Russian Journal of Genetics, Vol. 38, No. 3, 2002, pp. 277–285. Translated from Genetika, Vol. 38, No. 3, 2002, pp. 357–367.

ANIMAL GENETICS

Phylogeny of Mitochondrial DNA in Salmonids of the Subfamily Salmoninae: Analysis of the Cytochrome b Gene Sequences

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Received February 2, 2001; in final form, June 18, 2001

Abstract—On the basis of comparison of the cytochrome b gene nucleotide sequences from genetic databases, the possible phylogenetic relationships of mitochondrial DNA (mtDNA) among all major lineages of Salmoninae (*Brachymystax, Parahucho, Salvelinus, Salmo, Parasalmo*, and *Oncorhynchus*) were examined. Three different phylogenetic methods (UPGMA, NJ, and ML) yielded phylogenetic trees of essentially the same topology: (((*Brachymystax, Parahucho*), *Salvelinus, Salmo*), (*Parasalmo, Oncorhynchus*)). The results obtained using the maximum parsimony method were less clear. Apparently, the divergence of the main salmonid lineages occurred during a relatively short time period; hence, the number of synapomorphs marking the order of their divergence was extremely low. This may account for the relative failure to use the maximum parsimony method of phylogenetic reconstruction. The problem of concordance of mtDNA and species phylogenetic schemes is discussed. Their discrepancy in salmonids may be caused by interspecific introgressive hybridization.

INTRODUCTION

Phylogenetic studies of salmonids from the subfamily Salmoninae based on mitochondrial DNA (mtDNA) have been started by Berg and Ferris [1] who examined phylogenetic relationships among members of four genera: chars *Salvelinus*, salmons *Salmo*, Pacific trouts *Parasalmo*, and salmons *Oncorhynchus*. The later works [2–10] and others) were as a rule focused on establishing mtDNA phylogenetic relationships among different species within one of these genera. Because of this, the other three genera were used only as outgroups, while mtDNA phylogeny received little attention. Earlier [11], we have analyzed phylogenetic relationships of mtDNA among six genera: *Brachymystax, Hucho, Parahucho, Salvelinus, Parasalmo*, and *Oncorhynchus*. We have shown that these genera form two stable groups: ((*Brachymystax, Hucho*), *Salvelinus*), and (*Parasalmo, Oncorhynchus*). The position of Sakhalin taimen *Parahucho* remained unclear. Therefore, the mtDNA phylogeny of Salmoninae at the generic level appeared as an unsolvable trichotomy of these groups.

At present, sufficient amount of data has been accumulated for, first, solving this problem and, second, resolving the extremely controversial issue (see [1, 3, 12]) of the phylogenetic position of *Salmo* mtDNA. These data include nucleotide sequences of the cytochrome b mitochondrial gene established in sequencing mtDNA of various salmonid fishes (Matsuda *et al.*, 1995[1]) [13–15]. As recently shown [16], this gene can be used to estimate phylogenetic mtDNA relationships of fishes not only at the intra- and interspecific levels, but also at the level of families and even larger taxa. Here, we present analysis of possible phylogenetic relationships of mtDNA among members of all major Salmoninae lineages based on cytochrome b nucleotide sequences.

MATERIALS AND METHODS

Complete sequences of the cytochrome b gene of nine Salmoninae species from several genetic databases (Table 1) served as the material for this study. As an outgroup, we used nucleotide sequences of this gene from two members of whitefish Coregoninae, which are phylogenetically closest to Salmoninae and belong to another salmonid subfamily, Salmonidae. In addition, to test monophyletic origin of Salmoninae, we combined data for taxonomically distant fishes (two species of carp Cyprinidae and one species of cod Gadidae) to form a second outgroup.

For alignment of cytochrome b gene nucleotide sequences, we used program package ClustalW, version 1.7 [21]. The phylogeny reconstruction of cytochrome b gene haplotypes was carried out using different procedures: (1) unweighted pair-group method with arithmetic mean (UPGMA), (2) neighbor-joining, (NJ) (3) maximum likelihood (ML), and (4) maximum parsimony (MP) methods. In the former three approaches, phylogenetic tree topology is inferred from various genetic distances. The fourth approach is based on analysis of discrete states (in our case, the presence/absence

[1] The manuscript by M. Matsuda, T. Oshiro, T. Kobayashi, R. Ueshima, H. Yonekawa, and M. Sakaizumi, "Molecular Perspective on Phylogenetic Relationships of Salmonid Species," 1995, has not been published (M. Matsuda, personal communication).

