A POSSIBLE CYTOGENETIC ANALOGY TO GENOMIC «SPECIATION ISLANDS» AS REVEALED BY CHROMOSOME STUDY OF A NATURAL HYBRID VOLE

© *N. Sh. Bulatova*, *S. V. Pavlova*

A. N. Severtsov Institute of Ecology and Evolution RAS, *Moscow*, *119071*;
e-mail: *ninbul@mail.ru*; *svetpavlova@yandex.ru*

Chromosome analysis in mammals over the last half century has largely focused on species identification. A growing number of hybrid zones of karyotypically differentiated cryptic taxa have been described. A good example is provided by two karyoforms of the 46-chromosome common vole (long known as «arvalis» and «obscurus») that make contact longitudinally from the north to the south of European Russia. The hybrid F1 karyotype displays genomic markers which distinguish arvalis and obscurus and which are cytogenetically detectable as minor variants. The apparent insignificance of the genomic region of heterozygosity associated with these markers perhaps does not prevent hybrid chromosome pair formation at meiosis but may reflect a site resistant to gene flow. A cytogenetic analogy with so-called «speciation islands», based, for example, on the study of the corvine hybrid zone and represented by a small number of limited genomic sites (occupying less than 1 % of the genome) (Poelstra et al., 2014), may be relevant.

Key words: chromosome hybrid zone, common vole, FISH, molecular-cytogenetic markers, telomeric sequences.

Abbreviations: AR — arvalis, FNA — fundamental number of autosomal arms, ITS — interstitial telomeric site, NOR — nucleolus organizing region, OB — obscurus.

Chromosome analysis is an effective tool for species identification in mammals represented by the field of cytotaxonomy and the modern discipline of comparative cytogenetics but ultimately founded on the most universal method of metaphase chromosome preparation by Ford and Hamerton (1956). At a within-species level there are a growing number of chromosome races and hybrid zones between them described since the 1960s (Searle, 1993 and references herein). The contact of the two widely distributed karyoforms of the 46-chromosome common vole (the «*arvalis*» and «*obscurus*» forms of *Microtus arvalis*) has been detected in Eastern Europe by systematic chromosome studies accumulated accurately and finished exactly at the joint (Meyer et al., 1996, 1997). The findings of the hybrid F_1 karyotype and varied recombinant karyotypes marks the position of the narrow chromosomal hybrid zone between the two forms (Bulatova et al., 2010a).

The hybrid zone between the «*arvalis*» and «*obscurus*» karyoforms was first localized in the Vladimir region of Central European Russia in a continuous area, without geographic barriers, within 24 km of the main highway near Kovrov (Meyer et al., 1997). The two pure karyotypes called here AR (arvalis) and OB (obscurus), both 2N = 46, can easily be distinguished: one karyotype with 8 diagnostic acrocentrics, or 4 pairs (AR, FNA = 80) and the other karyotype with 20 diagnostic acrocentrics, or 10 pairs (MOB, FNA = 68). A series of intermediate karyotypes with acrocentric numbers varying between the two parental forms are found in the Vladimir hybrid zone, including the expected number of 14 acrocentrics for the F_1 hybrid (FNA = 74; Bulatova et al., 2010a). DNA studies have revealed a 4.6 % divergence between AR and OB for the mitochondrial cytochrome *b* gene (Lavrenchenko et al., 2009) that corresponds to the lower limits for species-level differences in *Microtus* (Jaarola et al., 2004). Within the hybrid zone all individuals so far examined have had the AR mitotype (Bulatova et al., 2010b).

Additional data reveal the level of cytogenetic differentiation of the two 46-chromosome genomes. Full euchromatin G-band homology between the AR/OB karyotypes has been reported repeatedly with conventional G-banding (e. g. Meyer et al., 1996) and confirmed with high resolution G-banding analysis (Mazurok et al., 2001). Besides a diploid number of 2N = 46, the two karyotypes share five large bi-armed elements among the autosomes, a metacentric X-chromosome of medium size and groups of small metacentrics and small acrocentrics. Among the largest pairs of chromosomes, a complex evolutionary origin can be inferred from banding and chromosome painting data with homology to acrocentrics of the 54-chromosome ancient karyotype such as that seen in one of the sibling species, *M. rossiaemeridionalis* (also known as *M. subarvalis*) (Mazurok et al., 2001; Lemskaya et al., 2010). The large subtelocentric No 5 is polymorphic for centromere position in the OB karyotype and has been described as heterozygous subtelocentric/acrocentric or occasional homozygous acrocentric (e. g., Malygin, Sablina, 1994; Meyer et al., 1996).

