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GENERAL BIOLOGY

Voles of the *Microtus arvalis* Group in Zones of Ecological Risk: Interspecies Hybridization

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During the past four decades, chromosomal twin species *Microtus arvalis* sensu stricto Pallas, 1779 ($2n = 46$) and *M. rossiaemeridionalis* Ognev, 1924 ($2n = 54$) have been the focus of attention of zoologists, evolutionists, and ecologists. Phylogenetic relationships between them remain unclear in many aspects, because the independence of these species confirmed by hybridization experiments and cytogenetic analysis is combined with a high degree of phenotypic likeness, similar biotopes, and sympatric distribution. Although different researchers karyotyped at least one thousand animals from natural populations of sympatric voles *M. arvalis* and *M. rossiaemeridionalis*, no interspecies hybrids have been discovered until recently [1–3].

In the experiments on crossing *M. arvalis* with *M. rossiaemeridionalis* performed by Meier [1, 3] and Malygin [2], a significant decrease in fertility of nonconspecific pairs was observed. However, the authors of [1–3] obtained hybrids that were infertile in backcross analysis performed on more than 100 males and 100 females of the F1 generation. As expected based on the Holden rule, gametogenesis was suppressed to a higher degree in the hybrid males than in females. Spermatogenesis was blocked at the stage of primary spermatocytes. Conjugation of chromosomes in the pachytene and diakinesis was disturbed much more pronouncedly than could be expected based on the homology of the major portion of chromatin of the parent species, as revealed by differential staining [1, 2, 4]. Meier did not find any pregnant F1 female [3]. Although in some of them, oogenesis proceeded to the stage of the Graafian vesicle, ovulation never occurred [5].

Thus, under the laboratory conditions, the degree of reproductive isolation of the common and East European voles is sufficiently high. However, there are grounds to believe that the effectiveness of crossing between closely related species may vary depending on the biotic and abiotic environmental conditions [6]. The relationships between the closely related species exposed to ecological stress are of special interest. This is the case with *M. arvalis* and *M. rossiaemeridionalis*, which are sympatric in some Ural regions significantly contaminated with chemical and radioactive agents. In addition, this is the eastern edge of the *M. rossiaemeridionalis* range, which also increases the probability of environmental stress. Of 15 localities found in the Middle and Southern Urals (51°08′ to 57°21′ N and 53°28′ to 62°08′ E), sympatric coexistence of twin species of the common vole was observed in 5 of these, hybrid females being found in 2 of them. In the previous work, we described in detail 14 localities studied and reported that *M. arvalis* (the *obscurus* form) is predominant in the Urals and that the East European vole, which is characterized by more pronounced commensalism, predominantly inhabits regions with an increased content of radionuclides. In 1998, we caught a female with the karyotype comprised of 50 chromosomes, whose hybrid character was confirmed by G-banding, in such an area (the outskirts of the village Ozernyi, Rezhevsk raion, Sverdlovsk oblast, 57°19′ N, 61°14′ E) [4]. It lived under extremely adverse conditions (in the locality with an area of about 300 m^2 covered with sparse birch forest and weeds and surrounded by an anthropogenic desert 800–1000 m in width where thorium-containing wastes were stored for more than 40 years). In addition, Ozernyi is situated in the region of radiogeochemical anomaly characterized by an increased content of radium, thorium, and uranium in the natural media (including the soil), which is tens to hundreds times higher than the background level. The background γ radiation in the locality of capturing was 107 μR/h). Therefore, it can be assumed that interspecies crossing was induced by unusual conditions, which disturbed the mechanisms of reproductive isolation of the twin species [4]. Our new data confirm this assumption.

In June 2000, we found a new sympatric area of these twin species in the outskirts of the village Bainy (Bogdanovich raion, Sverdlovsk oblast, 56°42′ N, 62°08′ E), which is located in the tail part of the East Ural radioactive track, which emerged as a result of an accident at the Mayak chemical industrial complex in 1958. The initial contamination with ^{90}Sr was 1.7 Ci/km^2; currently it is, on average, 0.3 Ci/km^2 [7], which is approximately fourfold higher than the global level [8]. However, the radioactivity distribution is

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Content of heavy metals in the liver of the twin species *M. arvalis* and *M. rossiaemeridionalis* from different localities of the Urals

Species	Locality of capture	Number of animal	Content of metals in the liver, μg/g of dry weight			
			zinc	copper	lead	cadmium
M. arvalis	Outskirts of village Bainy	12	68.8 ± 3.1	11.7 ± 0.5	0.2 ± 0.07	0.2 ± 0.03
	Predural'e reserve, Perm oblast	12	84.1 ± 2.9	13.3 ± 0.5	2.1 ± 0.84	0.5 ± 0.12
	Biostation of UGU, Sverdlovsk oblast	13	79.1 ± 8.1	10.7 ± 1.2	1.2 ± 0.35	0.1 ± 0.02
	Village Talitsa; Sverdlovsk oblast	9	70.2 ± 7.9	12.5 ± 0.5	3.1 ± 0.65	0.1 ± 0.02
M. rossiae-meridionalis	Outskirts of village Bainy	12	61.4 ± 1.5	10.7 ± 0.6	1.0 ± 0.27	0.2 ± 0.05
	Botanical Garden, Yekaterinburg	35	83.6 ± 3.0	12.7 ± 0.8	2.0 ± 0.36	0.4 ± 0.05
	Village Kristalka, Orenburg oblast	13	75.0 ± 5.7	10.6 ± 1.4	4.2 ± 2.28	0.5 ± 0.15
	Village Starobogdanovka, Orenburg oblast	10	106.5 ± 15.9	14.4 ± 3.0	3.3 ± 2.98	0.3 ± 0.13