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Table 1. Sources of data on nucleotide sequences of the mitochondrial cytochrome b gene in salmonids and other fish species

Sequence no. in GenBank (EMBL, DDBJ)	Haplotype designation	Species	Reference
U12143	SSALA	*Salmo salar* Linnaeus (Atlantic salmon)	[13]
D58400	STRUT	*Salmo trutta* Linnaeus (brown trout)	Matsuda *et al.* (1995)
D58396	PPERR	*Parahucho perryi* Brevoort (Sakhalin taimen)	The same
D58399	SFONT	*Salvelinus fontinalis* Mitchill (brook char)	"
D58398	SLEUC	*Salvelinus leucomaenis* Pallas (white-spotted char)	"
D58401	PMYKI	*Parasalmo mykiss* Walbaum (mykiss trout)	"
D58402	OMASO	*Oncorhynchus masou* Brevoort (masu salmon)	"
AF125212	OKETA	*Oncorhynchus keta* Walbaum (chum salmon)	[14]
AF125213	BLENO	*Brachymystax lenok* Pallas (lenok)	[14]
AJ251591	CPOLL	*Coregonus autumnalis pollan* Thompson (Irish pollan)	[15]
AJ251589	CLAVA	*Coregonus lavaretus baikalensis* Dybovski (Baikalian whitefish)	[15]
AB006953	CARAS	*Carassius auratus* Linnaeus (goldfish)	[17]
X61010	CYPRI	*Cyprinus carpio* Linnaeus (common carp)	[18]
X99772	GADUS	*Gadus morhua* Linnaeus (Atlantic cod)	[19]

* Generic affiliation of Pacific trouts is given according to the last review of the freshwater ichtyofauna of Russia [20].

of four nucleotide types in a particular position of cytochrome b gene sequences) on the assumption of the minimal number of possible evolutionary changes.

Genetic distances between cytochrome b gene haplotypes were measured as the proportions of different nucleotides p. The UPGMA and NJ trees were obtained from matrices of distances p using the PAUP program package, version 4b4a [22]. In addition to p, UPGMA and NJ trees were constructed on the basis of other, more complicated genetic distances, which accounted for different probabilities of six nucleotide substitution types (JC, K2, HKY85, TamNei; see [22] for details). However, this virtually did not affect the final results. The stability of clustering of the trees obtained was tested in 1000 bootstrap cycles [23] using the same software package.

Phylogenetic maximum likelihood analysis was based on the HKY85 nucleotide substitution model [24] using the NucML procedure from the MOLPHY package, version 2.3b3 [25], in which the transition/transversion ratio (Ts/Tv) was estimated empirically using the "–topt" option. The bootstrap estimates of the ML tree branching nodes were obtained by the RELL method in 10 000 local bootstrap cycles ([25], p. 49).

Maximum parsimony analyses were performed with the PAUP program package using heuristic search (TBR branch swapping; MulTrees option in effect) with ten random stepwise additions of taxa. Robustness of the inferred MP trees was tested by bootstrapping (as implementation in PAUP with 1000 pseudoreplications each).

Uniformity of the rate of nucleotide substitution accumulation in the cytochrome b gene of different lineages of Salmoninae haplotypes was tested by the relative rate test [26] using procedures RRTree [27] and PHYLTEST [28].

RESULTS

Our analysis has shown that 303 out of 1140 nucleotide positions in the cytochrome b gene exhibited variation. Most of the nucleotide substitutions (267 of 303; see Table 2) were at the third codon position. Only two substitutions were found at the second position (both in masu salmon). The first codon position accounted for 34 subsitutions. At 100 nucleotide positions, mutational substitutions were detected only in one of the Salmononae taxa studied. These substitutions were not phylogenetically informative. At 203 nucleotide positions, mutational substitutions were observed in two or more salmonid species and thus were phylogenetically significant.

