

SHORT
COMMUNICATIONS

Nucleolar Organizing Regions (NORs) of Common Vole Chromosomes as Nuclear Markers of Genome Differentiation in Data from a Hybrid Zone of Two Karyoforms, *arvalis* and *obscurus*

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Received August 12, 2016

Abstract—Contrasting chromosome location of nucleolar organizing regions (NORs), a poorly studied differentiating feature of two karyoforms of the 46-chromosome common voles, *arvalis* and *obscurus*, is demonstrated in the heterozygous karyotype of a natural hybrid from the *arvalis/obscurus* contact zone in European Russia. It was deduced from the karyotype analysis that the *obscurus* form was parental in this case and the *arvalis* form was defined as maternal via the markers of the mitochondrial genome. The similarities in the levels of chromosome polytypy and the lineage differentiation of the *arvalis/obscurus* and *obscurus* mitochondrial genomes according to the new and earlier published data is noticeable.

Keywords: AgNOR, biodiversity, hybridization, karyotype, *Microtus*

DOI: 10.1134/S1022795417060035

In the last decade, the genome studies of mammals have become available and they show a significant increase in biodiversity owing to the genetically defined taxa. This is a direct consequence of the application of new molecular methods based on classical genetics [1]. The methodology of N.I. Vavilov concerning species was already synthesized with genetics in the 1930s, i.e., before the genomic era. Founded on studies of botanical objects, though on global cultivated flora scale, it highlighted the essence of the Linnaean species as a system and led to the need “...to distinguish the main categories associated with the geographical and physiological isolation and sometimes accompanied by the differences in number and in individuality of chromosomes” (Vavilov, 1930–1931, cited by [2]) in the theory and practice of biological species. In this way, it is possible to consider the phenomenon of chromosomal polytypy, which is often observed in mammals and appeared in karyoforms and chromosomal races [3]. Robertsonian chromosomal races are easily identifiable by karyotype and they are studied better than other examples [4]. Common voles from the *arvalis* group (genus and subgenus *Microtus* of the Eurasian herbivore rodents) are an example of a non-Robertsonian complex, which has been of interest in karyosystematics for almost a half century. Now it is important for genomics because of the discovery of cryptic taxa at the chromosomal and genomic levels and the prospects of studying their relevance to each other [5, 6].

In the European territory of Russia, the group *arvalis* is composed of twin species with different diploid chromosome number *M. arvalis* ($2n = 46$) and *M. rossiaemeridionalis* ($2n = 54$); at the same time, the 46-chromosome species is represented by two karyoforms replacing each other from west to east, *arvalis* and *obscurus* [7]. In the contact zone, discovered on the Russian Plain, the two forms converge with the formation of a parapatric hybrid zone [8]. Judging by the diversity of the hybrid karyotypic combinations [5, 8] and by the data from experimental breedings [7] *arvalis* × *obscurus* hybrids are fertile.

Both the original 46-chromosome forms and hybrids are occurred in the contact area [8]; they are recognized cytogenetically by the chromosomal arm number (fundamental number (FN) and autosome fundamental number (FNA)) owing to the intrachromosomal differences in six pairs of chromosomes (centromere shift [9]). Although outside the contact zone *arvalis* and *obscurus* were differentiated for mitochondrial genome markers at the level of 4.6%, which was close to the lower level of interspecies differences in *Microtus*, in a narrow interval of the transition zone between the two parental forms, all specimens, nevertheless their karyotypes, belonged to only one category of mitotypes—*arvalis* [5].

In two karyoforms of 46-chromosome karyotype from 22 pairs of autosomes, the majority are biamed (metacentric, submetacentric or subtelocentric); the

