## GENERAL BIOLOGY

## **Evolutionary Consequences of Parasite Invasion:** A Case Study of Atlantic salmon *Salmo salar* L.

V. S. Artamonova<sup>*a*</sup>, O. V. Khaimina<sup>*b*</sup>, A. A. Makhrov<sup>*a*</sup>, V. A. Shirokov<sup>*c*</sup>, B. S. Shulman<sup>*d*</sup>, and I. L. Shurov<sup>*c*</sup>

Presented by Academician D.S. Pavlov March 17, 2008

Received March 19, 2008

**DOI:** 10.1134/S0012496608060136

Researchers in different fields, including genetics, ecology, and paleontology, regularly discuss the hypothesis on a considerably accelerated evolution of populations under unfavorable ecological conditions. However, most attention of researchers studying real populations under these conditions is paid to deleterious processes leading to extinction, whereas favorable evolutionary consequences are rarely taken into consideration (see monographs [1, 2]).

Invasion of alien species is a common factor deteriorating the habitat conditions of natural populations [3]. Here, we consider evolutionary processes in the population of the Atlantic salmon (*Salmo salar* L.) of the Keret' River (the White Sea Basin) (Fig. 1), where *Gyrodactylus salaris* Malmberg, a parasite that has caused a drastic decrease in the sizes of 55 Norway populations of the Atlantic salmon, was first detected in 1992.

Since 1992, juvenile salmon from the Keret' have been examined for *G. salaris* infection practically every year; in this period, the parasite was not detected only in 2004 (in 2005–2007, it was again found every year) [4, our unpublished data].

The Atlantic salmon population of the Keret' drastically decreased in the early 1990s (apparently, because of the parasite invasion). During the past decade and a half, the stock survived almost exclusively due to the activities of the Vyg and Kem' fish hatcheries: most spawners coming to the Keret' originated from these fish hatcheries, their proportion in some years being a high as 90% (according to data reported by the Karelian Department of Pisciculture).

Wild juvenile Atlantic salmon from the river were electrofished or angled. The ages of these fish were determined from growth rings on the scales, and the fins were examined under a binocular microscope to evaluate the infection by *G. salaris*. Fish-hatchery juveniles were collected at the Vyg Fish Hatchery in 2001 (underyearlings) and 2004 (two-year-olds). Table 1 shows the main characteristics of the samples studied.

The numbers of wild Atlantic salmon in the Keret' were small throughout the period of the study; therefore, we could collect only small samples of juveniles for analysis. In some years, there were rapids where Atlantic salmon were absent; sometimes, all juveniles captured in one locality were of the same age (Table 1). Smolts or downstream migrants (fish migrating to the sea) are shown as a separate row in the table because it was unknown from which rapids they had come.

Tissue samples for genetic analysis from white muscles and the liver were fixed and stored in ethanol (1 : 5). Total cell DNA was isolated from the samples with the use of a DIAtom<sup>TM</sup>DNAPrep100 kit (IzoGen, Moscow, Russia) as recommended by the manufacturer. An approximately 1400-bp mitochondrial DNA (mtDNA) fragment containing parts of the genes encoding *16S* RNA and NADH dehydrogenase I (ND-I) was analyzed by the PCR–RFLP method. We used the methodology and designations of haplotypes described in [5]. The heterogeneity of haplotype frequencies was estimated according to [6] with the use of the CHIRXC software [7].

We found four mtDNA haplotypes in the Atlantic salmon population of the Keret' River: *ABAAA*, *ABAAB*, *DBBAB*, and *DBBBB*. One specimen had heteroplasmy (it combined the *DBBAB* and *DBBBB* haplotypes).

Comparison of pooled samples of wild juveniles captured in different years (Table 2) showed that the

<sup>&</sup>lt;sup>a</sup> Vavilov Institute of General Genetics, Russian Academy of Sciences, ul. Gubkina 3, Moscow, 119991 Russia

<sup>&</sup>lt;sup>b</sup> Russian State Hydrometeorological University, Malookhtinskii pr. 98, St. Petersburg, 195196 Russia

<sup>&</sup>lt;sup>c</sup> Northern Research Institute of Fisheries, Petrozavodsk State University, nab. Varkausa 3, Petrozavodsk, 185031 Russia

<sup>&</sup>lt;sup>d</sup> Zoological Institute, Russian Academy of Sciences, Universitetskaya nab. 1, St. Petersburg, 199034 Russia



**Fig. 1.** (a) The geographical location of (1) the Keret' River and (2) the Vyg Fish Hatchery. (b) The scheme of the Keret' River: (1) a fish trap; (2–8) rapids: (2) Morskoi; (3) Kolupaevsky; (4) Maslyany; (5) Krasnobystry; (6) Varatsky; (7) Sukhoi; (8) Murash.

frequency of the *DBBAB* haplotype increased with time. The samples studied significantly differed in the haplotype frequency (p < 0.001). Comparison of samples of fish-hatchery juveniles from different generations showed a similar trend (p < 0.001).

