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ANIMAL GENETICS

Hybridization between Atlantic Salmon *Salmo salar* L. and Brown Trout *S. trutta* L. upon Artificial Propagation

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Abstract—Samples of *Salmo salar* and *S. trutta* were examined in 12 Russian fish hatcheries. With protein markers, hybrids of the two species were found in three hatcheries of the Baltic Sea basin. Some fishes had a phenotype intermediate between the *S. salar* and *S. trutta* phenotypes by morphological traits, but did not differ genetically from one of the parental species. Possible consequences of hybridization and ways to prevent it are discussed.

INTRODUCTION

Two species of the genus *Salmo*—Atlantic salmon *S. salar* and brown trout *S. trutta*—are closely related and often inhabit the same water systems. Natural hybridization of the two species is hindered by several biological barriers (in particular, assortative mating). Hybrids of the two species are rare in rivers that are only slightly affected by anthropogenic factors (see [1] for references).

However, numerous river ecosystems have changed as a result of damming, navigation, and pollution with wastes. In addition to other consequences, this had a negative impact on salmon populations, of which many were endangered. Hence it was of particular importance to employ hatcheries in preserving such populations. The problem is especially urgent in the Baltic Sea basin, where fish originating from hatcheries currently account for almost 90% of the commercial stock [2].

Hatchery reproduction eliminates the natural barriers preventing interspecific hybridization. Yet hybridization of *S. salar* and *S. trutta* on their joint reproduction did not received due attention for a long time; moreover, in some cases such hybridization was performed on purpose. As early as in the mid-19th century, V.P. Vrasskii obtained hybrids of the two species at the Nikol'skii fish hatchery [3]. There are fragmentary data on hybrids reared near St. Petersburg in the late 19th century [4]. In the 1930s, hybrid were reared in the Neva River [5]. Artificial hybridization of *S. salar* and *S. trutta* was also conducted in the adjacent regions: in Finland (the Saima Lake, Ladozhskoe Lake basin) [6] and in Baltic countries [7–9].

Latter, experiments on hybridization ceased, but, when spawners of one species were lacking, those of the other were still used in some cases [10]. Hybrids accounted for 31.4% of the brood stock of the Narvskii hatchery in 1979 and for 18.8%, in 1980. Hatcheries of other Russian regions had virtually no hybrids of *S. salar* and *S. trutta* at that time [11].

In the 1980s, morphology of gill rakers, shape of some bones, and weight variation of ovulated eggs were used to identify and cull hybrids (see [10] for review). In the 1990s, new methods came into use for identifying hybrids by several criteria, including exterior traits, scale morphology, and color phenes [12]. Some results are shown in Table 1. Spawners with pronounced hybrid features were excluded from the brood stock.

The objective of this work was to test 12 Russian hatcheries propagating *S. salar* for the presence of hybrids with the use of genetic markers.

MATERIALS AND METHODS

Seven fish hatcheries operate in the White Sea basin, and one functions in the Barents Sea basin (Fig. 1). There are four hatcheries in the Russian region of the Baltic Sea basin: these are on the Neva, Svir', Narva, and Luga rivers of the Leningrad oblast (Fig. 2). The progeny of spawners of the Shuya River, which flows into the Onega Lake, is obtained in the Kemskii hatchery. We examined the young sampled at the above hatcheries (Table 2). In addition, tissue specimens were obtained from a few spawners captured in rivers of the Baltic Sea basin for the purposes of propagation (Table 3).

We used the adipose fin in the case of spawners from the Shuya River and white muscle tissue in the case of all other spawners and the young. Specimens were transported in liquid nitrogen and stored at –70°C until use. Electrophoresis was carried out as described previ-

Table 1. Numbers of *S. salar* (numerator) and *S. trutta* (denominator) spawners tested for exterior features, scale morphology, and color phenes