AR/OB karyotypic differences have been recorded in previous studies including Y chromosome morphology (Meyer et al., 1996) and the alternative presence/absence of nucleolar-organizing regions (NORs) in large chromosomes of the

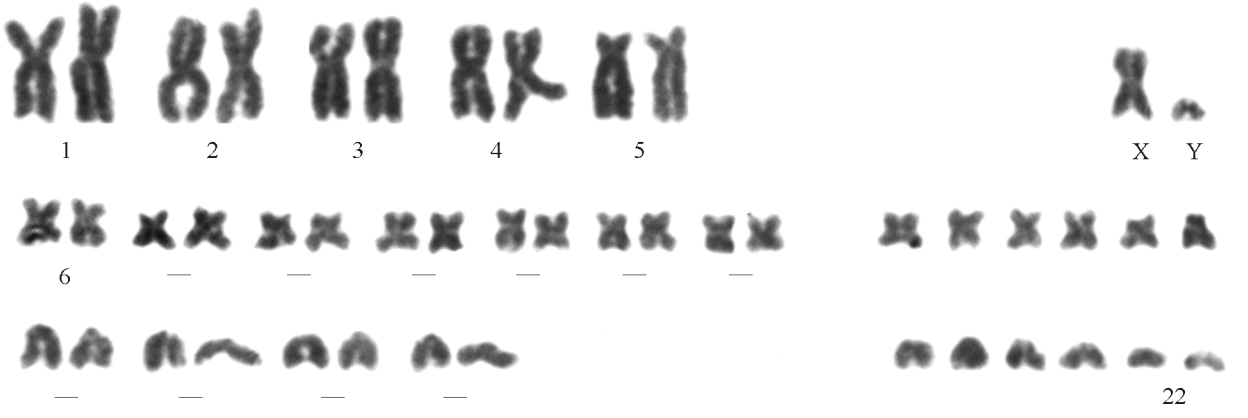

Fig. 1. Karyogram of a male from the hybrid zone of two 46-chromosome common voles (AR, OB).

1 to 22 — autosome pair numbers, XY — sex chromosomes. 6 heterozygous pairs of the diagnostic group are placed unpaired separately in each category (small metacentrics or acrocentrics), in addition to homozygous pairs — 7 metacentric (OB diagnosis) and 4 acrocentric (AR diagnosis). Conventional Giemsa staining.

OB/AR karyotypes, respectively (Mazurok et al., 2001). Variation in heterochromatic regions between the karyoforms is also suspected, but so far is poorly studied.

Karyotypic differences produced by chromosome rearrangements in 6 small chromosomes have been identified on the basis of chromosome differential staining and interpreted as centromeric shifts (Mazurok et al., 2001). The expectations of possible negative meiotic effect on hybrid reproduction have, however, not been supported in laboratory crosses (Meyer et al., 1997). The AR/OB hybrid zone, thus, represents a case rather different in a mammalian context from the more widely reported Robertsonian chromosomal hybrid zones (Searle, 1993).

New interesting minor chromosome markers distinguishing AR and OB have been revealed by FISH (Bulatova et al., 2013). FISH analysis shows a specific chromosome distribution (+ presence, – absence) of standard telomeric and rDNA sequences marking, first, a pericentromeric ITS signal and, second, telomeric NOR signals on the larger chromosomes of the AR (+/–) and OB (–/+) genomes.

In this communication, new details of a natural F_1 hybrid from the AR/OB hybrid zone are presented.

