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Geographic Variation and Diversity in the Mitochondrial DNA of the Medaka, *Oryzias latipes*, as Determined by Restriction Endonuclease Analysis

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ABSTRACT—Analysis of mitochondrial DNA (mtDNA) restriction fragment length polymorphism in Japanese wild populations of the medaka, *Oryzias latipes* revealed a large number of mtDNA haplotypes that form three distinct clusters (clusters A, B and C). The average nucleotide diversities among these three clusters are 8.9% (A versus B), 8.4% (A versus C), and 7.3% (B versus C). Cluster A consists of seven haplotypes and was subdivided into two subclusters. The nucleotide diversity in cluster A is low, ranging from 0.3% to 1.4% (mean 0.8%). Cluster B has 55 haplotypes and was subdivided into 11 subclusters. The nucleotide diversity in cluster B is high, ranging from 0.1 to 4.8% (mean 1.5%). Cluster C consists of only one haplotype, found in two sites of the Kanto district. The geographic distributions of mtDNA haplotypes in clusters A and B appear fully concordant with the previously described ranges of the Northern Population and the Southern Population defined by allozymes. Moreover, the distributions of mtDNA haplotypes in the subclusters show strong geographical associations. The distribution patterns of mtDNA haplotypes suggest some migration events of the medaka.

INTRODUCTION

The medaka, *Oryzias latipes*, is an egg-laying freshwater fish native to East Asia. Its small size, ease of breeding and shortness of generation time make it a useful experimental animal for genetic research. It inhabits marshes, ponds, and brooks amid rice fields in alluvial plains. As with other freshwater fish, it is thought that land is a barrier to the migration of *O. latipes*, and that local populations of this species are thus confined to their own watershed and isolated from one another. Because this species has little commercial value, it seems that its natural distribution has not been disturbed by human action (e.g., by fish breeding and discharge *etc.*).

Geographic variations in the biochemical characters of medaka have been demonstrated in the allozymes encoded in the nuclear genome (Sakaizumi *et al.*, 1983). The results of such analyses show that the Japanese wild populations of medaka are divided into two genetically different groups: the Northern Population from the northern coast of the Sea of Japan, and the Southern Population distributed in eastern, western, and southern Japan. The Southern Population is further divided into 5-7 subgroups. The genetic diversity estimated between the two populations is large enough to be considered characteristic of interspecific comparisons. However, male and female progeny from crosses between the two populations are fully fertile (Sakaizumi, 1986; Sakaizumi *et al.*, 1992).

Allozymes are coded by a different allele at a given locus in the nuclear genome. In contrast to the nuclear DNA, the rapid pace of mitochondrial DNA (mtDNA) nucleotide substitution, coupled with the special mode of maternal non-recombining inheritance, offers advantages for phylogenetic analysis. Thus, the mtDNA of various genera and species has been studied (reviewed in Avise, 1991; Meyer, 1993).

The main purpose of the present study was to survey mtDNA polymorphism in the Japanese wild population of medaka, which has large intraspecific divergences. The phylogenetic reconstructions are based on the restriction fragment length polymorphisms (RFLPs) of the mtDNA. This analysis revealed 63 mtDNA haplotypes and showed that the mtDNAs of Japanese medaka were divisible into three clusters,

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which were further divided into subclusters. The distribution of mtDNA haplotypes indicated strong geographical associations. The migration events inferred from the distribution are discussed.

MATERIALS AND METHODS

Sample collection

In the years 1979 to 1991, we collected wild specimens of *O. latipes* at 188 different sites in Japan (Fig. 1). The collection sites are listed in Table 1. One fish was analyzed at most of the sites; two were examined at the sites with an asterisk (*).

Five inbred strains were also examined. The HNI strain of the medaka was established from the wild population of Niigata. The HdrR and the HO5 strains were established from orange-red stocks. The HB32C and the HB12C strains were established from wild stocks (Hyodo-Taguchi and Egami, 1985).

DNA extraction

The head, intestines and fins were removed and discarded from adult medaka. The remaining tissue was placed in 500 μl of 100 mM EDTA, 50 mM Tris (pH 8.0), 100 mM NaCl, 1% sodium dodecyl sulfate

Table 1. Site numbers, collection site and mtDNA haplotype numbers

	Collection site	Haplotype		Collection site	Haplotype
1*.	Higakubo, Momoishi	7	50.	Takada, Mobara	46
2.	Sakaenuma, Kamikita	1	51.	Shimoiino, Futtu	15
3*.	Mena, Higashidori	1, 4	52.	Kuzuma, Kisarazu	15
4.	Koyanagi, Aomori	5	53.	Kawado-cho, Chiba	15
5.	Minori-cho, Hirosaki	1	54.	Hagiyamashinden, Sakura	21
6*.	Fukkoshi, Noshiro	1, 4	55.	Chiyoda-ku, Tokyo	17
7.	Nakadai, Ootate	1	56.	Setagayaku, Tokyo	20
8.	Teramura, Yuwa	1	57.	Kamiimai, Toyoda	19
9.	Tsutsumi, Yokote	1	58.	Ryoke, Nagano	15
10.	Deto-machi, Honjo	1	59.	Nakatsuna, Oomachi	15
11.	Kisakata, Kisakata	1	60.	Kogawa, Suwa	16
12.	Shimokawa, Tsuruoka	1	61.	Fukushima, Suwa	16
13.	Senjudo, Yamagata	1	62.	Shibusaki, Suwa	18
14.	Sanjogata, Inawashiro	1	63.	One, Kobuchizawa	16
15.	Kamo-Utashiro, Ryoutsu	1	64.	Shinden, Fijiyoshida	16
16.	Nabekata, Niigata	1	65.	Nanbara, Iida	18
17.	Yoneoka, Jyoetsu	1	66*.	Kawahara, Odawara	32
18*.	Honkaihotsu, Oshima	2, 5	67.	Odoi, Kannami	12
19.	Okisaki, Himi	1	68.	Oya, Shizuoka	12
20.	Kawashiri, Nanao	3	69.	Ninomiya, Iwata	32
21.	Yuwaku-machi, Kanazawa	3	70.	Kozakai, Kozakai	33
22.	Iburibashi-machi, Kaga	3	71.	Juroku-cho, Ogaki	20
23.	Kaminoda, Sabae	1	72.	Kose, Saori	20
24.	Maruyama, Tsuruga	1	73.	Kamaganiji, Nagashima	19
25.	Kogasaki, Obama	1	74.	Ishinden, Tsu	32
26.	Ichiba, Maizuru	1	75.	Kuzaki, Toba	47
27.	Suzu, Miyazu	1	76.	Sakazaki, Isobe	16
28.	Kotsubo, Ine	1	77.	Tategami, Ago	32
29*.	Miyajima, Toyooka	6	78.	Funatsu, Umiyama	42
30*.	Yunokawa, Hakodate	16	79.	Sano, Shingu	42
31.	Saichi-moriai, Kesennuma	16	80.	Minachi, Hongu	51
32.	Akogi, Ichinoseki	15	81.	Kujinokawa, Kushimoto	57
33*.	Gamo, Sendai	16	82.	Tanoi, Hikigawa	57
34*.	Obama, Soma	15, 16	83.	Moto-machi, Tanabe	57
35.	Taira-fujima, Iwaki	17	84.	Musata, Wakayama	55
36.	Kawawada, Mito	16	85.	Ikedashimo-cho, Izumi	63
37.	Higashi-kinokura, Naka	16	86.	Samita, Kawai	50
38.	Kashima, Urizura	15	87.	Ota-cho, Ueno	49
39.	Kamisaruuchi,Kawachi	16	88.	Kiko-cho, Ueno	55
40*.	Imai, Mibu	15, 29	89.	Kamigori, Ueno	63
41.	Sayado, Mooka	46	90.	Nodayama-cho, Hikone	55
42.	Katori, Sawara	29	91.	Oto, Kinomoto	55
43.	Kamishinshikushinden, Nagareyama	16	92.	Nakasuji, Ayabe	55
44.	Kita-ku, Tokyo	29	93.	Kaibara, Kaibara	55
45.	Hasuda, Hasuda	29	94.	Ikawadani-cho, Kobe	62
46.	Shimogosoya, Yoshimi	55	95.	Kusumoto, Higashiura	55
47.	Hinatashinden, Tatebayashi	29	96.	Torikaiura, Goshiki	52
48.	Kamiyokota, Ogawa	15	97.	Tugi, Himeji	54
49.	Harayokoji, Narutou	17	98.	Kanoharada, Himeji	55