The haplotypes of Atlantic salmon and brown trout and of masu salmon and lenok exhibited respectively the smallest (66 subsitutions) and the greatest (157 substitutions) differences in the cytochrome b gene nucleotide sequences among the Salmoninae species (Table 3). In the intergeneric comparisons the haplotypes of Pacific salmon *Oncorhynchus* and Pacific trout *Parasalmo* exhibited the highest similarity ($\bar{p} = 8.9 \pm 0.8\%$). The pairwise comparison of the cytochrome b gene haplotypes in members of the genera *Parahucho*, *Salvelinus*, and *Salmo* revealed a slightly higher level of divergence ($\bar{p}$ = 11.0–11.2%). The haplotype of lenok *Brachymystax* was similar to this group ($\bar{p}$ = 11.0–12.7%). The greatest differences were found in pairwise intergeneric comparisons with Pacific salmon and trout haplotypes ($\bar{p}$ = 12.1–12.8%).

Table 2. Nucleotide composition and number of variable sites in the mitochondrial cytochrome b gene of salmonids

Parameter	Codon position			
	1	2	3	1–3
Nucleotide frequency, %				
T	23.7	40.0	23.1	28.9
C	25.6	26.3	40.5	30.8
A	23.4	19.8	31.1	24.8
G	27.3	13.9	5.3	15.5
Total number of nucleotide positions	380	380	380	1140
of them:				
stable	346	378	113	837
with unique substitutions (in one haplotype)	18	2	80	100
variable (in two and more haplotypes)	16	–	187	203

Amino-acid sequences of the cytochrome b gene in carp and cod species, on the one hand, and salmonids, on the other, were drastically different (for 31–47 positions; the positions were reconstructed using utility nuc2ptn from the MOLPHY package). The cytochrome b sequences of different Salmonidae representatives typically differed only in one to five amino-acid residues. The species of Pacific salmons and trouts provided the exception to this trend. The cytochrome b of mykiss and chum salmon differed from that of Coregoninae species and members of other Salmoninae genera by three to seven amino-acid sites. This protein showed an even greater difference in masu salmon: it differed by 7 to 8 amino-acid positions from cytochromes b of mykiss and chum and by 11 to 14 positions from those of the other Salmonidae species. The relative rate test (using the RRTree procedure) demonstrated that the rate of accumulation of nonsynonymous mutations in the lineage leading to haplotype OMASO was significantly higher ($P = 0.002$–0.012) than in the other salmonid lineages, which were homogeneous in this respect. The test for the synonymous substitution accumulation rate revealed a significant ($P = 0.033$) difference only for the BLENO–PPERR pair (the rate in the lineage leading to the latter haplotype was higher). The simultaneous consideration of the both substitution types using the PHYLTEST program did not show any significant deviations in their accumulation rates in different Salmoninae haplotype lineages.

In the NJ tree constructed from the *p* distance matrix, the cytochrome b gene haplotypes of Salmoninae were grouped into two distinct clusters (figure), one of which was composed of haplotypes of whitefish Coregoninae, and the other, by those of salmonids proper, Salmoninae. The latter group exhibited a sequential branching order: first, *Parasalmo* and *Oncorhynchus*, then *Salmo* and *Salvelinus*, and finally *Parahucho* and *Brachymystax*. A test for significance of the NJ tree branching nodes by bootstrap showed the following. The cluster including haplotypes of all the Salmoninae representatives examined was highly statistically significant (Table 4). The clusters of Pacific salmons and trouts (PMYKI + OMASO + OKETA), salmons *Salmo* (STRUT + SSALA), and chars *Salvelinus* (SFONT + SLEUC) were also statistically significant. The cluster including haplotypes of *Salmo, Salvelinus, Parahucho*, and *Brachymystax* was reproduced in 597 out of 1000 bootstrap cycles. However, the remaining branching nodes of the NJ tree related to Salmoninae haplotypes turned out to be very unstable. In view of this, only the following associations within Salmoninae at the generic level can be considered significant: (1) cluster of *Parasalmo* and *Oncorhynchus* haplotypes and (2) cluster of haplotypes of *Salmo, Salvelinus, Parahucho*, and *Brachymystax* species.