Karyotype analysis was carried out in the southern part of the established AR/OB hybrid zone in Vladimir region, on the OB side of the hybrid zone. The basic procedure of mammal chromosome preparation of Ford and Hamerton (1956) was followed by conventional Giemsa metaphase chromosome staining, C-banding and meiotic divisions' fixation using standard protocols (in a modification of the methods from the laboratory of A. S. Graphodatsky http://www.bionet.nsc.ru/labs/chromosomes/intr_engl.htm). The FISH procedure introduced earlier by Bulatova et al. (2013) for common voles were applied. Digital images of several full metaphases were analyzed in each case.

The hybrid origin of an adult vole male (specimen code 12—26) was demonstrated chromosomally in contrast to 3 other specimens collected in the same locality showing the pure «*obscurus*» karyotype (OB codes 12—20, 21, 22). Also from this locality (Shevinskaya village) a single individual of the sibling species (*M. rossiaemeridionalis*, 2N = 54) and several specimens of *M. oeconomus* (2N = 30) were identified from karyotype.

Fig. 1 presents the karyogram of the hybrid male, showing that it is typical for an F_1 hybrid, with combination of metacentric and acrocentric small diagnostic chromosomes in a homozygous (7 and 4 pairs, respectively) and a heterozygous state (6 single homologues in each group, metacentric or acrocentric). The Y-chromosome of this specimen is one of the acrocentrics, not recognizable without C-banding among all other acrocentrics, thus, showing its attribution to the OB

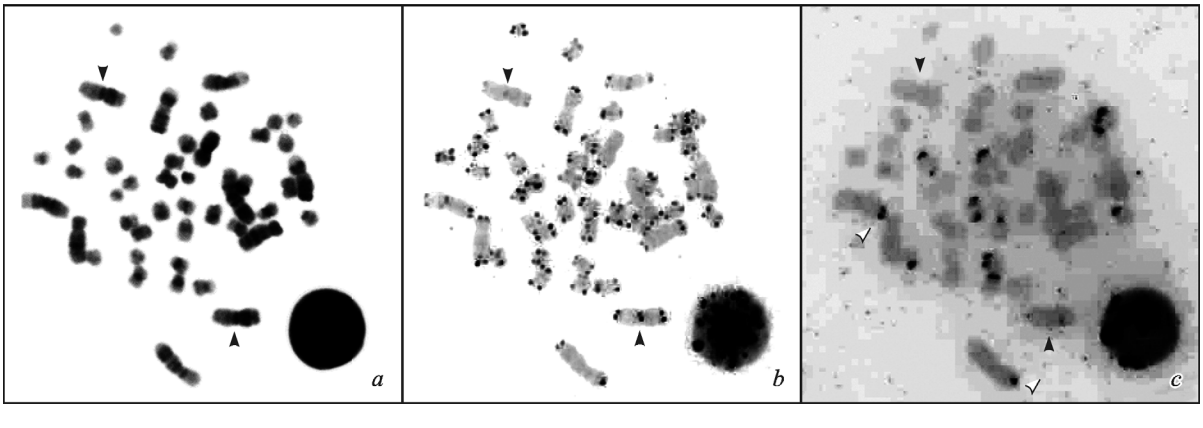

Fig. 2. Molecular-cytogenetic probes identified successively in metaphase chromosomes of the hybrid specimen against DAPI control (*a*) : FISH (inverted signals) for tDNA (*b*) and rDNA (*c*).

Black arrowheads indicate homologs of the remarkable large metacentric heterozygous for ITS signal (positive in AR genome). *White arrowheads* indicate two large metacentrics heterozygous for the rDNA site (OB genome markers).

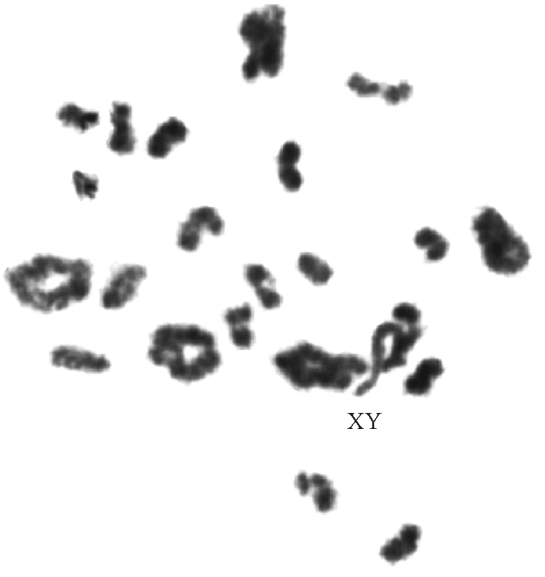

Fig. 3. Regular meiotic configuration of autosome homologs and sex bivalent in metaphase I-diakinesis of the F1 AR × OB hybrid.