(SDS), and 100 μg/ml of Proteinase K, then minced and incubated at 55°C overnight. The homogenate was extracted twice with buffer-equilibrated phenol, once with a 1:1 mixture of phenol:chloroform, and once with chloroform. DNA was precipitated with isopropyl alcohol, rinsed with ethanol, and resolved in TE buffer (1 mM EDTA, 10 mM Tris pH 8.0).

Isolation of mtDNA for probe

Approximately 50 grams orange-red medaka (about 200 individuals) were homogenized twice by a whirling blender for 10 sec, then the mtDNAs were prepared by the SDS-phenol method. Mitochondria and crude mtDNAs were prepared as described by Yonekawa *et al.* (1978). Mitochondrial DNAs were further purified by CsCl-ethidium bromide density-gradient centrifugation at 36,000 rpm for 40 hr. The fractions containing closed circular and open circular mtDNAs were collected separately.

Restriction analysis

The isolated closed circular mtDNA was used as a radioactive probe in Southern blotting to detect mtDNA restriction fragments obtained by restriction endonuclease digestions from total cellular DNA. Six restriction enzymes, *Sma* I, *Bgl* II, *Xba* I, *Dra* I, *Pst* I, and *Eco* RV were used for RFLPs analysis. The restriction fragments were assigned molecular weights in comparison to a size standard of lambda

Table 1. Continued

Collection site	Haplotype	Collection site	Haplotype
99. Futaomote, Ikeda	55	148. Honjo, Iwami	41
100. Tsushima, Okayama	55	149. Shimazu, Amino	34
101. Yata, Saeki	55	150. Misaka, Omiya	34
102. Koigakubo, Tessei	55	151. Shitaru, Kamiagata	23
103. Hatajiki, Miyoshi	53	152. Komoda, Izuhara	23
104. Takasugi-cho, Miyoshi	55	153. Yamada, Hisayama	48
105. Inokuchi, Kamiura	55	154. Taku, Munakata	28
106. Siwaishinden, Yoshiumi	55	155. Imai, Yukuhashi	25
107. Ato, Yasuura	53	156. Minato, Shiida	25
108. Ikushima-cho, Takamatsu	56	157. Mikekado, Buzen	25
109. Kokubi-cho, Tokushima	31	158. Kusaji, Bungotakada	25
110. Tomioka-cho, Anan	11	159. Kamegawa, Beppu	40
111. Tai, Yuki	58	160. Ikeda, Saeki	33
112. Okugawachi, Hiwasa	48	161. Mushika-cho, Nobeoka	24
113. Asakawa, Kainan	14	162. Uwae, Takanabe	36
114. Doi, Aki	32	163. Tonokori-cho, Saito	10
115. Asakura, Kochi	32	164. Shioji, Miyazaki	9
116. Nii, Tosa	32	165. Kihara, Kiyotake	10
117. Saga, Tosasaga	32	166. Takamatsu, Kushima	10
118. Gudo, Nakamura	33	167. Minamikata, Kushima	13
119. Kozukushi, Sukumo	32	168. Kawahigashi, Higashikushira	10
120. Fussaki, Misho	55	169. Kamiobaru, Kushira	10
121. Uchiko, Uchiko	55	170. Noma, Nakatane	10
122. Dogo, Matsuyama	55	171. Oasato, Kikai	60
123. Ozu, Iwakuni	43	172. Taira, Nago	60
124. Usanagi, Hirao	39	173. Odon, Gushikami	60
125. Koigahama, Kudamatsu	32	174. Sato, Sato	60
126. Daido, Hofu	36	175. Suguchiike, Sato	60
127. Kiwa, Ube	36	176. Oura, Oura	60
128. Ozuki, Kotsuki	26	177. Yamada, Hiyoshi	60
129. Yamakawa, Sanyo	30	178. Takae-cho, Sendai	60
130. Yoshida, Yamaguchi	38	179. Ichihino, Hiwaki	60
131. Uga, Toyoura	26	180. Euchi, Takaono	61
132. Kawatana, Toyoura	56	181. Kotsunagi, Minamata	61
133. Kandakami, Hohoku	45	182. Taraki, Taraki	61
134. Igami, Yuya	27	183. Tomioka, Reihoku	60
135. Higashifukagawa, Nagato	29	184. Shimomiyabaramyo, Kazusa	60
136. Tamae, Hagi	37	185. Tsunehiro, Kashima	61
137. Nago, Abu	30	186. Arita, Arita	59
138. Esaki, Tamagawa	26	187. Sakioka-cho, Sasebo	22
139. Toda, Masuda	26	188. Mukata, Fukue	22
140. Chiwa, Hamada	34	Inbred strains	
141. Tsuchi, Goutsu	44	HNI	1
142. Kawado, Sakurae	8	HO5	32
143. Nagahisa, Oota	35	HB12C	32
144. Yawata-cho, Matsue	34	HB32C	32
145. Oshinozu-cho, Yonago	34	Hd-rR	32
146. Tsuma, Tsuma	34		
147. Koyama-cho, Tottori	35		

Asterisk (*) shows the site where we have examined two individuals.

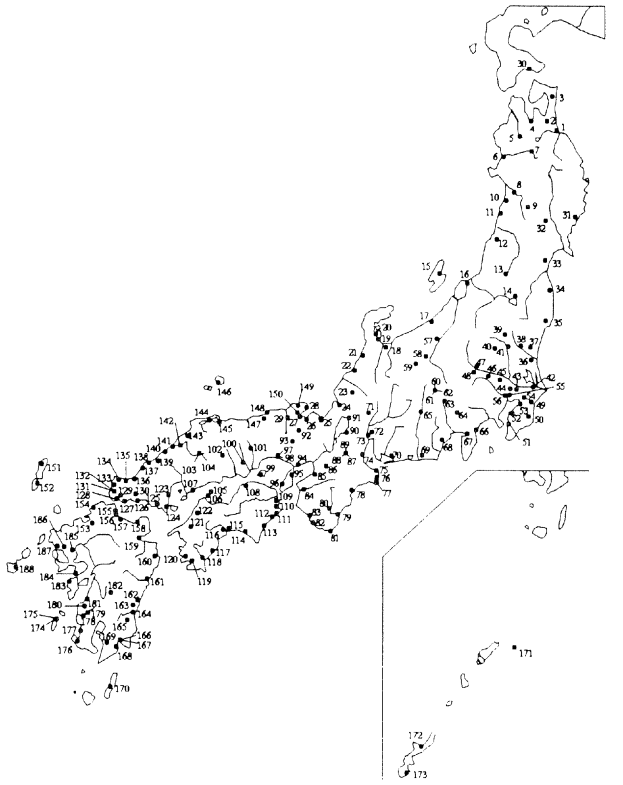

Fig. 1. Location of collection sites for medaka. The numbers refer to the locations in Table 1.

phage DNA digested with *Eco* T14I. Each distinct restriction fragment pattern produced by any of the six endonucleases was assigned an upper-case letter code in alphabetical order of the detection (e.g. A, B). Thus, each individual was finally assigned a six-letter composite mtDNA haplotype.