As shown in Table 3, Salmoninae have a great number of transition-type nucleotide substitutions (Ts) in the cytochrome b gene. Since the transition number is only slightly lower in a pairwise haplotype comparison within the subfamily than in their comparison not only to whitefish but even to taxonomically more distant species of carp and cod, the number of transition within Salmoninae is close to the maximum possible level. This means that multiple replacements occurred in the same position of the cytochrome b gene in different Salmoninae species. Apparently, these replacements have low phylogenetic significance due to their reccurrence. Conversely, Table 3 shows that the number of transversion-type replacements (Tv) gradually increases with the distance between the taxa compared. Therefore, the use of only this type of replacements for estimating distances and constructing NJ trees seem to produce more reliable results than the simultaneous analysis of both transitions and transversions.

The exclusion of transitions from analysis did not change the general topology of the tree:

(outgroup, (((BLENO, PPERR, (STRUT, SSALA)), (SFONT, SLEUC)), (PMYKI, (OKETA, OMASO)))).

However, the second cluster was rearranged: the *Salvelinus* group of haplotypes occupied an outer position. As expected, the bootstrap value for the cluster

Table 3. Number of nucleotide differences between cytochrome b gene haplotypes in salmonids and other fish species (above diagonal: transition-type; below diagonal: transversion-type)

Haplotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. GADUS	–	145	149	157	156	159	164	162	167	158	160	161	162	169
2. CARAS	136	–	100	142	141	130	138	142	146	142	144	135	142	140
3. CYPRI	136	34	–	131	134	122	128	123	128	129	126	123	127	129
4. CLAVA	123	119	123	–	36	120	127	123	129	112	120	125	129	126
5. CPOLL	122	120	124	7	–	127	128	125	122	116	120	126	131	128
6. BLENO	122	118	122	49	52	–	95	104	99	113	123	109	119	102
7. PPERR	118	120	122	61	64	30	–	90	90	92	102	102	106	107
8. SFONT	116	122	128	55	58	32	36	–	56	89	100	94	107	112
9. SLEUC	116	124	132	59	64	34	34	16	–	85	99	96	104	99
10. STRUT	118	126	130	57	60	28	30	36	36	–	51	100	104	97
11. SSALA	111	121	127	50	53	25	31	31	31	15	–	102	113	103
12. PMYKI	113	113	127	50	53	37	43	43	45	45	36	–	88	65
13. OMASO	122	120	128	55	58	38	44	42	42	38	35	27	–	75
14. OKETA	118	120	126	49	52	32	34	36	36	34	29	23	20	–

Table 4. Bootstrap estimates (%) of significance of branching nodes on phylogenetic trees of cytochrome b haplotypes in salmonids

Method of phylogeny reconstruction	Branching node (see figure)										
	a	*b*	*c*	*d*	*d**	*e*	*e**	*f*	*g*	*h*	*h**
NJ; Ts and Tv	99	100	60	–	–	–	–	100	100	51	–
NJ; only Tv	95	91	89	–	–	–	–	100	91	59	–
UPGMA; Ts and Tv	100	100	69	–	–	–	–	100	100	–	75
UPGMA; only Tv	100	93	66	–	55	–	59	100	98	69	–
ML	88	90	88	56	–	55	–	100	100	–	54
MP; weight Tv : Ts = 1 : 1	94	98	–	–	–	–	–	100	100	–	56
MP; weight Tv : Ts = 1 : 2	97	92	–	–	–	–	–	100	100	57	–
MP; weight Tv : Ts = 1 : 4	75	69	–	–	–	–	–	100	99	–	–

Note: – indicates that this node was reproduced less than in 50% of bootstrap replications; *d**, *e**, *h** are branching nodes for clusters (PPERR + BLENO + STRUT + SSALA), (BLENO + STRUT + SSALA) and (PMYKI + OKETA), respectively.

that included *Salmo, Salvelinus, Parahucho*, and *Brachymystax* was noticeably higher in this variant of phylogenetic reconstruction (Table 4), which was caused by elongation of the branch connecting nodes *a* and *c*. In contrast to the previous variant, here the length of this branch was significantly higher than zero ($P = 0.048$, the interior-branch test from PHYLTEST). On the other hand, this was compensated by shortening of other internodes in this cluster, which was expressed in the trichotomy of haplotypes BLENO, PPERR, (STRUT + SSALA). The bootstrap estimates for cluster (OMASO + OKETA) were slightly higher. At the same time, the significance level of clusters of Pacific salmons and trouts (PMYKI + OMASA + OKETA) and salmons from the *Salmo* genus (STRUT + SSALA) somewhat decreased.