Year	Fish hatchery		
	Narvskii	Luzhskii	Svirskii
1994	141 (0.234) / 29 (0.276)	–	13 (0.231) / 72 (0.458)
1995	125 (0.456) / 2 (0.0)	46 (0.239) / 197 (0.289)	3 (0.333) / 49 (0.755)
1996	161 (0.062) / 4 (0.75)	34 (0.154) / 157 (0.605)	10 (0.5) / 42 (0.762)
1997	213 (0.174) / 3 (1.0)	2 (0.5) / 61 (0.164)	9 (0.111) / 51 (0.49)
1998	514 (0.027) / 13 (0.385)	55 (0.091) / 79 (0.418)	21 (0.095) / 60 (0.133)
1999	684 (<0.01) / 14 (0.143)	77 (0.13) / 95 (0.231)	18 (0.0) / 26 (0.115)
2003	–	–	7 (0.0) / 41 (0.19)

Note: The proportion of fish expressing hybrid features are indicated in parentheses. The data were obtained by the monitoring group of the St. Petersburg State University.

ously [1]. We studied six diagnostic loci, which code for esterase D (*ESTD**), esterase (*EST-2**), glucose-6-phosphate isomerase (*GPI-3**), mannose-6-phosphate isomerase (*MPI**), and phosphoglucomutase (*PGM-1**, *PGM-2**). At least five of the loci were examined in samples from the hatcheries of the Baltic Sea basin, where F_1 hybrids were detected earlier. Only the *ESTD** locus was examined in the other samples.

RESULTS

Electrophoretic patterns of the proteins examined did not differ from published ones (Fig. 3). When tests involved several loci differentiating *S. salar* and *S. trutta,* coincident results were always obtained in all tests. Some fishes had the hybrid genotypes at all loci under study and were identified as F_1 hybrids, while some others had only the genotypes characteristic of one species and were identified as representatives of the corresponding parental species. We did not detect F_2 hybrids or backcrosses, which would display the hybrid genotype at some loci and the genotype of a parental species at some others.

By morphological traits, a phenotype intermediate between the *S. salar* and *S. trutta* phenotypes was observed in a few fishes. Yet these fishes did not differ genetically from one of the parental species.

Interspecific hybridization was not detected in the hatcheries of the White and Barents sea basins, where only *S. salar* is propagated. Hybrids were found among the young sampled in the Luzhskii, Svirskii, and Narvskii hatcheries (Table 2). One hybrid was found among the spawners used at the Narvskii hatchery (Table 3). Thus, hybridization takes place in the hatcheries that propagate (or propagated earlier) both *S. salar* and *S. trutta.*

Table 2. Young samples tested for genetic markers

River of spawner capture	Hatchery	Year of sampling, fish age	Number		
			S. salar	F_1 hybrids	*S. trutta*
Kola	Taibol'skii	2001, 1+	100	0	0
Umba	Umbskii	2000, 1+	101	0	0
Umba	Umbskii	2001, 2+	50	0	0
Kola	Kandalakshskii	2001, 1+	206	0	0
Kola	Knyazhegubskii	2001, 1+	55	0	0
Shuya	Kemskii	1995, 2.	50	0	0
Keret'	Vygskii	1995, 2.	50	0	0
Keret'	Vygskii	2001, 0+	50	0	0
Keret'	Vygskii	2001, 2+	73	0	0
Keret'	Vygskii	2002, 1.	140	0	0
Keret'	Kemskii	2002, 1.	70	0	0
Onega	Onezhskii	2000, 1–2+	48	0	0
Onega	Solzenskii	2000, 1+	50	0	0
Solza	Solzenskii	2001, 1+	40	0	0
Emtsa	Solzenskii	2002, 0+	51	0	0
Svir'	Svirskii	1995, 1+	30	37	0
Svir'	Svirskii	1996, 1+	11	0	0
Svir'	Svirskii	1997, 0+	8	0	0
Neva	Nevskii	1996, 0+	7	0	0
Luga	Luzhskii	1996, 0–2+	17	14	6
Narva	Narvskii	1995, 0+	29	1	0
Narva	Narvskii	1998, 0+	30	0	0

