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## PECULIARITIES OF GENETIC DIFFERENTIATION OF THE DOLLY VARDEN *SALVELINUS MALMA* (*SALMONIDAE*) INHABITING RIVERS OF THE FAR EAST

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### Abstract

Eleven river populations of Dolly Varden were analysed in the Primorye region using the mitochondrial DNA (mtDNA) RFLP method. A conclusion was made of existence of at least three superpopulation groupings (those of Amur, southern Primorye and north-eastern Primorye) in this territory. Primorye populations ("southern" form) and Kamchatka population ("northern" form) of *S. malma* showed considerable genetic difference (more than 2% nucleotide substitutions) that is comparable with *S. malma*-*S. leucomaenis* interspecific genetic distance. In the Sakhalin population there were found the "primorye" variant of mtDNA, as well as the variant typical of the "kamchatka" which was observed in 6 of 28 samples. The "kamchatka" variant of mtDNA was found also in *S. malma* from southern Kurils but its frequency was much less (0.08% of samples). The data obtained raise the problem of "southern" and "northern" forms of *S. malma* and superespecies *S. malma*-*S. alpinus* complex.

### Introduction

A representative of salmonid fishes – *Salvelinus malma* (Dolly Varden) has wide circumpolar area. In the Far-Eastern Russian region two subspecies – *S. malma malma* and *S. m. krashennikovii* were described by some authors (see Gritsenko et al., 1998; Osinov, Pavlov, 1998). According to their geographic distribution these two taxonomic groups are referred to as "northern" and "southern" forms. It is stated that the "northern" form lives in Kamchatka, while the "southern" form inhabits Primorye and Sakhalin rivers. Phylogenetic relationships between distinct forms of *S. malma* and inside *S. malma*-*S. alpinus* complex are debatable. A limited number of publications concerns genetic diversity in Far-Eastern populations of Dolly Varden. In these works main attention was paid to allozyme marker analysis of some populations from Sakhalin and Kamchatka. The aim of the present study was to estimate intra- and interpopulational heterogeneity of *S. malma* from Primorye, Sakhalin, Southern Kurils and Kamchatka using mitochondrial DNA (mtDNA) as the genetic marker.

### Material and Methods

Eleven samples of *S. malma* (landlocked form) were collected from Primorye rivers. Two samples were taken from continental rivers belonging to the Amur basin (r. Ussuri, upper waters, n=28; r. I Zvilinka, n=15) and other fishes were from the East coast (from south to north-east: r. Artemovka, n=15; r. Shkotovka, n=14; r. Partizanskaya, n=7; r. Kievka, n=6; r. Milogradovka, n=16; r. Margaritovka, n=11; r. Avvakumovka, n=17; r. Tayezhnaya, n=6; r. Maximovka, n=11). The landlocked *S. malma* was collected also in the Sakhalin from the Belaya river (tributary of the Naiba river, n=28) and in the Kurils (two streams in the Iturup island, n=25). Anadromous form of *S. malma* (n=17) was collected from the Kamchatka river near Azabachye lake. The white spotted charr *S. leucomaenis* (n=3) was collected from the Edinka river (northern Primorye). mtDNA was isolated by the standard alkaline method. The method of detection of restriction fragments length polymorphisms of mtDNA (RFLP) was used. Three (*Bgl*II, *Nco*I, *Eco*91 I) of 26 enzymes tested were applied to inter- and intrapopulation analysis and two enzymes – *Pvu*II and *Xba*I – to the interpopulation comparison. Fifteen enzymes were used in interregional (Primorye-Sakhalin-Kamchatka) comparison of *S. malma* populations and in interspecific (*S. malma* – *S. leucomaenis*) comparisons. The restriction endonuclease reaction and detection of mtDNA fragments (by UV light) were carried out according to standard methods.

## Results

The use of three "marker" enzymes detected mtDNA polymorphism in each of nine of the *malma* populations inhabiting the rivers of the Primorye eastern coast. The two Amur populations were genetically identical and fully monomorphic, although a large number of fishes has been analyzed (totally 43). On the whole, five clonal lines of mitochondrial genotypes were found in the Primorye *malma*. One of them was present in all populations analysed, varying in frequency from 8 to 100%. The maximum number of mtDNA clones (4) was revealed in the population from the Milogradovka river. In the dendrogramme of genetic similarity eleven Primorye populations separated into two pronounced clusters. One of them included two Amur populations and two southern Primorye populations (r. Artemovka, r. Partizanskaya). Grouping of populations in the second cluster mainly corresponded to geographic distances with the exception of the Shkotovka river population (southern Primorye) which fell into the second instead of the first one. Employment of two additional enzymes – *Xba*I and *Pvu*II revealed variants of mtDNA patterns that were present only in the Amur populations. These variants seem to be possible genetic characters that differentiate populations of the western and eastern slopes of the Sikhote-Alin mountains. The results suggest that there exist at least three superpopulation groupings (those of amur, southern-primorye and north-eastern-primorye) in the Primorye territory. However, on the whole interpopulation mtDNA variability was small (0.2–0.3%). Genetic heterogeneity in the *malma* population from Kamchatka proved to be even less: only one of the 15 enzymes revealed a variant genotype in one fish of 17 analysed. Only 4 of 15 enzymes gave an identical pattern of mtDNA digestion for Kamchatka and Primorye *malma* samples. The parallel analysis of mtDNA, of *S. leucomaenis* (as an outgroup species) and *S. malma* from two regions gave the following values of genetic distances (percent of nucleotide substitutions): *malma* (Primorye)-*malma* (Kamchatka) – 2.1; *malma* (Primorye) – *leucomaenis* – 2.6; *malma* (Primorye) – *malma* (Kamchatka) – *leucomaenis* – 3.6. Two monomorphic clonal lines of mtDNA were found in the Sakhalin sample of *S. malma* collected at the same time in a small stream. One line (80% of samples) fully corresponded to the "primorye" variant and the second line was the same as the "kamchatka" variant. A screening of mtDNA of 25 Kuril fishes by the two enzymes discriminating the Primorye and Kamchatka Dolly Varden revealed the "kamchatka" variant to be present in two specimens and the "primorye" variant in the rest (without polymorphism).

## Discussion

The results of this work suggest that the Primorye *malma* populations form at least three superpopulation groupings according to genetic similarity. This is in good agreement with genogeographic schemes obtained by us for other salmonids of Primorye (*Oncorhynchus keta*, *O. masou*, *Brachymystax lenok*) existing in similar geographic localities (Ginatulina et al., 1998). In a more extensive geographic analysis of *S. malma* populations (Primorye-Kamchatka) two main clonal lines of mitochondrial genotypes (with weak heterogeneity within them) were detected. Genetic difference between them is high (more than 2%) as compared with interspecific *S. malma* - *S. leucomaenis* distances. The morphologically "northern" form (Kamchatka) had the first variant, while the typical "southern" form (Primorye) possessed the second variant. At the same time in fishes from Sakhalin-Kuril region morphologically ascribed to the "southern" form both variants were present. The scale of genetic differences points to a rather large age of their divergence. It is interesting to note that comparative analysis of mtDNA from North American *S. malma* and three subspecies of arctic charr *S. alpinus* gave even less estimates of interspecies divergence (0.44; 0.45; 1.91%) (Grewe et al., 1990). Our results together with literature data force to return to the problems concerning evolution, systematics and history of area formation of charrs within *S. malma* - *S. alpinus* complex.

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