Analysis of data

Percentages of nucleotide sequence divergence (p value) between mtDNA haplotypes were estimated from the shared restriction fragment (Nei and Li, 1979). Relationships among mtDNA haplotypes were assessed by unweighted pair group method using arithmetic averages (UPGMA) and neighbor joining (NJ) clustering using the NEIGHBOR program, version 3.5c of PHYLIP (Felsenstein, 1993).

RESULTS

Mitochondrial DNA haplotypes of wild population of medaka

Polymorphisms in the cleavage fragment patterns of medaka mtDNA were revealed by the six restriction enzymes. The fragment patterns revealed by each enzyme are illustrated in Fig. 2. Five different fragment patterns were found for *Sma* I (A-E), six for *Bgl* II and *Xba* I (A-F), eight for *Dra* I and *Pst* I (A-H), and 10 for *Eco* RV (A-J).

Altogether, 63 mtDNA haplotypes were recognized, as presented in Table 2. Each haplotype was designated with a number representing a haplotype. Thirty haplotypes were found in unique sites. Fifteen haplotypes shared two sites. Six haplotypes shared three sites. Three haplotypes shared four sites. Nine haplotypes shared more than six sites (Table 2).

Genetic distance between mtDNA haplotypes and phylogenetic analysis

The estimated pairwise sequence divergence among haplotypes ranged from 0.1 to 12.4% (Appendix 1). A phylogenetic tree based on these sequence divergences was constructed by the UPGMA method (Fig. 3). The tree showed that the 63 mtDNA haplotypes found among the Japanese wild populations were divisible into three clusters: haplotypes #1 to #7 (cluster A), haplotype #46 (cluster C), and the other 55 haplotypes (cluster B). Cluster C was more closely related to cluster B than to cluster A. Cluster A was further separated into two subclusters (A-1 and A-2) and cluster B into 11 subclusters (B-1 to B-11). We accepted a depth of 0.48% as a standard of subclusters, except for subcluster B-11. In subcluster B-11, it was clear that haplotype #22 was closely related to haplotypes #59, #60, and #61.

The estimated nucleotide sequence divergence among clusters ranged from 5.5% to 11.4%. Between clusters A and B, the range (mean ± standard deviation) was 7.0%-11.4% (8.9 ± 0.87%); between clusters A and C, 8.1%-8.6% (8.4 ± 0.18%); and between clusters B and C, 5.5%-11.2% (7.3 ± 1.14%).

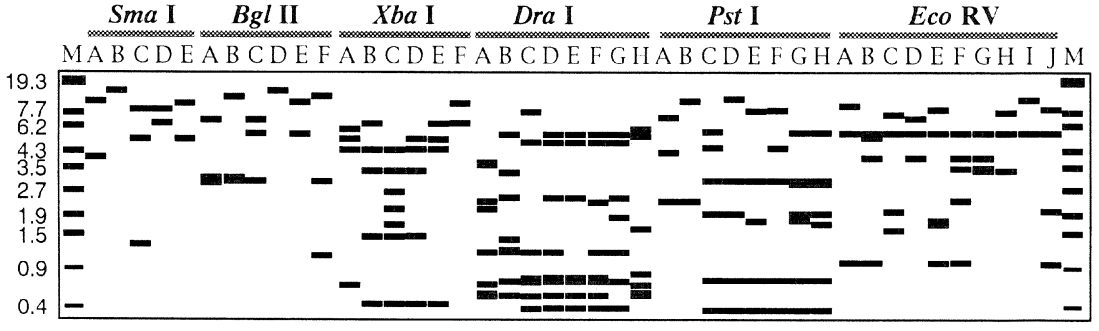

Fig. 2. Diagram of the restriction patterns revealed by each of six restriction enzymes. Restriction fragment length polymorphisms of medaka mtDNAs were digested with six endonucleases. M shows DNA size marker, lambda phage DNA digested with *Eco* T14I. The numbers indicate fragment length (kb).

Table 2. Mitochondrial DNA haplotypes, fragment patterns (indicated by six letters), number of sites in which the haplotype was found, and number of individuals detected. Enzyme order: *Sma* I, *Bgl* II, *Xba* I, *Dra* I, *Pst* I, *Eco* RV.

Haplotype	Fragment patterns	No. of sites	No. of individuals
#1	AAAAAA	22	22
#2	AAAABA	1	1
#3	ABAABA	3	3
#4	ACAAAA	2	2
#5	BAAAAA	2	2
#6	BBAABA	1	2
#7	BCAAAA	1	2
#8	BDBDCF	1	1
#9	BDBDFB	1	1
#10	BDBDFD	6	6
#11	BDBDGD	1	1
#12	BDDDCD	2	2
#13	BDEDFD	1	1
#14	BFBGGB	1	1
#15	CDBBCB	10	10
#16	CDBBDB	13	15
#17	CDBBDD	3	3
#18	CDBCDB	2	2
#19	CDBDCB	2	2
#20	CDBDCD	3	3
#21	CDBDDD	1	1
#22	CDEDFJ	2	2
#23	DDBBCD	2	2
#24	DDBBFB	1	1
#25	DDBCCA	4	4
#26	DDBCCB	4	4
#27	DDBCCE	1	1
#28	DDBCFA	1	1
#29	DDBCFB	6	6
#30	DDBCFD	2	2
#31	DDBCGB	1	1
#32	DDBDCB	10	11
#33	DDBDCD	3	3
#34	DDBDCF	6	6
#35	DDBDCG	2	2
#36	DDBDFB	3	3
#37	DDBDFD	1	1
#38	DDBDGB	1	1
#39	DDBECD	1	1
#40	DDBEGD	1	1
#41	DDBGCF	1	1
#42	DDCDCB	2	2
#43	DDEECD	1	1
#44	DDEDCF	1	1
#45	DDECCB	1	1
#46	DDFHIC	2	2
#47	DEBDCB	1	1
#48	EDBBGD	2	2
#49	EDBCGB	1	1
#50	EDBDCB	1	1
#51	EDBDCD	1	1
#52	EDBDEB	1	1
#53	EDBDFB	2	2
#54	EDBDGA	1	1
#55	EDBDGB	19	19
#56	EDBDGD	2	2
#57	EDBDHB	3	3
#58	EDBFGD	1	1
#59	EDEBFI	1	1
#60	EDEDFH	11	11
#61	EDEDFI	4	4
#62	EDEDGB	1	1
#63	EEBDGB	2	2

The intra-cluster divergence in cluster B is two times as large as that in cluster A (Fig. 3). Specifically, the estimated pairwise sequence divergence in cluster A and cluster B ranged from 0.3 to 1.5% (mean: 0.8 ± 0.37%) and from 0.1 to 5.0% (mean: 1.5 ± 0.66%), respectively.

We constructed an NJ tree using the same data set. The results also demonstrated that the mtDNA haplotypes were divisible into three clusters and that clusters A and B could be subdivided into two and ten subclusters, respectively. The patterns of clustering revealed by the UPGMA and NJ methods were essentially identical.

Geographic distributions of the mtDNA haplotypes

Figure 4 shows the geographic distribution of the mtDNA haplotypes of each subcluster. The data analysis revealed strong geographical associations for the mtDNAs of clusters and subclusters. The features of the haplotypes of each (sub) cluster are as follows.