DISCUSSION

First, we would like to consider the resolution of our method for diagnosing hybrids. The proteins examined all differ in electrophoretic mobility between *S. salar* and *S. trutta.* Although some *S. trutta* alleles of the *ESTD** and *EST-2** loci code for proteins with the same electrophoretic mobility as in *S. salar*, these alleles have never been detected in *S. trutta* populations inhab-

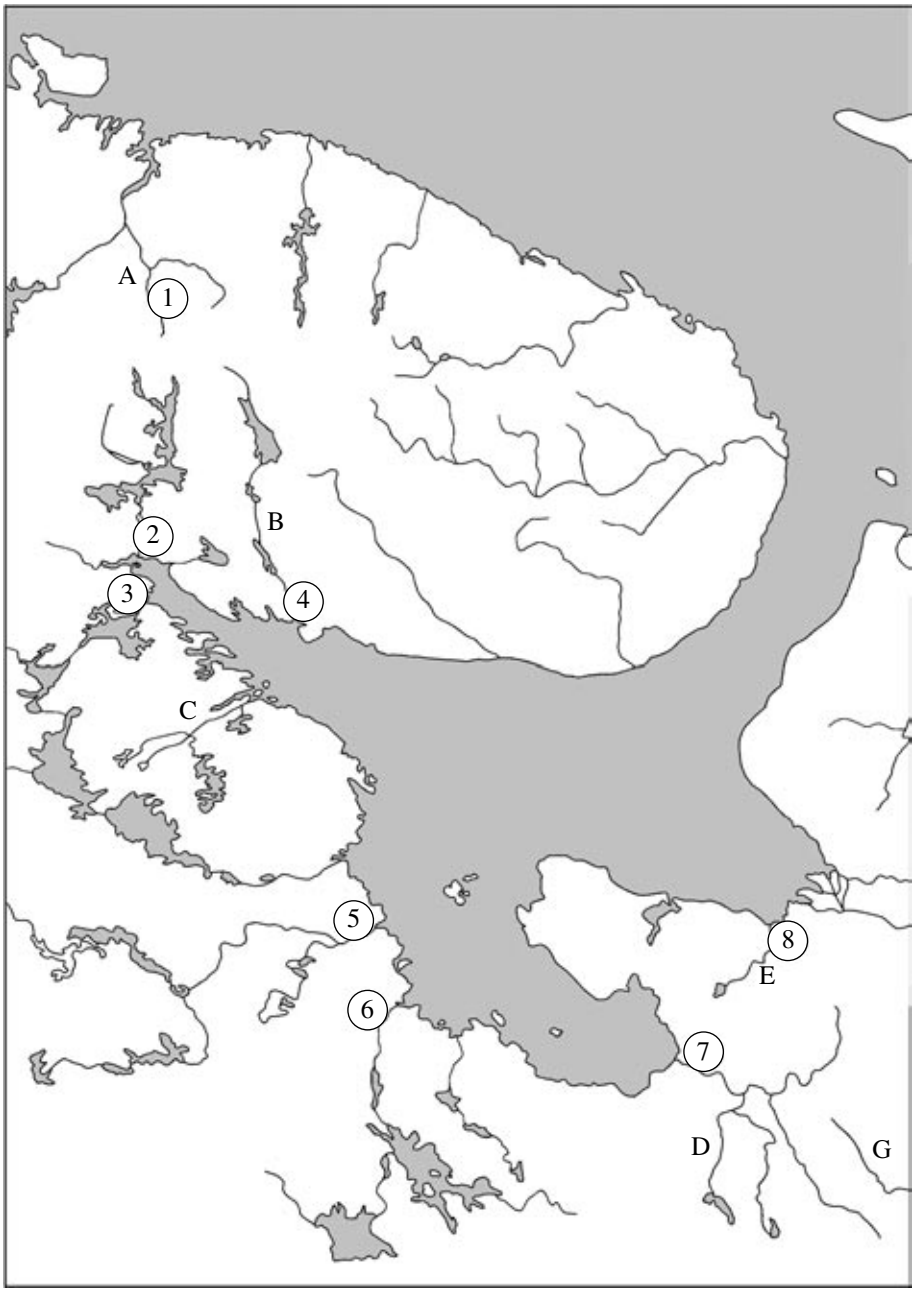

Fig. 1. Hatcheries and their base rivers in the Barents and White sea basins. Hatcheries: 1, Taibol'skii; 2, Kandalakshskii; 3, Knyazhegubskii; 4, Umbskii; 5, Kemskii; 6, Vygskii; 7, Onezhskii; and 8, Solzenskii. Rivers: A, Kola; B, Umba; C, Keret'; D, Onega; E, Solza; and G, Emtsa.