mosaic, as follows from the differences in the content of ^{90}Sr in the muscles and bones of the karyotyped animals. We found that the content of ^{90}Sr in the common vole was less than 27 Bq/kg dry weight, which corresponds to the global level. In the East European vole, it was 165 Bq/kg dry weight, which is close to the concentration of ^{90}Sr in the tissues of voles living near the Mayak complex [9]. β-Radiometric analysis was performed at the Department of Radiochemistry of Ural State Technological University. In 2000, 15 common and 12 East European voles were captured in a floodplain birch, asp, and alder thicket and in a wet herb–grass meadow. During a second capturing in these localities performed in June 2001, we captured 72 common voles, 1 East European vole, and 1 female with a hybrid karyotype ($2n = 50$). The latter was comprised of 6 large bi-armed chromosomes, 8 small metacentric chromosomes, and 36 acrocentric chromosomes, which corresponds to the sum of haploid sets of *M. arvalis* and *M. rossiaemeridionalis*. The morphology of the cranium indicates that the hybrid female was an underyearling (i.e., its age was no more than three months).

As mentioned above, although the hybrid female from Ozernyi was phenotypically normal, it had underdeveloped ovaries and uterus and, most likely, was completely sterile [4]. Another situation was observed in the case of the hybrid female captured in the outskirts of Bainy. In its ovaries, we found six degraded corpora lutea, and we detected two placental sites in its uterus with markedly thickened walls. This indicates that preimplantation mortality was 66.7%, whereas in *M. arvalis*, it was, on average, 8.8% ($n = 17$), and in the only pregnant *M. rossiaemeridionalis* it was absent. It remained unclear whether the pregnancy was finished by parturition or the implanted embryos were resorbed. In any case, our finding indicates that, in hybrid females, a complete egg maturation to the degree of ovulation may occur and that some eggs contain chromosomal sets that are sufficiently balanced to produce the embryos that retain viability at least to the stage of implantation. It appeared that, under natural conditions, at least some hybrid females are able to conceive and carry the embryos, although the preimplantation mortality of the embryos is very high.

The state of the reproductive system of two hybrid voles found in the Ural localities is indicative of a significant variability in the reproductive status of F1 females obtained by crossing between *M. arvalis* and *M. rossiaemeridionalis*. This situation is consistent with the modern view that the fertility of interspecies hybrids considerably depends on the allelic and epistatic interactions between the parental genomes [10]. The genetic background of the parents largely determines the completeness of synapsis of the chromosomes in meiosis and, therefore, the extent of balance of the genotypes of the resultant gametes, maturation of the gametes, and subsequent fertilization. Production of completely balanced gametes in the hybrid females is unlikely but cannot be excluded completely (in particular, because of the homology of many chromosomal segments in *M. arvalis* and *M. rossiaemeridionalis* [1, 4]). Apparently, in very rare cases, the production and maturation of genetically balanced eggs and emergence of progeny as a result of backcrosses may occur in the hybrid females.

It is conceivable that the genetic processes specific for Ural populations of twin species of common vole cause the disturbances of prezygotic and postzygotic reproductive isolation and interspecies hybridization, which were not observed in the other regions of the area, although, in the sympatric localities of the Urals, we captured only 165 (140 common and 25 East European) voles, which is much lesser than in the sympatric localities of the other part of the area. Near Ozernyi, the voles experience not only radiation, but also the biotic stress, whereas near Bainy, the environmental conditions are favorable for the voles. We did not detect here the anthropogenic chemical contamination (the content of heavy metals in the liver of the voles did not exceed the average levels characteristic of this region) (see the table). However, an increased content of radionuclides in the natural media in both localities allows us to sug-

gest that hybridization of *M. arvalis* and *M. rossiaemeridionalis* was related to radiation-induced mutations. Although the mutagenic effect of ionizing radiation in this area is not high when calculated per generation (the absorbed dose calculated for the bone marrow of the voles was no more than 1 cGy for 1 year), its action was so long-term (70 to 80 generations) that the populations could accumulate the mutations that attenuate the genetic control of the mechanisms of isolation of the common and East European voles. In addition, it is possible that some individuals absorbed much higher doses as a result of the mosaic distribution of radionuclides in the environment.

Thus, using *M. arvalis* and *M. rossiaemeridionalis* as an example, we showed that the hybridization potential of closely related mammalian species may be different under the natural and experimental conditions. We suggest that, in a stressful ecological situation, a gene flow between twin species may occur, which, even if extremely limited, would enable retaining of their phenotypical similarity.

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