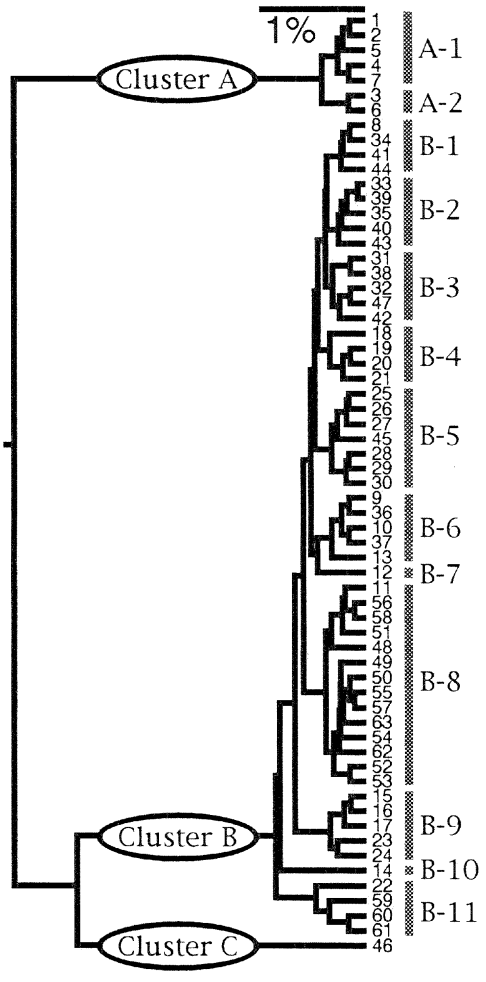

Fig. 3. Phylogenetic tree. The UPGMA phenogram of 63 mtDNA haplotypes found among Japanese medaka. The phenogram was derived from a matrix of percentage nucleotide sequence divergence estimates based on the restriction fragment length polymorphisms among the haplotypes (Appendix 1).

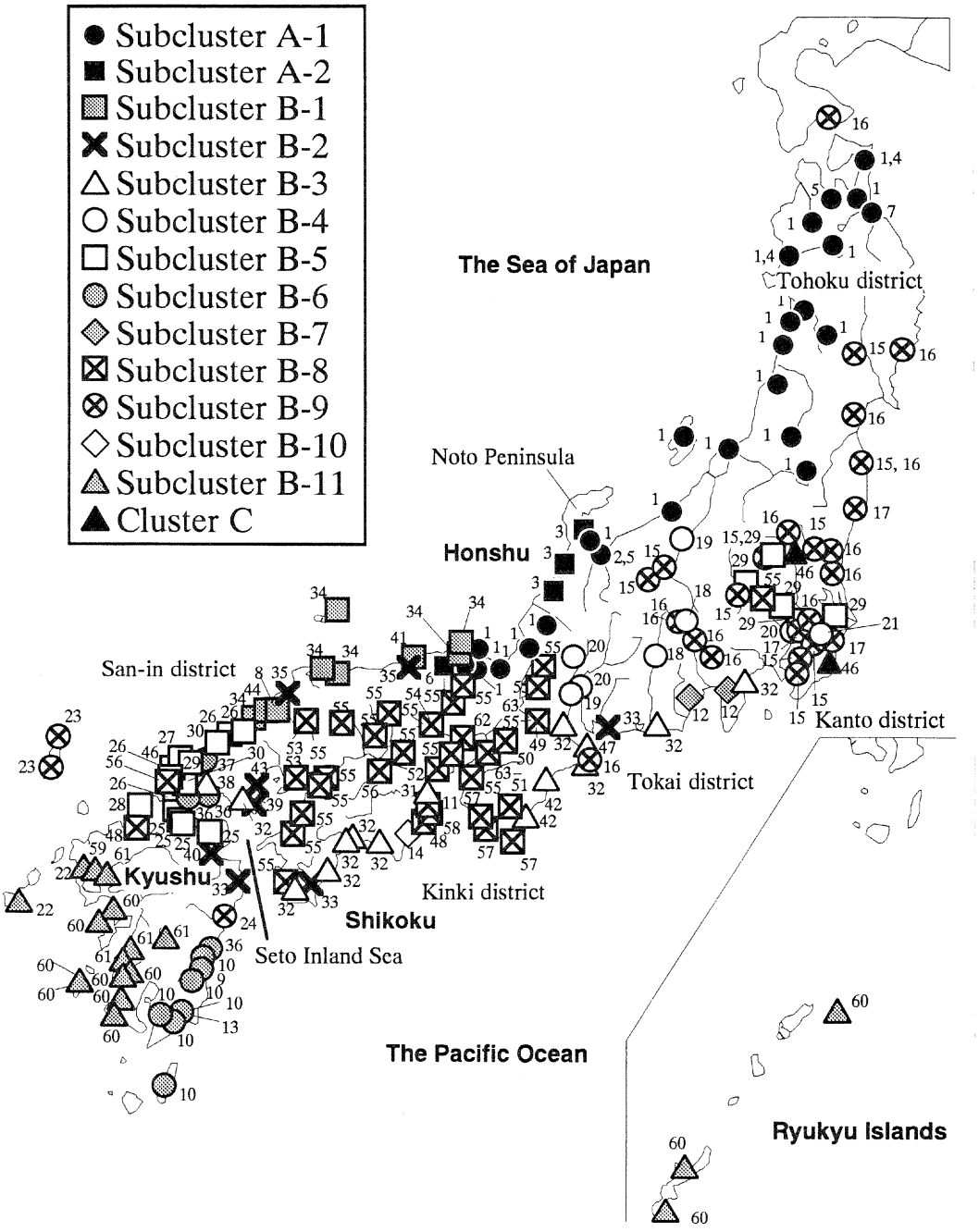

Fig. 4. Geographic distribution of mtDNA haplotypes. Geographical distribution of mtDNA haplotypes of clusters and subclusters inferred from the UPGMA analysis. Numbers indicate the haplotype numbers. One symbol represents one fish, except for the ten sites which are marked with an asterisk (*) in Table 1.

Cluster A was separated into two subclusters (A-1 and A-2). The mtDNA haplotypes of cluster A were distributed in northern Japan along the Sea of Japan. The mtDNAs of subcluster A-1 had a large distribution area. Haplotype #1 in particular had a large distribution area. The mtDNAs of subcluster A-2 were found only in three sites at the western part of the Noto Peninsula.

Cluster B was divided into 11 subclusters (B-1 to B-11). The mtDNA haplotypes of cluster B were found in southern Japan along the Pacific coast southward from Iwate Prefecture and along the Sea of Japan westward from Kyoto Prefecture. The mtDNAs of subcluster B-1 were found only in the San-in district. Most of the mtDNAs of subcluster B-2 were found mainly in the western region of the Seto Inland Sea. The mtDNAs of subcluster B-3 were found at the Pacific coast. The mtDNAs of subcluster B-4 were found in the central part

of Honshu island, and also in the Kanto district. The mtDNAs of subcluster B-5 were mainly distributed at the western edge of Honshu. However, five specimens from the Kanto district also had mtDNAs of subcluster B-5. All five of the haplotypes found in the Kanto district were haplotype #29. The mtDNAs of subcluster B-6 were distributed in the southeastern area of Kyushu and at the western edge of Honshu. The mtDNA haplotype of subcluster B-7 was a minor haplotype in this study. Two specimens from two sites (No. 67 and 68) showed haplotype #12. The mtDNAs of subcluster B-8 had a large distribution area and a large number of haplotypes. The haplotypes were found mainly in the eastern region of the Seto Inland Sea district. In addition, the haplotypes were found in the Kanto district (haplotype #55) and at the western edge of Honshu (#48 and #56). The mtDNAs of subcluster B-9 had two distribution areas. The two areas were a range from the Kanto district to the Tohoku district and northern Kyushu. The mtDNAs found in the two areas were different. Haplotypes #15, #16, and #17 were found in the Kanto-Tohoku district. Haplotypes #23 and #24 were found in northern Kyushu. The mtDNA haplotype of subcluster B-10 was a minor haplotype, haplotype #14 from one site, Kainan (No. 109). The mtDNAs of subcluster B-11 were found in the western area of Kyushu. The medaka from the Ryukyu Islands also had the mtDNA of the subcluster B-11. We found only haplotype #60 in the southern region of the distribution range.