karyotype (Meyer et al., 1996; Bulatova et al., 2010a). Therefore, the X, though morphologically indistinguishable between the two karyotypes, belongs to the AR genome in this F_1 hybrid male.

The FISH procedure revealed additional heterozygosity. The signal for the standard telomeric sequence ($TTAGGG_n$) shows a typical telomeric distribution in all the chromosomes, except for one. The ITS (interstitial telomeric site) signal marked the centromeric region of only one homologue of the two large metacentrics with identical G-bands (Fig. 2, *a*, *b*). Referring to our previous data (Bulatova et al., 2013), the presence of the centromeric ITS is a characteristic of the AR genome. Conversely, rDNA signals on the same preparation reveal two heterozygous large pairs (Fig. 2, *c*). This is in correspondence with the results of our previous finding of rDNA markers on the large chromosomes of the OB genome and earlier reported NOR localization data (Mazurok et al., 2001).

Despite the karyotypic peculiarities, meiotic preparations do not show any irregularity at metaphase I-diakinesis stage prepared from the same hybrid (Fig. 3).

In concert with genetic data, the two 46-chromosome karyotypes of AR and OB show free hybridization permitted by a regular meiotic process. On the contrary, subchromosome minor differences between AR and OB karyotypes might be the indicators of small regions of the nuclear genome that are resistant to massive gene flow. In this respect, a cytogenetic analogy with «speciation islands» is relevant, based, for example, on the study of the corvine hybrid zone and represented by a small number of limited genomic sites (occupying less than 1 % of the genome) (Poelstra et al., 2014).

The experimental part of the study connected with the use of the FISH method was carried out in the Laboratory of Prof. A.S. Graphodatsky, with kind support of Drs S. Romanenko and N. Serdyukova. The results were in parts presented at the International Conference Chromosoma 2015 in continuation of a previous presentation by the same authors at Chromosoma 2012. We acknowledge scientific and linguistic help from Prof. J. B. Searle of Cornell University, Ithaca, New York. Grant support from the Russian Foundation for Basic Research was appreciated.

References

Bulatova N. Sh., *Golenishchev F. N.*, *Koval'skaya Yu. M.*, *Emelyanova L. G.*, *Bystrakova N. V.*, *Pavlova S. V.*, *Nadzhafova R. S.*, *Lavrenchenko L. A. 2010a*. Cytogenetic study of the parapatric contact zone between two 46 chromosomal forms of the common vole in European Russia. Rus. J. Genetics. 46 (4) : 443—448.

Bulatova N. Sh., *Pavlova S. V.*, *Romanenko S. A.*, *Serdyukova N. A.*, *Golenishchev F. N.*, *Malygin V. M.*, *Lavrenchenko L. A. 2013*. Molecular cytogenetic markers of cryptic species and hybrids of the common vole superspecies complex *Microtus arvalis s. l.* Tsitologiya. 55 (4) : 268—270.

Bulatova N. Sh., *Potapov S. G.*, *Lavrenchenko L. A. 2010b*. Genomic versus chromosomal polytypy in studies of mitochondrial and nuclear DNA markers in the *Microtus arvalis* group. Rus. J. Genetics. 46 (5) : 586—594.

Ford C. E., *Hamerton J. L. 1956.* A colchicine, hypotonic citrate, squash sequence for mammalian chromosomes. Stain Technol. 31 : 247—251.

Jaarola M., *Martínková N.*, *Gündüz I.*, *Brunhoff C.*, *Zima J.*, *Nadachowski A.*, *Amori G.*, *Bulatova N.*, *Chondropoulos B.*, *Fraguedakis-Tsolis S.*, *González-Esteban J.*, *López-Fuster M.*, *Kandaurov A.*, *Kefelioğlu H.*, *da Luz Mathias M.*, *Villate I.*, *Searle J. B. 2004*. Molecular phylogeny of the speciose vole genus *Microtus* (Arvicolinae, Rodentia) inferred from mitochondrial DNA sequences. Mol. Phylogenet. Evol. 33 : 647—663.