iting the same rivers as *S. salar* [13]. Thus, the loci under study can be used to identify *S. salar, S. trutta,* and their hybrids.

Analysis of a single diagnostic locus is sufficient for detecting F_1 hybrids. Hence it is safe to say that we identified all F_1 hybrids present in our samples. In addition, approximately half of F_2 hybrids and backcrosses can be revealed with the use of one locus, and more than 95% of such fishes, with five loci, provided that the inheritance is Mendelian [14].

However, the progeny of interspecific hybrid may display a mode of inheritance other than Mendelian. Hybrid fish may produce gametes identical to those of the parental species; e.g., this is the case in hybridogenesis, when several generations of hybrid females produce gametes with the chromosome set of one parental species [15–17]. Such "restoration" of the parental chromosome sets is probably due to spatial isolation of the parental sets in hybrid cells. In particular, isolation of chromosome sets is known to *S. salar* and *S. trutta*

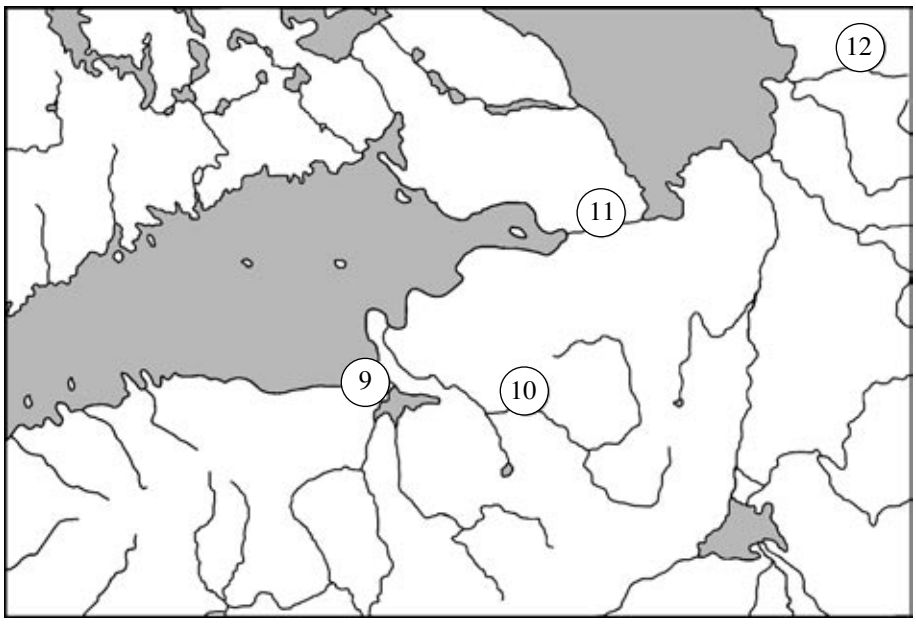

Fig. 2. Hatcheries of the Baltic Sea basin: 9, Narvskii; 10, Luzhskii; 11, Nevskii; and 12, Svirskii.