Cluster C had only one haplotype, #46. The mtDNA of two specimens found at two sites in the Kanto district showed haplotype #46.

Haplotypes of the inbred strains of medaka

In this study, five inbred strains were analyzed. The results showed that all inbred strains except for the HNI strain shared the same haplotype, #32. The HNI strain was haplotype #1. This result coincides with the mtDNA haplotype of the wild fish captured at Niigata (Site No. 16).

DISCUSSION

Marked intraspecific diversity among mtDNAs

Intraspecific mtDNA divergences of 7.4% in anchovy (Magoulas *et al.*, 1996) and 8.5% in sunfish (Avise *et al.*, 1984) have been reported as the highest degrees of intraspecific divergences. In the medaka, the pairwise comparisons between haplotypes of clusters A, B and C demonstrated large divergence; 7.3%-12.4%. In particular, the mean sequence divergence between clusters A and B is 9.0%. The large divergence among these three clusters suggests that the events separating these clusters are very old.

The rate of base substitution of mammalian mtDNA has been estimated to be 2.0% per million years (Brown *et al.*, 1979). At this rate, cluster A and cluster B would have shared a common ancestor 4-5 million years ago (sequence divergence estimated approximately 8-10%).

Concordance between mtDNA haplotypes and allozyme genotypes in the medaka

Allozymic variations have been studied at 21 loci in Japanese wild populations of the medaka collected at 53 sites, and the Japanese wild population of medaka was divided into two genetically distinct groups, the Northern Population and the Southern Population. Allozymic analyses demonstrated that the Northern Population is less variable than the Southern Population (Sakaizumi *et al.*, 1983).

The rigid isolation between the mtDNAs of cluster A and those of cluster B coincides perfectly with the previously defined two population ranges— A is associated with the Northern Population and B with the Southern Population. The characteristics of cluster A's mtDNAs also show lower intra-cluster polymorphism than do cluster B's mtDNAs (Fig. 3). Such concordance between mtDNA haplotypes and allozyme genotypes would be expected if the Northern Population and the Southern Population had evolved in complete isolation in the past.

Mitochondrial DNA haplotype of cluster C

Haplotype #46 in cluster C is a rare variant in the Japanese wild population of medaka. This cluster is more closely related to cluster B than to cluster A (mean estimated divergence 7.7 versus 8.9), but divergent from cluster B (pairwise divergence ranged from 5.8% to 12.1%). The fish with this mtDNA haplotype have an allozymic genotype similar to that of the fish with haplotypes of cluster B (the Southern Population type; data not shown), despite the such large sequence divergence in mtDNA estimated. Thus, we suspect that the "old" mtDNA haplotypes which diverged in the ancestor of the medaka may have persisted in a limited area (the Kanto district). Examinations of the frequency and distribution, and detailed molecular analysis are in progress to elucidate the origin and dispersal of this haplotype.

Migration of the Japanese wild population of medaka

In the present study, we divided the mtDNA haplotypes in Japanese medaka into three clusters, and clusters A and B were subdivided into two and 11 subclusters, respectively. The distribution pattern of haplotypes of each subcluster showed a strong geographical association and unique distribution pattern (Fig. 4). The distribution pattern presumably reflects the migration history of Japanese wild medaka. We are especially mindful of the distributions of single haplotypes and those of haplotypes in a subcluster. We can thus propose three migration scenarios from the distribution patterns of mtDNA haplotypes. These scenarios are "to the Tohoku district", and "to the Ryukyu Islands", and "to the Kanto district". These scenarios are based on a hypothesis that migration occurred from a region where we found high variation in mtDNAs to a region with low variation in mtDNAs.

All specimens found in the Tohoku district at the sea of Japan coast had mtDNA of subcluster A-1, those at the Pacific coast had mtDNA of subcluster B-9. In the southern regions of the Tohoku district, we found mtDNAs of more than one

subcluster. This observation leads to a scenario in which the medaka in the southern region of the Tohoku district have recently expanded their range into the northern region.

We also propose a scenario from the distribution pattern of mtDNAs of the subcluster B-11 in western Kyushu and the Ryukyu Islands. Subcluster B-11 consists of four haplotypes (Fig. 3). Haplotype #60, of these four haplotypes, is found in the southern part of western Kyushu and the Ryukyu Islands. The other three haplotypes were found in the northern part of western Kyushu. This observation leads to a scenario in which the medaka in the southern part of the region have recently expanded their range into the Ryukyu Islands.

In the Kanto district, two features were notable; the high frequency of the subcluster B-9 mtDNA haplotypes, and a small number of haplotypes from each other subcluster of cluster B. Regarding the first feature, we found that mtDNA haplotypes

Appendix 1. Percentage of pairwise sequence divergence among the 63 composite mtDNA haplotypes (*p* values).
Estimates were derived from a matrix of shared restriction fragments and the numbered mtDNA haplotypes are defined in Table 1.