The observed trend cannot be explained by stochastic processes. This has been demonstrated by analysis of three samples of wild fish of the 2002 generation captured in 2003 (0+), 2004 (1+), and 2005 (2+) at the Morskoi Rapids, where some fish were found to be infected with *G. salaris* in 2003 and 2005. The proportion of carriers of the *DBBAB* haplotype significantly increased (p < 0.001) from year to year in this generation at the Morskoi Rapids (Fig. 2). On the other hand, comparison of two samples of juveniles of the same generation from the Sukhoi Rapids captured in 2003 (0+) and 2004 (1+), where *G. salaris* were absent in that period, showed that the frequency of the *DBBAB* 

Year of study	Rapids	Pres- ence/ab- sence of <i>G. salaris</i>	Numbers of specimens of different ages			
			0+	1+	2+	3+
2001	Morskoi	+	0	7	0	0
	Sukhoi	+	16	5	0	0
	Varatsky	+	1	1	0	0
	Murash	-	1	0	0	0
2003	Morskoi	-	10	5	2	0
	Sukhoi	-	29	0	0	0
	Varatsky	-	0	10	0	0
2004	Morskoi	-	4	34	1	0
	Sukhoi	-	0	7	0	0
	Varatsky	-	17	0	0	0
2005	Morskoi	+	2	15	9	2
	Varatsky	+	9	8	0	0
	Kolupaevsky	+	11	1	0	0
	Maslyany	+	0	12	0	0
	Krasnobystry	+	16	3	2	0
	Smolts	+	0	2	10	0

**Table 1.** Characteristics of samples of wild Atlantic salmon from the Keret' River

haplotype in this group of fish did not change with time. Thus, selection for carriers of the *DBBAB* haplotype occurred in the Atlantic salmon population of the Keret' River in the presence of the parasite.

The results of our earlier studies on the Atlantic salmon population of the Keret' River show that, although selection for a certain mtDNA haplotype is the main factor of gene pool rearrangement, the effects of other factors, including the gene drift, interspecific hybridization, and, probably, mutations, enhance under adverse ecological conditions.

Judging from the distribution of allozyme markers in groups of fish, there were rapids where only one pair spawned in some years. Because of the small size of the spawning stock, samples of juveniles collected at the same rapids in different years sometimes considerably differed from one another in the frequencies of alleles of some protein loci [8], which indicates gene drift.

The finding of heteroplasmy in the salmon population of the Keret' River suggests an increase in the mutation rate: heteroplasmy had not been found in Atlantic salmon before (see [9] for review). At the same time, we do not know yet whether heteroplasmy has resulted from a point mutation in an mtDNA molecule of the egg or a mutant mitochondrion was transferred to the egg in the spermatozoon. In addition, the carrier of heteroplasmy originated from a fish hatchery; therefore, it cannot be excluded that artificial fertilization rather than contact with *G. salaris* was the main cause of heteroplasmy, although such phenomena are not known from the previous experience of breeding of Atlantic salmon.

We found natural hybrids between the Atlantic salmon and a closely related species, the brown trout (*S. trutta*), in the Keret' River in 1995 [10]. Hybridization between these species is considerably more common in Norwegian rivers, which are also invaded by *G. salaris*. In Norway, triploid hybrids (offspring of female interspecific hybrids and male Atlantic salmon) have also been found [11]. Triploid forms reproducing by gynogenesis are common among fishes; probably, we are witnessing the emergence of such a form.

A high resistance of the hybrids to the parasite favors their spread over Norwegian rivers invaded with *G. salaris* [11]. However, as can be seen from our data, selection has a different "application point" in the Atlantic salmon population of the Keret': here, the frequency of the *DBBAB* haplotype increases, whereas this haplotype is almost absent in Atlantic salmon of Norway [5].

Selection (including selection for resistance to parasites) has often been observed to cause rapid changes in the genetic structure of populations; however, almost all these observations were carried under artificial conditions or during formation of experimental populations in natural habitats (see [12-15] for reviews).

Origin of the juveniles	Year of study	Sample size	DBBAB	ABAAA	DBBBB	ABAAB
Wild	2001	31	0.32	0.42	0.06	0.19
Wild	2003	56	0.48	0.52	0.00	0.00
Wild	2004	63	0.79	0.21	0.00	0.00
Wild	2005	104	0.75	0.24	0.01	0.00
Fish-hatchery	2001	47	0.02	0.92	0.06	0.00
Fish-hatchery	2004	54	0.81	0.19	0.00	0.00

Table 2. Mitochondrial haplotype frequencies in samples of juvenile Atlantic salmon studied in different years



**Fig. 2.** Mitochondrial haplotype frequencies in samples from the 2002 generation captured at the Morskoi Rapid.

Thus, many data presented here are unique in that they describe a situation that has happened in a natural population. In addition, our data clearly demonstrate that not only extinction, but also rapid adaptive evolution of species may occur under unfavorable ecological conditions. On the one hand, this phenomenon is favorable for the species that rapid adaptation may save from extinction. However, it should be borne in mind that novel forms, in particular, those of hybrid origin, are integrated into natural ecosystems and affect them in an unpredictable manner.

## ACKNOWLEDGMENTS

We are grateful to V.F. Bugaev, V.E. Gilepp, E.P. Ieshko, V.A. Ignatenko, V.A. Movchan, and A.S. Rezanov for the assistance in collecting the material.

This study was supported by the programs of the Russian Academy of Sciences "Biodiversity and Gene Pool Dynamics" and Biological Resources of Russia: Fundamental Principles of Rational Use."

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