arvalis form has only four acrocentric pairs (FNA = 80); *obscurus* form has ten acrocentrics pairs (FNA = 68). The karyotype of the F1 hybrid shows an intermediate number of acrocentrics: 14 by the sum of the haploid sets (FNA = 74); in the hybrid zone, other options in the number of acrocentrics were also observed, almost the entire range of values from 8 ("pure" *arvalis*) to 20 ("pure" *obscurus*) in recombinants [8]. It is possible to identify the karyotype of the paternal parent of the hybrid by the differences in the shape of the Y chromosome (small acrocentric in *arvalis* and larger acrocentric because of the heterochromatin in *obscurus*); i.e., the direction of crossing can be determined by the karyotype of hybrid male [8]. Meiotic configurations of the hybrid bivalents were not defective in specimens from the hybrid zone, as in the F1 offspring from experimental crosses [7, 10]. This showed that in this case some structural rearrangements that affect the shape of the chromosomes appeared to be absolutely insufficient for the formation of cytogenetic isolation of *arvalis* and *obscurus*. At the same time, on the large pairs of these chromosome forms, there were some point differences, which were a possible cytogenetic analogy to genomic "speciation islands" [10]. Four pairs of large metacentrics, homologous in two karyoforms according to G banding, were evolutionarily new in the *arvalis* group and a unique feature (apomorphy) in the 46-chromosome karyotype. Their occurrence was associated with tandem pairwise acrocentric fusion of the ancestral 54-chromosome set as in the *M. rossiaemeridionalis* karyotype [9]. In two karyoforms, an alternative presence/absence of such molecular cytogenetic markers as standard sequences of telomeric (tDNA) and ribosomal DNA (rDNA) was recently identified [11], in addition to the previously known cytological characteristic associated with rDNA, the area of the nucleolar organizing regions (NORs) [9]. Heterozygosity for the relevant molecular markers was shown in the natural hybrid karyotype [10], but the NORs, which were also useful for the identification of the hybrid karyotype, were not localized. This gap is bridged in the present paper.

Material for the cytogenetic study was obtained from the hybrid zone of the *arvalis* and *obscurus* karyoforms previously found in a number of localities south of the city of Kovrov in Vladimir oblast, about 250 km east of Moscow. After a preliminary analysis, the Laboratory of Microevolution of Mammals at the Institute of Ecology and Evolution (Dr. Sci. (Biol.) L.A. Lavrenchenko, head) carried out regular monitoring in accordance with accepted academic procedures. Karyotyping of four voles and identification of a hybrid via mitochondrial genome markers (*cytb*) was performed according to the same protocols which were applied after the discovery of this hybrid zone [5, 8]. The differentially stained metaphase chromosomes were studied by C-heterochromatin banding to identify the male Y chromosome and localization of NORs selec-

tively stained by silver (AgNORs) according to the procedures previously used to study the chromosomes of common voles [7–9].

Out of the four karyotyped individuals (journal codes 12-20, 12-21, 12-22, and 12-26), in the first three (two males and a female), we identified the *obscurus* karyotype typical of the Russian part of the range (ten pairs of acrocentrics, FNA = 68), and the fourth specimen (a male) had the karyotype of the F1 hybrid. This male had 15 acrocentrics, including the acrocentric Y chromosome identified by a dense C banding as a marker of the *obscurus* form (Fig. 1a) and 14 autosomes, which was consistent with the karyotype of the F1 hybrid. All other chromosomes were banded, including five large pairs and a group of small metacentrics (FNA = 74).

NORs in the hybrid were constantly detected on the telomeres of the two largest metacentrics (two arrows in Fig. 1b), varying in size and belonging to two different pairs (heterozygosity for NORs). Out of three voles of the *obscurus* form studied together with the hybrid, we managed to obtain NOR banding only for the male 12-22. Four arrows in Fig. 1c indicate the location of the NORs on the telomeres of two large homozygous pairs of chromosomes. NORs showed the features of the paternal *obscurus* form in the karyotype of the F1 hybrid, as was demonstrated for the Y chromosome. NORs were also observed on small acrocentrics as bright spots near the centromeres on the shorter arm. Without special study, it was not possible to understand whether small carriers of NORs were homozygous or heterozygous; therefore, we do not discuss them here. For the hybrid 12-26 using mtDNA markers, we detected the *arvalis* genome as maternal, which emphasized the hybrid status of the individual, since nuclear markers presented karyotypic characteristics of the paternal *obscurus* genome.

Until now, information about NORs on the large metacentrics was absent not only for common vole hybrids but also for the natural *obscurus* form of the Russian populations. Heterozygosity for NORs on the telomeres of only two large pairs of voles from the hybrid zone could be considered either as a specific feature of the paternal karyotype or as a characteristic of the entire border *obscurus* population near the hybrid zone. This needs to be clarified in the future. According to early reports, often not specifying the geographical origin of the voles from the vivarium, it was known that the telomeric NORs were present in at least three major pairs of chromosomes in homozygous and heterozygous conditions in the karyotype *obscurus* [7] and they were not identified in the karyotype *arvalis* either by silver staining or via specific DNA sequences of ribosomal genes [9]. From more specific data from Armenia [12] and Turkey [13], in the south of the range of the form *obscurus*, AgNOR staining was indeed present on three large pairs of this karyoform. Other options with a lower number of