Lavrenchenko L. A., *Potapov S. G.*, *Bulatova N. S.*, *Golenishchev F. N. 2009.* A genetic study of natural hybridization between two forms of the common vole (*Microtus arvalis*) with the use of molecular and cytogenetic methods. Dokl. Biol. Sci. 426 : 222—224.

Lemskaya N. A., *Romanenko S. A.*, *Golenishchev F. N.*, *Rubtsova N. V.*, *Sablina O. V.*, *Serdukova N. A.*, *O'Brien P. C. M.*, *Fu B.*, *Yiğit N.*, *Ferguson-Smith M. A.*, *Yang F.*, *Graphodatsky A. S. 2010.* Chromosomal evolution of Arvicolinae (Cricetidae, Rodentia). III. Karyotype relationships of ten *Microtus* species. Chromosome Res. 18 : 459—471.

Malygin V. M., *Sablina O. V. 1994*. Karyotypes. In: Common vole: the sibling species. M.: Nauka. 7—32.

Mazurok N., *Rubtsova N.*, *Isaenko A.*, *Pavlova M.*, *Slobodyanyuk S.*, *Nesterova T.*, *Zakian S. 2001*. Comparative chromosome and mitochondrial DNA analyses and phylogenetic relationships within common voles (*Microtus*, Arvicolidae). Chromosome Res. 9 : 107—120.

Meyer M. N., *Golenishchev F.*, *Radjabli S .I.*, *Sablina O. V. 1996.* Voles (subgenus *Microtus* Schrank) of Russia and adjacent territories. Proc. Zool. Inst. (St. Petersburg). 232 : 32—141.

Meyer M. N., *Golenishchev F.*, *Bulatova N.*, *Artobolevsky G. V. 1997*. On the distribution of two *Microtus arvalis* chromosomal forms in European Russia. Zool. Zhurn. 76 : 487—493.

Poelstra J. W., *Vijay N.*, *Bossu C. M.*, *Lantz H.*, *Ryll B.*, *Müller I.*, *Baglione V.*, *Cunneberg P.*, *Wikelski M.*, *Grabherr M.*, *Wolf J. B. W. 2014.* The genomic landscape underlying phenotypic integrity in the face of gene flow in crows. Science. 344 : 1410—1414.

Searle J. B. 1993. Chromosomal hybrids in eutherian mammals. In: Hybrid zones and the evolutionary process. (Ed. R. G. Harrison). New York: Oxford Univ. Press. 309—353.

Received 1 XII 2015

ВОЗМОЖНАЯ ЦИТОГЕНЕТИЧЕСКАЯ АНАЛОГИЯ ГЕНОМНЫХ «ОСТРОВАМ ВИДООБРАЗОВАНИЯ» ПО ДАННЫМ ХРОМОСОМНОГО ИЗУЧЕНИЯ ГИБРИДА ПОЛЕВОК

Н. Ш. Булатова, *С. В. Павлова*

Институт проблем экологии и эволюции им. А. Н. Северцова РАН, Москва, 119071;
электронные адреса: ninbul@mail.ru; svetpavlova@yandex.ru

При изучении гибрида двух различающихся по кариотипу форм у грызунов надвидового комплекса обыкновенная полевка из зоны их природного контакта показано, что различия в хромосомной локализации стандартных геномных маркеров, таких как сайты теломерной и рибосомной ДНК, могут рассматриваться как видоспецифические. В свете проблемы генетической изолированности вида подобные участки хромосом, не связанные непосредственно с хромосомной стерильностью гибридов, могут являться индикаторами геномных сайтов, характеризующих резистентность к генному потоку и потому интересных в аспекте видообразования.

К л ю ч е в ы е с л о в а: FISH, молекулярно-цитогенетические маркеры, обыкновенная полевка, теломерные последовательности, хромосомная гибридная зона.