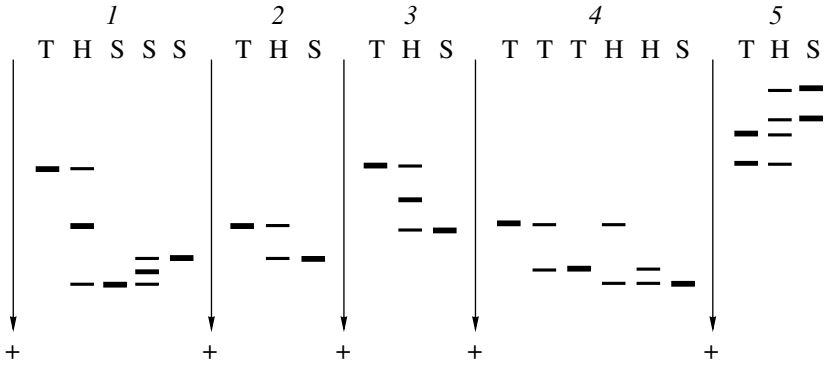

Fig. 3. Schemes of the electrophoretic phenotypes determined by diagnostic loci (*1*) *ESTD**, (*2*) *EST-2**, (*3*) *GPI-3**, (*4*) *MPI**, (*5*) *PGM-1**, and *PGM-2** in (S) *S. salar,* (T) *S. trutta*, and (H) their hybrids.

[16]. Moreover, a case has been reported that all progeny was hybrid when a *S. salar* female was crossed with an F_1 male, which presumably produced only gametes with the *S. trutta* set [11].

Note that some *S. salar* × *S. trutta* hybrid females gynogenetically produce diploid eggs. The progeny of such females may include diploid and triploid hybrids [19–23]. A triploid hybrid has been found in the progeny of a *S. trutta* female and an F_1 male, suggesting spontaneous gynogenesis for the female [20].

Spontaneous gynogenesis has been observed in several fish species, with some paternal chromosomes occasionally being incorporated in the genome of the progeny [24]. For instance, this has been reported for tropical fish *Poecilia formosa* [25] and assumed for *S. salar* and *S. trutta* [26]. As for other salmons, there is experimental evidence that microchromosomes may transfer a color-determining gene from one species to another [27].

It is noteworthy in this connection that a few fishes with the genotype characteristic of *S. salar* or *S. trutta* and the phenotype (color phenes, some other traits) characteristic of their hybrids were found in our samples obtained from hatcheries of the Baltic Sea basin. Similar results have earlier been reported for spawners of the Narvskii and Svirskii hatcheries [12, 28]. It is possible that fishes with the hybrid phenotype carried DNA fragments of another species in the genome or, alternatively, the specifics of the color and morphology reflect intraspecific diversity [29]. This problem requires further investigation. Detailed studies of the remote consequences of *S. salar*–*S. trutta* hybridization is of interest for elucidating the mechanisms of specia-

Table 3. Spawner samples tested for genetic markers

River of capture	Hatchery	Year of sampling	Number		
			S. salar	F_1 hybrids	*S. trutta*
Shuya	Kemskii	1994	22	0	0
Svir'	Svirskii	1995–1998	1	0	17
Neva	Nevskii	1996	12	0	0
Luga	Luzhskii	1995–1998	1	0	17
Narva	Narvskii	1995–1998	25	1	2

tion, because several fish taxa are known to be of a hybrid origin (see [15, 30, 31] for review).

Note than detection of hybrids in the samples from the Narvskii and Svirskii hatcheries allows another interpretation of earlier findings. For instance, it is possible that "winter" *S. salar* individuals found in the Narva River [32] were actually interspecific hybrids [33]. Some morphological features described for *S. salar* from the Svir' River [34] may be ascribed to hybrids present in the sample examined.

Comparison of our results and published [11] data demonstrates that the proportion of hybrids in the Narva population appreciably decreased in the recent 15 years. This is probably due to the facts that spawners with the most marked hybrid features were excluded from reproduction by 1990s the monitoring group of the St. Petersburg State University and that *S. trutta* ceased to be propagated in the Narva River.

To completely eliminate the possibility of interspecific hybridization in fish propagation in the Baltic Sea basin, it is necessary to further monitor the specific purity of spawners with the use of genetic methods in particular. Such monitoring is essential, because the life cycle of *S. salar* and *S. trutta* may be rather long, up to nine years.

Development of methods for diagnosing hybrids and preventing hybridization is of importance with many animals and plants and is among the most urgent problems of conservation genetics [35, 36]. Our results demonstrate that accidental interspecific crossing on artificial propagation is a possible way of generating hybrids.

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