Haplotype	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	#13	#14	#15	#16	#17	#18	#19	#20	#21	#22	#23	#24	#25	#26	#27	#28	#29	#30	#31
#2	0.3																														
#3	0.8	0.5																													
#4	0.3	0.7	1.0																												
#5	0.3	0.7	1.3	0.7																											
#6	1.3	0.9	0.4	1.4	0.9																										
#7	0.7	1.1	1.4	0.3	0.3	1.0																									
#8	9.0	8.8	8.6	8.8	7.9	7.5	7.8																								
#9	8.7	8.6	8.3	8.6	7.6	7.2	7.5	0.7																							
#10	9.8	9.6	9.4	9.6	8.5	8.1	8.3	0.9	0.4																						
#11	10.0	9.9	9.6	9.9	8.7	8.3	8.6	0.8	1.1	0.8																					
#12	10.0	9.9	9.6	9.9	8.7	8.3	8.6	0.8	1.1	0.8	0.7																				
#13	8.5	8.3	8.1	8.3	7.4	7.0	7.2	1.3	0.8	0.4	1.2	1.2																			
#14	10.2	10.1	9.9	10.1	9.0	8.6	8.8	1.6	1.7	2.2	1.2	2.1	2.8																		
#15	9.2	9.1	8.8	9.1	9.1	8.7	9.0	1.4	1.5	2.0	1.9	1.9	2.6	2.4																	
#16	9.1	9.0	8.7	9.0	9.0	8.6	8.8	1.9	1.7	2.2	2.1	2.4	2.8	2.7	0.3																
#17	10.1	10.0	9.8	10.0	10.0	9.6	9.9	2.1	2.2	1.8	1.7	2.0	2.3	3.3	0.7	0.3															
#18	8.8	8.7	8.5	8.7	8.7	8.3	8.6	1.7	1.4	2.0	1.9	2.2	2.6	2.4	1.3	0.9	1.4														
#19	9.1	9.0	8.7	9.0	9.0	8.6	8.8	0.8	0.8	1.3	1.2	1.2	1.8	1.7	0.5	0.9	1.3	0.7													
#20	10.1	10.0	9.8	10.0	10.0	9.6	9.9	0.9	1.3	0.9	0.8	0.8	1.3	2.2	0.9	1.3	0.9	1.1	0.3												
#21	10.0	9.9	9.6	9.9	9.9	9.5	9.8	1.4	1.4	1.0	1.0	1.3	1.5	2.4	1.3	0.9	0.6	0.7	0.7	0.3											
#22	7.9	7.8	7.5	7.8	7.8	7.4	7.6	2.0	1.4	1.6	2.6	2.6	1.2	3.6	1.9	2.1	2.3	1.9	1.2	1.4	1.6										
#23	10.1	10.0	9.8	10.0	10.0	9.6	9.9	1.5	1.9	1.4	1.4	1.4	2.0	2.9	0.7	1.0	0.7	2.3	1.3	0.9	1.4	2.7									
#24	9.0	8.8	8.6	8.8	8.8	8.5	8.7	1.8	1.0	1.4	2.3	2.3	2.0	2.9	0.7	0.8	1.2	2.0	1.3	1.8	2.0	2.0	0.7								
#25	7.8	7.6	7.4	7.6	7.6	7.2	7.5	1.3	1.6	1.8	1.8	1.8	2.5	2.7	1.8	2.3	2.6	1.1	1.1	1.3	1.8	2.1	1.6	1.9							
#26	8.8	8.7	8.5	8.7	8.7	8.3	8.6	1.1	1.1	1.6	1.6	1.6	2.2	2.1	1.3	1.8	2.3	0.7	0.7	1.1	1.6	2.2	1.4	1.4	0.4						
#27	8.8	8.7	8.5	8.7	8.7	8.3	8.6	1.4	1.8	2.0	1.9	1.9	2.6	2.8	1.9	2.4	2.7	1.3	1.2	1.4	1.9	2.2	1.7	2.0	0.4	0.5					
#28	7.6	7.5	7.2	7.5	7.5	7.1	7.4	1.8	1.2	1.4	2.3	2.3	1.9	3.4	2.3	2.6	2.8	1.3	1.6	1.8	2.0	1.6	2.1	1.4	0.4	0.8	0.8				
#29	8.7	8.6	8.3	8.6	8.6	8.2	8.5	1.6	0.8	1.2	2.1	2.1	1.7	2.7	1.8	2.0	2.6	0.9	1.1	1.6	1.8	1.8	1.9	1.0	0.8	0.4	0.9	0.4			
#30	9.8	9.6	9.4	9.6	9.6	9.2	9.5	1.8	1.2	0.8	1.6	1.6	1.3	3.4	2.3	2.6	2.1	1.3	1.6	1.1	1.3	2.0	1.4	1.4	0.9	0.8	1.0	0.5	0.4		
#31	9.0	8.8	8.6	8.8	8.8	8.5	8.7	1.5	1.6	2.1	1.1	2.0	2.7	1.6	1.7	1.9	2.4	0.8	1.0	1.5	1.7	2.7	1.8	1.8	0.7	0.3	0.8	1.1	0.7	1.1	
#32	9.0	8.8	8.6	8.8	8.8	8.5	8.7	0.7	0.7	1.1	1.1	1.1	1.6	1.6	0.9	1.3	1.8	1.1	0.3	0.7	1.1	1.7	0.9	0.9	0.7	0.3	0.8	1.1	0.7	1.1	0.7
#33	10.0	9.9	9.6	9.9	9.9	9.5	9.8	0.8	1.1	0.8	0.7	0.7	1.2	2.1	1.3	1.8	1.4	1.6	0.7	0.3	0.7	1.9	0.6	1.4	0.9	0.7	1.0	1.3	1.1	0.8	1.1
#34	9.1	9.0	8.7	9.0	9.0	8.6	8.8	0.3	1.1	1.3	1.2	1.2	1.8	2.0	1.3	1.7	1.9	1.5	0.7	0.8	1.2	1.8	1.0	1.3	0.8	0.7	0.9	1.3	1.1	1.3	1.0
#35	10.1	10.0	9.8	10.0	10.0	9.6	9.9	0.7	1.3	1.1	1.1	1.1	1.6	2.2	1.4	1.9	1.8	1.7	0.8	0.7	1.1	2.0	0.9	1.5	1.0	0.8	1.1	1.4	1.3	1.1	1.2
#36	8.8	8.7	8.5	8.7	8.7	8.3	8.6	1.1	0.4	0.8	1.6	1.6	1.2	2.1	1.3	1.5	2.0	1.3	0.7	1.1	1.3	1.3	1.4	0.6	1.1	0.7	1.3	0.8	0.4	0.8	1.1
#37	9.9	9.8	9.5	9.8	9.8	9.4	9.6	1.3	0.8	0.4	1.1	1.1	0.8	2.7	1.8	2.0	1.6	1.8	1.1	0.7	0.9	1.4	1.0	1.0	1.3	1.1	1.4	0.9	0.8	0.4	1.6