The UPGMA tree based on the matrix of *p* distances calculated using all types of nucleotide replacements has essentially similar topology:

(outgroup, (((PPERR, BLENO), ((STRUT, SSALA), (SFONT, SLEUC))), ((PMYKI, OKETA), OMASO))).

The differences primarily concerned the position of haplotype OMASO. In contrast to NJ tree, on the UPGMA tree the position of this haplotype was external with regard to the (PMYKI + OKETA) group. This transformation was confirmed by bootstrap (Table 4). The second cluster, which included haplotypes of *Salmo, Salvelinus, Parahucho*, and *Brachymystax*, also underwent rearrangement: on the UPGMA tree, the (BLENO + PPERR) cluster was outside of the cluster that united haplotypes of *Salmo* (STRUT + SSALA) and *Salvelinus* (SFONT + SLEUC) species. However,

the reproducibility of the branching order was extremely low (the latter cluster appeared in 36.2% of the bootstrap replications).

If the *p* distance matrix was calculated only on the basis of transversion-type nucleotide replacements, the new variant of the UPGMA tree differed from the original NJ tree only by rearrangements in the cluster of *Salmo, Salvelinus, Parahucho*, and *Brachymystax* species:

(outgroup, (((((STRUT, SSALA), BLENO), PPERR), (SFONT, SLEUC)), (PMYKI, (OKETA, OMASO)))).

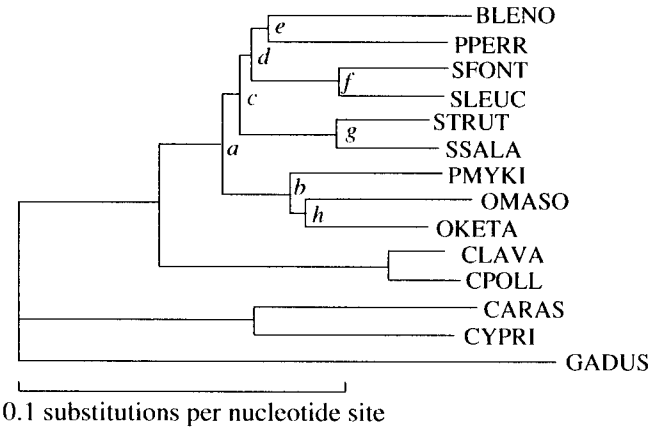
In this case, haplotypes of the *Salvelinus* species (SFONT + SLEUC) occupied the external position, then haplotype PPERR branched out, and then haplotypes of *Brachymystax* (BLENO) and *Salmo* (STRUT + SSALA) were divided. Note that these changes were reproduced in more than 50% of the bootstrap cycles (Table 4).

Since the nucleotide compositions at three codon positions of the cytochrome b gene were drastically different and the number of phylogenetically informative substitutions in the former two of them was very small (Table 2), we used only the data for the third codon position for the phylogeny reconstruction by maximum likelihood (ML) method. The topology of the ML tree obtained by combining branches of the originally star-shaped tree (star decomposition: [25], p. 48) completely coincided with that of the NJ tree shown in figure. However, further analysis of this tree configuration by the local rearrangement method ([25], p. 49) demonstrated that the most probable position of the OMASO haplotype was that occupied by this haplotype on the UPGMA tree, i.e., outside the (PMYKI + OKETA) group. This position of haplotype OMASO was preserved in 54% replications of local bootstrap (Table 4). As shown by bootstrap analysis, the reproducibility of ML tree branching nodes was in general somewhat higher than that for the NJ and UPGMA trees.

A search for a topologically optimal tree for all 105 possible combinations of the outgroup and five haplotype groups (PMYKI + OKETA + OMASO; STRUT + SSALA; SFONT + SLEUC; PPERR; BLENO) resulted in the same configuration:

(outgroup,((((BLENO, PPERR), (SFONT, SLEUC)), (STRUT, SSALA)), ((PMYKI, OKETA), OMASO))).

