytogenetic characterisation of the two races using various staining methods.

Materials and Methods

In October 2011 and April 2012, eleven animals were collected from several localities within the Moscow—Neroosa hybrid zone. All individuals were karyotyped by a standard mitotic technique from both bone marrow and spleen (Bulatova et al., 2009). G-banding was used for chromosome identification (Searle et al., 1991). The nuclear organiser regions (NORs) were detected by silver nitrate staining following Graphodatsky and Radjabli (1988). Meiotic preparations from the eight adult males caught in April were made following the method of Williams et al. (1971).

Fluorescent *in situ* hybridisation (FISH) for one individual was carried out in the Institute of Molecular and Cellular Biology SD RAS, Novosibirsk (Laboratory of Prof. A. S. Graphodatsky). Two probes were used for FISH: a telomeric DNA probe and plasmid pHr13 (human ribosomal plasmid), containing genes for both 18S- and 28S-rDNA. To generate the telomeric probe PCR was carried out in the absence of template using the primers $(TTAGGG)_5$ and $(CCCTAA)_5$ (Ijudo et al., 1991). The probes were labelled with biotin-11-dUTP or digoxigenin-11-dUTP by nick translation.

FISH was performed following G-banding of metaphase chromosomes according to published protocols (Graphodatsky et al., 2000) with some modifications. Images were captured with a ProgRes CCD (Jenoptic) camera, mounted on an Axioscope 2 plus (Zeiss) microscope with filter sets for DAPI, FITC and rhodamine, using VideoTesT-FISH 2.0. and VideoTesT-Karyo 3.1. (VideoTesT, St. Petersburg, Russia) software.

Results

Three main karyotypic variants were detected in the hybrid zone: the homozygotes of the pure parental races (Moscow and Neroosa) and complex Robertsonian heterozygotes (F_1 hybrids).

The majority of homozygous individuals (N = 6) had a Moscow race karyotype — *gm*, *hi*, *kr*, *no*, *pq* (2nA = 18, female $2n = 20$; males $2n = 21$). Silver nitrate staining revealed the terminal localisation of NORs on the chromosomal arms *o* and *q* of both *no* and *pq*, respectively, as well as in both arms of the metacentric *tu* (Fig. 1).

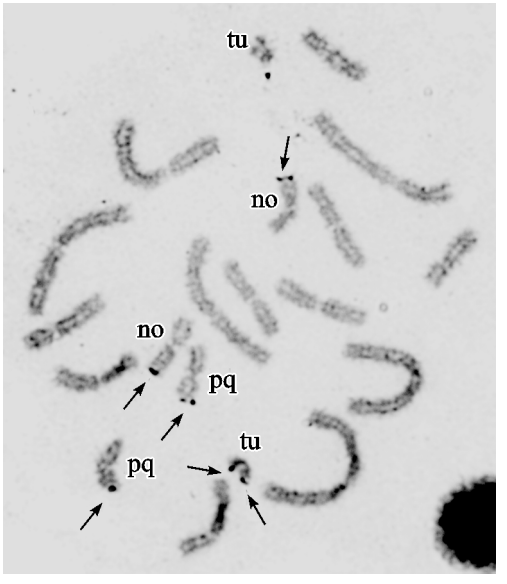

Fig. 1. Ag-stained karyotype of a common shrew belonging to the Moscow race.

Arrows indicate the localizations of NORs. Obj. 100×.