#38	9.1	9.0	8.7	9.0	9.0	8.6	8.8	1.0	1.1	1.6	0.7	1.5	2.1	1.2	1.3	1.4	1.9	1.2	0.7	1.0	1.2	2.1	1.3	1.3	1.1	0.7	1.2	1.6	1.1	1.6	0.3
#39	11.3	11.2	10.9	11.2	11.2	10.8	11.0	1.0	1.3	0.9	0.9	0.9	1.4	2.3	1.5	2.0	1.6	1.8	0.8	0.5	0.9	2.1	0.7	1.6	1.0	0.9	1.1	1.5	1.3	0.9	1.3
#40	11.4	11.3	11.0	11.3	11.3	10.9	11.2	1.4	1.8	1.3	0.5	1.3	1.8	1.8	1.9	2.1	1.7	1.9	1.2	0.8	1.0	2.6	1.1	2.0	1.4	1.3	1.6	2.0	1.8	1.3	0.8
#41	10.1	10.0	9.8	10.0	10.0	9.6	9.9	0.7	1.6	1.8	1.7	1.7	2.3	1.6	1.7	2.2	2.4	2.0	1.0	1.2	1.7	2.3	1.5	1.8	1.3	1.1	1.4	1.8	1.6	1.8	1.5
#42	10.1	10.0	9.8	10.0	10.0	9.6	9.9	1.2	1.2	1.7	1.6	1.3	2.2	2.1	1.4	1.8	2.3	1.6	0.8	1.2	1.6	2.2	1.4	1.4	1.2	0.8	1.3	1.7	1.2	1.7	1.2
#43	9.8	9.6	9.4	9.6	9.6	9.2	9.5	1.4	1.8	1.4	1.3	1.3	1.0	2.9	2.0	2.6	2.1	2.3	1.3	0.9	1.3	1.6	1.1	2.1	1.5	1.3	1.6	2.1	1.8	1.4	1.8
#44	8.0	7.9	7.6	7.9	7.9	7.5	7.8	0.7	1.6	1.8	1.7	1.7	1.3	2.5	1.7	2.2	2.4	2.0	1.0	1.2	1.7	1.4	1.5	1.8	1.3	1.1	1.4	1.8	1.6	1.8	1.5
#45	7.8	7.6	7.4	7.6	7.6	7.2	7.5	1.6	1.6	2.2	2.1	2.1	1.7	2.7	1.8	2.3	2.9	1.1	1.1	1.6	2.1	1.8	1.9	1.9	0.8	0.4	0.9	1.2	0.8	1.2	0.7
#46	8.6	8.5	8.2	8.5	8.5	8.1	8.3	8.5	7.2	7.1	8.3	9.5	7.0	11.2	7.8	7.6	7.5	7.4	7.6	7.5	7.4	5.8	6.7	5.9	6.4	6.5	6.5	5.5	5.7	5.5	6.7
#47	9.1	9.0	8.7	9.0	9.0	8.6	8.8	1.0	1.1	1.6	1.5	1.5	2.1	1.7	1.3	1.7	2.2	1.5	0.7	1.0	1.5	2.1	1.3	1.3	1.1	0.7	1.2	1.6	1.1	1.6	1.0
#48	10.2	10.1	9.9	10.1	10.1	9.8	10.0	1.9	2.3	1.9	0.9	1.8	2.4	2.3	1.0	1.2	0.8	2.4	1.7	1.3	1.5	3.2	0.8	1.6	2.7	2.4	2.8	3.4	3.1	2.6	1.9
#49	9.0	8.8	8.6	8.8	8.8	8.5	8.7	1.5	1.6	2.1	1.1	2.0	2.7	1.6	1.7	1.9	2.4	0.8	1.0	1.5	1.7	2.7	2.4	2.4	1.3	0.8	1.4	1.8	1.3	1.8	0.4
#50	9.0	8.8	8.6	8.8	8.8	8.5	8.7	0.7	0.7	1.1	1.1	1.1	1.6	1.6	0.9	1.3	1.8	1.1	0.3	0.7	1.1	1.7	1.5	1.5	1.3	0.8	1.4	1.8	1.3	1.8	1.2
#51	10.0	9.9	9.6	9.9	9.9	9.5	9.8	0.8	1.1	0.8	0.7	0.7	1.2	2.1	1.3	1.8	1.4	1.6	0.7	0.3	0.7	1.9	1.1	2.0	1.4	1.3	1.6	2.0	1.8	1.3	1.7
#52	9.0	8.8	8.6	8.8	8.8	8.5	8.7	1.5	0.7	1.1	1.1	2.0	1.6	1.6	1.7	1.6	2.1	1.4	1.0	1.5	1.4	1.7	2.4	1.5	2.2	1.7	2.3	1.8	1.3	1.8	1.2
#53	8.8	8.7	8.5	8.7	8.7	8.3	8.6	1.1	0.4	0.8	1.6	1.6	1.2	2.1	1.3	1.5	2.0	1.3	0.7	1.1	1.3	1.3	2.0	1.1	1.8	1.3	1.9	1.3	0.9	1.3	1.7
#54	8.0	7.9	7.6	7.9	7.9	7.5	7.8	1.2	1.6	1.8	0.8	1.7	2.3	1.6	1.7	1.9	2.1	1.7	1.0	1.2	1.4	2.0	2.1	2.4	1.3	1.7	1.7	1.8	2.2	2.4	1.2
#55	9.1	9.0	8.7	9.0	9.0	8.6	8.8	1.0	1.1	1.6	0.7	1.5	2.1	1.2	1.3	1.4	1.9	1.2	0.7	1.0	1.2	2.1	1.9	1.9	1.7	1.2	1.8	2.2	1.7	2.2	0.8
#56	10.1	10.0	9.8	10.0	10.0	9.6	9.9	1.2	1.6	1.1	0.3	1.1	1.6	1.6	1.7	1.9	1.5	1.7	1.0	0.7	0.8	2.3	1.5	2.4	1.9	1.7	2.0	2.4	2.2	1.8	1.2
#57	9.1	9.0	8.7	9.0	9.0	8.6	8.8	1.0	1.1	1.6	0.9	1.5	2.1	1.4	1.3	1.4	1.9	1.2	0.7	1.0	1.2	2.1	1.9	1.9	1.7	1.2	1.8	2.2	1.7	2.2	1.0
#58	9.0	8.8	8.6	8.8	8.8	8.5	8.7	1.5	1.9	1.4	0.6	1.4	2.0	1.9	2.0	2.2	1.8	1.7	1.3	0.9	1.1	2.7	1.8	2.8	1.9	1.7	2.0	2.4	2.2	1.8	1.2
#59	8.6	8.5	8.2	8.5	8.5	8.1	8.3	2.8	2.2	2.1	3.1	3.1	1.6	4.8	1.7	1.9	1.8	3.6	2.6	2.4	2.7	1.6	1.8	1.4	3.4	3.6	3.6	2.9	3.0	2.9	4.2
#60	8.6	8.5	8.2	8.5	8.5	8.1	8.3	2.1	1.5	1.4	2.3	2.3	1.0	3.8	2.7	2.9	2.8	2.7	1.9	1.8	2.0	1.0	2.8	2.4	2.6	2.7	2.7	2.1	2.2	2.1	3.2
#61	8.5	8.3	8.1	8.3	8.3	7.9	8.2	2.0	1.4	1.3	2.2	2.2	0.8	3.7	2.6	2.8	2.7	2.6	1.8	1.6	1.8	0.9	2.7	2.3	2.5	2.6	2.6	1.9	2.1	1.9	3.1
#62	8.0	7.9	7.6	7.9	7.9	7.5	7.8	1.5	1.6	2.1	1.1	2.0	1.6	1.6	1.7	1.9	2.4	1.7	1.0	1.5	1.7	1.7	2.4	2.4	2.2	1.7	2.3	2.8	2.2	2.8	1.2
#63	9.2	9.1	8.8	9.1	9.1	8.7	9.0	1.4	1.5	2.0	1.0	1.9	2.6	1.3	1.6	1.8	2.3	1.6	1.0	1.4	1.6	2.5	2.3	2.3	2.1	1.6	2.2	2.7	2.1	2.7	1.2
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	#13	#14	#15	#16	#17	#18	#19	#20	#21	#22	#23	#24	#25	#26	#27	#28	#29	#30	#31