Nevertheless, note that most generated trees did not statistically significantly differ from their "most likely" variant. Out of 105 possible tree variants, only 6 were significantly (at the 5% significance level) worse than the best of them ($\Delta \mathrm{Ln}L/\mathrm{SE} > 1.96$, where $\Delta \mathrm{Ln}L$ is the difference between the natural logarithms of two likelihood estimates and SE is its standard error; see [29]) and only 19 approached the threshold level ($1.71 < \Delta \mathrm{Ln}L/\mathrm{SE} < 1.96$). The phylogenetic trees of Salmoninae haplotypes in which haplotypes of *Parahucho* or *Salvelinus* occupied the external position had the lowest likelihood. According to our estimations based on



The NJ tree of cytochrome b gene haplotypes of salmonids and other fish species.

the fact that $\Delta \mathrm{Ln}L$ is proportional to the nucleotide sample size, and SE, to its square root ([29], p. 175), the differences between the best variant and 68 ML trees are expected to become statistically significant at the approximately twofold increase of the length of the sequenced DNA fragment. The significance of the remaining 30 trees can be demonstrated on the basis of data on even larger DNA fragment (4000–9000 bp in length).

Analysis of the phylogenetic relationships of the cytochrome b gene haplotypes in salmonid fishes using the maximum parsimony (MP) method yielded less clear results than the use of the approaches that are based on an analysis of branch lengths of phylogenetic trees (NJ, UPGMA, and ML). At equal phylogenetic weights of transversions and transitions (Tv : Ts = 1 : 1), the following two maximally parsimonious trees of *Salmoninae* haplotypes were obtained that have the same number of mutational steps (1226) but dramatically different topology:

(outgroup, BLENO, (((PPERR, (SFONT, SLEUC)), (STRUT, SSALA)), (PMYKI, (OMASO, OKETA)))));

(outgroup, ((STRUT, SSALA), (((PPERR, BLENO), (SFONT, SLEUC)),((PMYKI, OKETA), OMASO)))).

A twofold increase of the transversion weight (Tv : Ts = 1 : 2) produced two equivalent trees with the step number of 1610. One of them was topologically identical to the former of the above two trees. The configuration of the other tree was similar to that of the earlier variants of the NJ, UPGMA, and ML trees:

(outgroup, ((BLENO, (PPERR, ((SFONT, SLEUC), (STRUT, SSALA)))), (PMYKI, (OMASO, OKETA)))).

This variant turned out to be maximally parsimonious (the step number 2565) at the fourfold excess of transversions over transitions (Tv : Ts = 1 : 4). In view of the obvious prevalence of the transition-type in the

cytochrome b gene evolution (Table 3), this assumption seems likely. The obtained variant of the MP tree would also seem realistic. However, bootstrap analysis showed that the reproducibility of most branching nodes on all of these trees including the last one is very low (Table 4). In the framework of this approach, the phylogenetic relationships between the five major groups of cytochrome b gene haplotypes essentially remained unresolved.

DISCUSSION

Our results have shown that cytochrome b gene haplotypes of the Salmoninae species examined form a monophyletic unit, which is clearly divided into five groups: (1) Pacific salmons *Oncorhynchus* and trouts *Parasalmo*, (2) salmons *Salmo*, (3) chars *Salvelinus*, (4) Sakhalin taimen *Parahucho*, and (5) lenok *Brachymystax*. We could not find an unambiguous clustering order of these groups on phylogenetic trees constructed using different methods. An independent cluster of *Oncorhynchus* and *Parasalmo* haplotypes and another cluster combining haplotypes of *Salmo*, *Salvelinus*, *Parahucho*, and *Brachymystax* seem most likely. Within the latter cluster, haplotypes of *Parahucho* and *Brachymystax* tend to group together.