Only one individual with a homozygous karyotype of the Neroosa race was found — *go*, *hi*, *kr*, *mn*, *pq* (2nA = 18, male $2n = 21$) (Fig. 2, *a*; 3, *a*). For this specimen the active rDNA sites (which are equivalent to NORs) were visualised by FISH using an rDNA probe. Positive signals were observed at the terminal ends of the same chromosomal arms as in the Moscow race — *o*, *q*, *t* and *u* (Fig. 3, *c*). But in the last case only one homologue *go* had a bright signal of the NOR on the *o* arm, whereas arms *q*, *t* and *u* demonstrated active NORs on both homologous metacentrics. The hybridisation with the telomeric DNA probe revealed distinct signals at the telomeres of all chromosomes (Fig. 3, *b*). Also, positive signals were detected in pericentric regions of the race-specific metacentrics *go*, *hi*, *jl*, *kr*, *mn* and *pq*. Other metacentrics did not show any positive signals in the pericentric regions, though one metaphase cell displayed rather weak signals in the pericentric regions of both *bc* homologues.

Four common shrews were complex heterozygotes, F1 hybrids (Fig. 2, *b*), with both Moscow and Neroosa race metacentrics in their karyotypes — *gm/go/no/nm*, *hi*, *kr*, *pq* (2nA = 18, female $2n = 20$; males $2n = 21$). Therefore, they were expected to form a ring-of-four (RIV) configuration at meiosis I.

Indeed, such RIV configurations (*gm/go/no/nm*) were found in diakinesis/MI spreads of three hybrid males (Fig. 4, *b*).

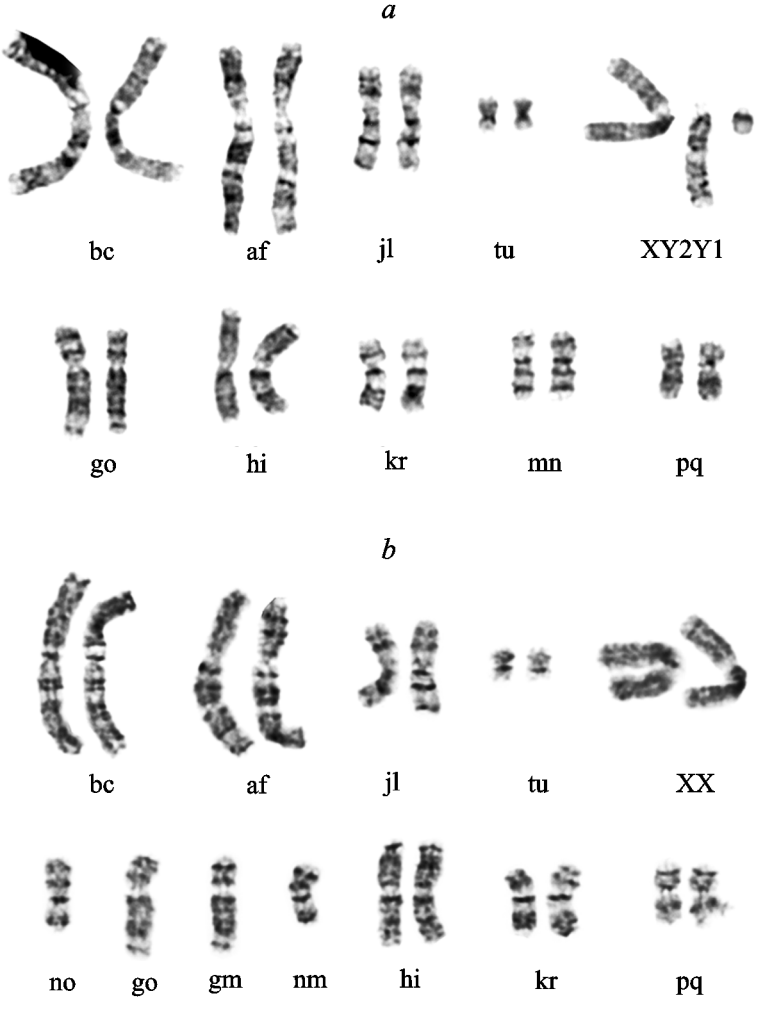

Fig. 2. G-banded karyotypes of the common shrew: the Neroosa race (*a*) and a Moscow-Neroosa F_1 hybrid (*b*).

Obj. 100×.