of subcluster B-9 were common in the Kanto district, and that the mtDNA haplotypes had split distribution ranges, i.e., the Kanto to the Tohoku district and northern Kyushu (Site No. 151, 152, and 161). In addition, there were no haplotypes common to both ranges; haplotypes #15, #16, and #17 in the Kanto and Tohoku district, and haplotypes #23 and #24 in northern Kyushu. This distribution pattern of the subcluster B-9 mtDNAs suggest that it was long ago when the medaka with the haplotype #15, #16, or #17 colonized the Kanto district. Regarding the second feature, we found five other mtDNA haplotypes in the Kanto district (haplotypes #20, #21, #29, #46, and #55). These five mtDNAs are classifiable into four (sub) clusters (subclusters B-4, B-5, and B-8, and cluster C). The four haplotypes of the three subclusters were also found in western Japan. Haplotypes #20 and #21 were found in the Tokai district, haplotype #29 at the western edge of Honshu,

#32	#33	#34	#35	#36	#37	#38	#39	#40	#41	#42	#43	#44	#45	#46	#47	#48	#49	#50	#51	#52	#53	#54	#55	#56	#57	#58	#59	#60	#61	#62
0.3																														
0.3	0.4																													
0.4	0.3	0.3																												
0.3	0.7	0.7	0.8																											
0.7	0.4	0.8	0.7	0.4																										
0.3	0.7	0.7	0.8	0.7	1.1																									
0.5	0.1	0.6	0.5	0.9	0.5	0.8																								
0.8	0.5	0.9	0.8	1.3	0.9	0.4	0.4																							
0.7	0.8	0.3	0.7	1.1	1.3	1.0	1.0	1.4																						
0.4	0.8	0.8	0.9	0.8	1.2	0.8	0.9	1.3	1.2																					
0.9	0.5	1.0	0.9	1.3	0.9	1.3	0.4	0.8	1.4	1.4																				
0.7	0.8	0.3	0.7	1.1	1.3	1.0	1.0	1.4	0.7	1.2	0.6																			
0.7	1.1	1.1	1.3	1.1	1.6	1.1	1.3	1.8	1.6	1.2	0.9	0.7																		
6.7	6.5	6.8	6.7	5.8	5.7	6.8	6.4	6.5	7.5	7.8	6.3	6.7	6.4																	
0.3	0.7	0.7	0.8	0.7	1.1	0.7	0.8	1.2	1.0	0.8	1.3	1.0	1.1	7.6																
1.9	1.5	2.0	1.9	2.4	2.0	1.4	1.7	1.2	2.5	2.4	2.2	2.5	3.1	8.6	2.3															
1.2	1.7	1.6	1.8	1.7	2.2	0.8	1.9	1.4	2.1	1.7	2.4	2.1	1.3	8.5	1.6	1.3														
0.4	0.8	0.8	0.9	0.8	1.3	0.8	1.0	1.4	1.2	0.9	1.4	1.2	1.3	8.5	0.8	1.3	0.7													
0.8	0.5	0.9	0.8	1.3	0.9	1.2	0.6	1.0	1.4	1.3	1.0	1.4	1.8	8.3	1.2	0.9	1.1	0.3												
1.2	1.7	1.6	1.8	0.8	1.3	0.8	1.9	1.4	2.1	1.7	2.4	2.1	2.2	7.5	1.6	1.3	0.7	0.7	1.1											
0.8	1.3	1.2	1.4	0.5	0.9	1.2	1.4	1.9	1.7	1.3	2.0	1.7	1.8	7.4	1.2	1.8	1.1	0.3	0.7	0.3										
1.2	1.4	1.3	1.5	1.7	1.9	0.8	1.6	1.1	1.8	1.7	2.1	1.8	2.2	8.5	1.6	1.0	0.7	0.7	0.8	0.7	1.1									
0.8	1.2	1.2	1.3	1.2	1.7	0.4	1.4	0.9	1.6	1.3	1.9	1.6	1.7	8.6	1.2	0.9	0.3	0.3	0.7	0.3	0.7	0.3								
1.2	0.8	1.3	1.2	1.7	1.3	0.8	1.0	0.6	1.8	1.7	1.4	1.8	2.2	8.5	1.6	0.6	0.7	0.7	0.3	0.7	1.1	0.4	0.3							
0.8	1.2	1.2	1.3	1.2	1.7	0.7	1.4	1.2	1.6	1.3	1.9	1.6	1.7	8.6	1.2	1.2	0.6	0.3	0.7	0.6	0.7	0.6	0.2	0.6						
1.5	1.1	1.6	1.5	2.0	1.6	1.0	1.3	0.8	2.1	2.0	1.8	2.1	2.2	8.5	1.9	0.8	0.7	0.9	0.6	0.9	1.4	0.7	0.6	0.2	0.8					
2.8	2.7	2.9	2.8	2.3	2.2	3.4	3.0	3.6	3.7	3.5	2.5	2.4	3.0	7.1	3.4	1.6	3.2	2.1	2.0	2.1	1.6	2.4	2.6	2.4	2.6	2.8				
2.1	2.0	2.2	1.8	1.6	1.5	2.6	2.2	2.7	2.8	2.7	1.7	1.8	2.2	7.1	2.6	2.6	2.4	1.4	1.3	1.4	1.0	1.8	1.9	1.8	1.9	2.1	1.1			
2.0	1.8	2.1	2.0	1.5	1.4	2.4	2.1	2.6	2.7	2.6	1.6	1.6	2.1	7.0	2.4	2.4	2.3	1.3	1.2	1.3	0.9	1.6	1.8	1.6	1.8	2.0	0.7	0.4		
1.2	1.7	1.6	1.8	1.7	2.2	0.8	1.9	1.4	2.1	1.7	1.4	1.2	1.3	8.5	1.6	1.3	0.7	0.7	1.1	0.7	1.1	0.7	0.3	0.7	0.6	0.9	2.1	1.4	1.3	
1.2	1.6	1.5	1.7	1.6	2.1	0.8	1.8	1.3	2.0	1.6	2.3	2.0	2.1	9.9	0.8	1.3	0.7	0.7	1.0	0.7	1.0	0.7	0.3	0.7	0.5	0.9	3.1	2.3	2.2	0.7
#32	#33	#34	#35	#36	#37	#38	#39	#40	#41	#42	#43	#44	#45	#46	#47	#48	#49	#50	#51	#52	#53	#54	#55	#56	#57	#58	#59	#60	#61	#62

and haplotype #55 in the Kinki district. The sole haplotype of the cluster C was found only in the Kanto district. Thus, haplotypes other than those of subcluster B-9 and cluster C were also found west of the Kanto district. Therefore, we suspect that the low variation of haplotypes in the four subclusters is the result of a recent expansion eastward (to the Kanto district). These two features lead to a scenario in which at least two migration events occurred in the Kanto district; first, medaka with mtDNAs of subcluster B-9 expanded their range into the Kanto district. Then, other haplotypes diverged in western Japan, and introgressed to the Kanto district recently. Consequently, the medaka found in the Kanto district are a "mixed population".

In this study, mtDNA RFLPs analysis suggests three clusters and two and 11 subclusters for wild populations of medaka, which have strong geographical associations. The geographical distributions of the mtDNA haplotypes suggest three migration events. However, this analysis also suggests the necessity for more research concerning the Southern Population. The phylogenetic relationships among the subclusters of cluster B, the separate distribution origin of haplotypes in subcluster B-9 and the origin of cluster C, and the migration histories in the San-in district and the Seto-Inland Sea area are not yet clear. Phylogenetic analysis using nucleotide sequence information is necessary to elucidate these issues. We are currently identifying the nucleotide sequences of mitochondrial cytochrome *b* gene. This project may clarify the relationships among the clusters and subclusters.

REFERENCES

Avise JC (1991) Ten unorthodox perspectives on evolution prompted by comparative population genetic findings on mitochondrial DNA. Annu Rev Genet 25: 45-69

Avise JC, Bermingham E, Kessler LG, Saunders NC (1984) Characterization of mitochondrial DNA variability in a hybrid swarm between subspecies of bluegill sunfish (*Lepomis macrochirus*). Evolution 38: 931-941

Brown WM, George Jr M, Wilson AC (1979) Rapid evolution of animal mitochondrial DNA. Proc Natl Acad Sci USA 76: 1967-1971

Felsenstein J (1993) PHYLIP (phylogeny inference package). University of Washingtion, Seattle

Hyodo-Taguchi Y, Egami N (1985) Establishment of inbred strains of the medaka *Oryzias latipes* and the usefulness of strains for biomedical research. Zool Sci 2: 305-316

Magoulas A, Tsimenides N, Zouros E (1996) Mitochondrial DNA phylogeny and the reconstruction of the population history of a species: The case of the European anchovy (*Engraulis encrasicolus*). Mol Biol Evol 13: 178-190

Meyer A (1993) Evolution of mitochondrial DNA in fishes. In "Molecular Biology Frontiers" Ed by PW Hochachka, TP Mommsen, Elsevier Science Publishers BV, Amsterdam, pp 1-38

Nei M, Li W (1979) Mathematical models for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76: 5269-5273

Sakaizumi M (1986) Genetic divergence in wild populations of medaka, *Oryzias latipes* (Pisces: Oryziatidae) from Japan and China. Genetica 69: 119-125

Sakaizumi M, Moriwaki K, Egami N (1983) Allozymic variation and regional differentiation in wild population of the fish *Oryzias latipes*. Copeia 1983: 311-318

Sakaizumi M, Shimizu Y, Hamaguchi S (1992) Electrophoretic studies of meiotic segregation in inter- and intraspecific hybrids among east Asian species of the genus *Oryzias* (Pisces: Oryziatidae). J Exp Zool 264: 85-92

Yonekawa H, Gotoh O, Motohashi J, Hayashi J-I, Tagashira Y (1978) Positioning of the A·T-rich regions in rat mitochondrial DNA by electron microscopy and analysis of the hysteresis of denaturation. Biochim Biophys Acta 251: 510-519

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