The position of *Parahucho* mtDNA was established with more certainty than in the previous study of salmonid mtDNA phylogeny based on restriction analysis of the total mtDNA genome [11]. In view of this, note that joining of the *Parahucho* haplotype to the cluster that included haplotypes of *Brachymystax* and *Hucho* also occurred earlier when trees were constructed from data averaged over taxa (the data were taken from Table 3 of [11]). Finally, it should be noted that if the present analysis included data on a sequenced 300-bp cytochrome b gene fragment of Danube taimen *Hucho hucho* L. (AF172397, GenBank), in all variants of phylogenetic reconstruction it occupied the same position as Siberian taimen *Hucho taimen* Pallas, which was a sister position with regard to *Brachymystax* ([11]: Figs 1, 2).

An mtDNA phylogenetic position of members of the genus *Salmo* has been so far under debate. Two studies based on mtDNA restriction analysis have shown that *Salmo* was closer to Pacific salmons *Oncorhynchus* and/or trouts *Parasalmo* than to chars *Salvelinus* [1, 3]. By contrast, an analysis of a 295-bp fragment of the cytochrome b gene sequence have lead to the opposite conclusion [12]. According to the results based on the complete nucleotide sequence of this gene, the latter variant seems more plausible.

As expected from the results obtained earlier, the relationships between cytochrome b gene haplotypes of *Oncorhynchus* and *Parasalmo* turned out to be unclear (Table 4). When analysis of the total mitochondrial genome using restriction enzymes was conducted or the cytochrome b gene fragment was examined, *Parasalmo* species typically were included in the *Oncorhynchus* species cluster (see [2, 11, 12]). According to analysis of a 1128-bp sequence of the control mtDNA region of salmonids, *Parasalmo* species occupied an external position with regard to the *Oncorhynchus* cluster, but bootstrap estimates of the reproducibility of the latter group were so low ([7]: Fig. 5c; [9]: Fig. 3) that *Parasalmo* and *Oncorhynchus* could not be confidently separated. Nevertheless, according to the most reliable current results obtained for sequenced mtDNA fragment of 2214 bp encoding four proteins and two tRNAs, a *Parasalmo* position on the mtDNA tree is definitely located within the *Oncorhynchus* cluster [5, 10].

The complications in reconstruction of the basal branches both at the base of the salmonid mtDNA phylogenetic tree and in its part related to the *Parasalmo–Oncorhynchus* group are probably not accidental and as we see it only to a slight extent explained by low efficiency of the approaches used. The relatively short length of the branches connecting the *a*, *c*, *d*, and *e* nodes of the tree presented in the figure suggests that the divergence of major salmonid lineages occurred in a relatively limited period of time, i.e., was an adaptive radiation [30]. This assumption is confirmed by the allozyme data [31–35] also demonstrating that these lineages are approximately equidistant. The fact that major lineages within the *Parasalmo–Oncorhynchys* group are similarly equidistant with regard to mitochondrial and allozyme distances (see references above) suggests the same mode of divergence.

Given "explosive" diversification of ancestral lineages of the modern *Salmoninae* genera, the number of synapomorphs marking the order of their divergence must be low. In addition, accumulation of recurrent mutations at potentially variable positions[2] theoretically must lead to the elimination of these few in number but phylogenetically important replacements. Apparently, under these conditions the distance-based methods of phylogenetic analyses (NJ, UPGMA, and ML) must produce a more stable clustering order than the maximum parsimony method, which was actually the case. In a similar study of mtDNA phylogeny of primates, the maximum parsimony methods ([36]: Fig. 11) yielded for genera *Homo*, *Pongo*, and *Gorilla* a variety of relationship schemes whereas different distance methods [24, 37] gave the same scheme: ((*Homo*, *Pongo*), *Gorilla*). Although this latter variant seemed at first statistically uncertain [29, 37], it was later accepted as true (see [38] and references therein). Hence, in our view, a nucleotide sequence analysis of a larger mtDNA fragment than the cytochrome b gene will better substantiate statistically the phylogeny of Salmoninae mtDNA presented in the figure.

[2] As a rule, this is the third codon position in genes for 13 proteins encoded by mtDNA (e.g., see Table 2). In salmonids these genes account for about 70% of mitochondrial genome. The distribution of mutational replacements in other genes (tRNA, 12S and 16S rRNA, the control region) in salmonids is also restricted by particular positions [5, 7, 10, 13].