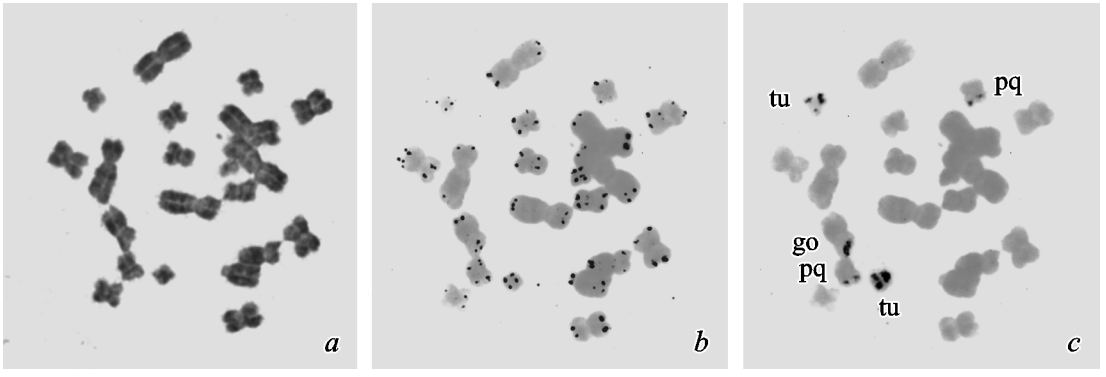

Fig. 3. Dual-colour FISH on G-banded chromosomes (*a*) of the common shrew (the Neroosa race), using biotinylated probe of rDNA (*b*) and digoxigenin-11-dUTP labelled telomeric probe (*c*).

Chromosomes counterstained with DAPI (*b* and *c* images are inverted). Obj. 100×.

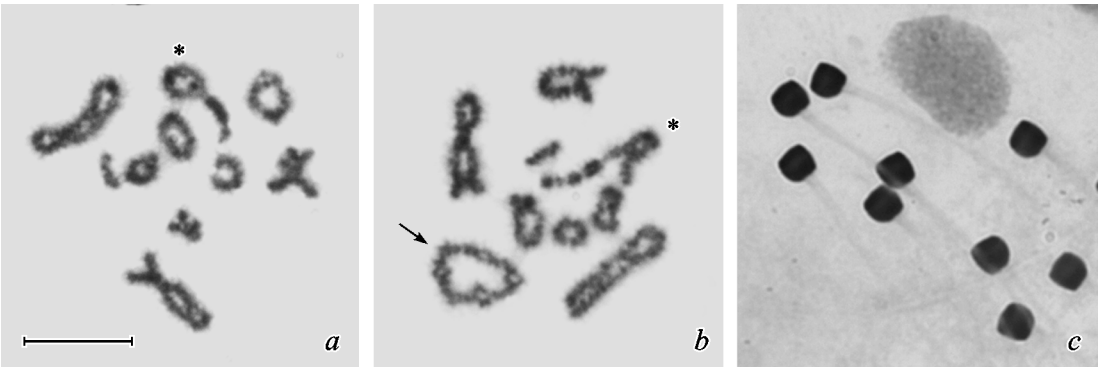

Fig. 4. Diakinesis/M I spreads of common shrews from the hybrid zone: (*a*) Moscow race and (*b*) Moscow-Neroosa F1 hybrid.

The sex trivalent (XY_1Y_2) is indicated by a *star*. An *arrow* marks the ring-of-four configuration (RIV); *c* — spermatozoa in meiotic preparations of the Moscow-Neroosa F1 hybrid. Bar: 10μm. Obj. 100×.

The number of meiotic configurations corresponded to a hybrid karyotype: seven autosomal bivalents *af*, *bc*, *hi*, *jl*, *kr*, *pq* and *tu*, the RIV multivalent comprising four monobrachial homologues (*gm/go/no/nm*), and the sex trivalent (*XY**1**Y**2*). Nine bivalents (*af*, *bc*, *jl*, *tu*, *gm*, *hi*, *kr*, *no* and *pq*) and the sex trivalent (*XY**1**Y**2*) were recorded in each spread of the Moscow race specimens (Fig. 4, *a*). In both homozygotes and complex heterozygotes numerous spermatozoa of normal appearance were observed (Fig. 4, *c*).

Discussion

The ranges of the Moscow and Neroosa races close to Ozery city meet at the Oka River which is about 250 m wide. Interracial hybrids together with individuals of the Moscow race were found on the left bank, whereas the specimen of the Neroosa race occupied the right bank of the river.

Due to the presence of four metacentrics with monobrachial homology in the hybrid karyotypes, a specific multivalent (RIV) is found at meiosis I. In the common shrew this configuration has only previously be demonstrated in hybrids between the Uppsala and Hällefors chromosomal races (Narain, Fredga, 1997).

In the present study no abnormal pairing or disturbances in the structure of RIV configurations in F_1 hybrids was observed. These data are in close correspondence with the results obtained from the Uppsala—Hällefors hybrid zone (Narain, Fredga, 1997) as well as with data from other chromosomal hybrid zones of *S. araneus* showing regularity of chain and ring configurations in complex heterozygotes (Searle, 1986; Mercer et al., 1992; Jadwiszczak, Banaszek, 2006; Pavlova et al., 2007). Moreover, our recent analysis of synaptonemal complexes (SCs) in pachytene spermatocytes of F_1 males from the Moscow—Neroosa hybrid zone did not reveal chromosome asynaptic regions or other pairing abnormality compared to pure Moscow race individuals (Matveevsky et al., 2012).

Robertsonian centric translocations and WARTs play an important role in the evolution of the *S. araneus* chromosomal races (Searle, Wójcik, 1998). Since pericentric regions of all race-specific metacentrics in the Neroosa race (*go*, *hi*, *jl*, *kr*, *mn*, *pq*) individual carried telomeric signals following FISH, we can suggest that telomeric sequences are not completely lost during centric fusion. Subsequent to fusion such sequences may be lost over time, however, based on the near absence of telomeric sequences on the metacentrics *af*, *bc* and *tu*, which were formed earlier in the evolution of the common shrew karyotype (Searle and Wójcik, 1998). Similar data were obtained by Zhdanova et al. (2007) who also found pericentric telomeric sequences in the Novosibirsk race metacentrics *go*, *hn*, *jl*, *ik*, *mp*, *qr*.

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ЦИТОГЕНЕТИЧЕСКИЙ АНАЛИЗ ГИБРИДНОЙ ЗОНЫ МЕЖДУ РАСАМИ МОСКВА И НЕРУССА ОБЫКНОВЕННОЙ БУРОЗУБКИ *SOREX ARANEUS*, РАЗЛИЧАЮЩИМИСЯ ОДНОЙ ХРОМОСОМНОЙ ПЕРЕСТРОЙКОЙ ТИПА WART

С. В. Павлова

Институт проблем экологии и эволюции им. А. Н. Северцова РАН, Москва;
электронный адрес: swpavlova@mail.ru

С помощью сравнительного цитогенетического анализа в гибридной зоне между хромосомными расами Москва и Нерусса были выявлены три кариотипические категории: гомозиготы обеих родительских рас и сложные гетерозиготы, т. е. гибриды F1. В мейозе у гибридов (на стадии диакинеза) была обнаружена мейотическая конфигурация в виде кольца из четырех элементов (ring-of-four, RIV), как и предполагалось исходя из кариотипической формулы. Каких-либо нарушений структуры этой конфигурации выявлено не было. Локализация сигналов теломерных повторов и рибосомной ДНК на хромосомах одной особи расы Нерусса была исследована с помощью двойной флуоресцентной гибридизации in situ (FISH).

Ключевые слова: кариотип, гибридная зона, мейоз, FISH, *Sorex araneus*.