In conclusion, let us consider the concordance between the phylogeny of salmonid mtDNA to the phylogeny of salmonids proper, i.e., of the carriers of the mtDNA. It is known [39] that the topologies of gene and species phylogenies do not coincide completely even when correctly reconstructed. Their expected concordance can be disrupted in case of high intraspecific mtDNA polymorphism at the divergence of populations of the ancestral species [40]. However, interspecific introgressive hybridization may lead to far more substantial discrepancy between the phylogenetic schemes of the genes and the species [41].

The number of mitochondria in a sperm of vertebrates is vanishingly small in comparison to their number in an egg. For instance, this ratio is about 1 : 10 000 000 in loach *Misgurnus* [42], 1 : 1 000 000 in amphibian *Xenopus* [43], and 1 : 10 000 in the murine genus *Mus* [44]. After the fertilization and subsequent propagation of embryonic cells these few copies of male mtDNA are usually lost for stochastic reasons. Because of this, mtDNA in vertebrates is strictly maternally inherited. Upon introgressive hybridization, when hybrid females backcrossed to the parental forms can produce relatively fertile progeny, mtDNA of one species may enter the mitochondrial gene pool of the other one. This is promoted by functional neutrality of the overwhelming majority of mutational substitutions in mtDNA sequences of related species.

Such cases of more or less extensive uni- or bidirectional mtDNA introgression are increasingly often found in detailed studies of mtDNA geographic variation in various Salmoninae species. We also have recorded a case of unidirectional mtDNA transfer from one lenok form to another and from lenok to Siberian taimen *Hucho* taimen ([11]; unpublished data).

Due to random processes, an alien mtDNA clone can be fixed and completely replace the original mtDNA cloned in a population of the recipient species [53]. If the species range in the future will be formed by immigration exactly from the area where the introgression of the alien mtDNA occurred, the original mitochondrial gene pool of the recipient species may be completely replaced. Consequently, the phylogenetic position of the recipient species inferred from mtDNA data will differ from its true position since on the phylogenetic tree it will be closer to the mtDNA donor species.

As a rule, nuclear genes far less intensively permeate the alien gene pool upon introgressive hybridization. Therefore, the simplest way to test an mtDNA phylogeny for its possible subjection to intogressive hybridization episodes is to compare it to phylogenetic schemes obtained by analysis of variation of nuclear genes.

In mtDNA phylogeny of salmonids, such comparison reveals several cases of possible interspecific mtDNA transfer with subsequent complete replacement of the original variants of mtDNA clones in the recipient species. For instance, a discrepancy between the phylogenetic positions of pink salmon *Oncorhynchus gorbuscha* Walbaum based on mtDNA ([2, 4, 5, 9, 10] and others), on the one hand, and on allozyme data [31, 32, 35, 54] and nuclear DNA sequences [55, 56], on the other, is most plausibly explained by a past mtDNA transfer from chum to pink salmon [57].

In our opinion, different positions of Pacific trout species *P. mykiss* and *P. clarkii* Richardson on mtDNA phylogenetic trees (generally these species, together with *O. kisutch* Walbaum and *O. tschawytscha* Walbaum, form a separate cluster within the cluster of Pacific salmons [2, 5, 11, 12]) and on phylogenetic trees based on variation of the nuclear DNA or its products (where they generally occupy an external position with regard to Pacific salmons [31, 32, 35, 54–56, 58] may also be explained by mtDNA transfer from an ancestral Pacific salmon species to an ancestral form of Pacific trouts.

Finally, mtDNA transfer from the members of the *S. alpinus* L.–*S.malma* Walbaum group to bull trout *S. confluentus* Suckley may account for [8, 59] similarity of these species in mtDNA [6, 8] and their significant divergence (*S. confluentus* is close to *S. leucomaenus*) in nuclear ribosomal DNA [60], allozymes [61], and karyotype [62].

Past introgressive hybridization probably explains some other principal differences between the salmonid mtDNA phylogeny and two most complete at the generic level phylogenetic schemes based on allozyme data [35] and/or intron sequence of a nuclear gene [55]. However, the latter two schemes are so different from one another that their comparison with the mtDNA phylogeny seems impossible unless this discrepancy is eliminated.

ACKNOWLEDGMENTS

This study is supported by the Russian Foundation for Basic Research, project nos. 97-04-48533, 98-04-63113, 99-04-63059, 00-04-49015.

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