Chapter 1
History and Features of Medaka

Medaka, *Oryzias latipes*, is a small egg-laying secondary freshwater fish native to East Asian countries, primarily Japan, Korea, Taiwan, and China. This fish is a member of the atherinomorpha taxon Beloniformes. Other members of order Beloniformes are halfbeaks and garfish, many of which are marine fish. This suggests that the common ancestors of medaka and relatives were marine fish and some species of this group adapted to the freshwater environment. This is one possible reason why there are several species adapted to freshwater or seawater within the same genus (Inoue and Takei, 2003).

1.1 History

Medaka has been reared as an ornamental fish since the Edo period. Figure 1-1 shows the Ukiyoe painting called "Medaka Scooping," published in 1767–1768. Two girls are scooping medaka and putting them in a small glass tank. In 1835, Motohisa Mori described three medaka strains: wild type, orange-red type, and white type (see also Figure 1-7). The orange-red type strain has a mutation at the *b* locus and the white-type strain is a double mutant at the *b* and *r* loci. As it is not feasible to isolate a double mutant from wild type medaka, it is likely that relatively large numbers of the orange-red type strain were cultured at the end of Edo period.

Medaka was first described in Siebold's *Fauna Japonica*, and originally assigned to the genus *Poecilia* by Temminck and Schlegel in 1846. After this description, medaka has been a favorite experimental animal for researchers in Japan and other countries. Several important achievements have been made with the color mutants of medaka described by Motohisa Mori. Aida (1921) found the sex-limited inheritance of the *r* locus that controls the expression of orange pigment cells (xanthophores). Yamamoto (1953, 1975) established the d-rR strain and demonstrated the artificial induction of sex reversal with estrogen and androgen to fish of the d-rR strain. The d-rR strain showed body-color dimorphism, with the male orange-red and the female white. Because the *R* allele is on the Y chromosome and the *r* allele is on the X chromosome, genetic sex can be distinguished by body color. Studies of medaka sex determination and differentiation finally resulted in the identification of the primary sex-determination gene, *DMY* (Matsuda et al., 2002; Nanda et al., 2002). This gene is the second "primary sex determination gene" isolated in vertebrates, and is the functional equivalent of the *Sry* gene in mammals. The establishment of an efficient method for making transgenic medaka was also an important achievement (Ozato et al., 1986), and the establishment of several inbred lines from genetically different natural populations is unique to medaka (Hyodo-Taguchi and Egami, 1985). From around 2000, several important studies to establish genetic/genomic resources have been archived. A large-scale Expressed Sequence Tag (EST) analysis was done by Kimura et al. (2004). There

Figure 1-1. Ukiyoe painting "Medaka Scooping" by Harunobu Suzuki, 1767–1768. Two girls are scooping medaka and putting them into a small glass tank. Courtesy of http://www.japanism.net/.

are now over 210,000 cDNA/EST sequences deposited in the public DNA database (DDBJ/EMBL/Genbank), and summarized up to about 39,000 unique sequences. A genome-wide linkage map was also established (Naruse et al., 2000, 2004a). The medaka genome sequencing project commenced in 2002 and the draft genome sequence was published by Kasahara et al. (2007). All genetic/genomic data are now open to the public through the UT genome browser (http://medaka.utgenome.org/), Ensembl genome browser (http://www.ensembl.org/Oryzias_latipes/index.html), and UCSC genome browser (http://genome.ucsc.edu/cgi-bin/hgGateway). In addition to these genome resources, mutagenesis screening for the isolation of mutants with a specific phenotype during embryonic development has been conducted by several groups. About 500 mutants with specific phenotypes have been established, and projects to identify the causal gene of the mutants have been conducted in several laboratories

(Furutani-Seiki et al., 2004; Yokoi et al., 2007). Since these studies and activities, medaka has become a representative model for vertebrates.

1.2 Phylogeny

1.2.1 Phylogeny and distribution of medaka and relatives

Teleostei is the most diversified group in the vertebrates, comprising over 26,800 species in 4278 genera, 448 families, and 40 orders (Nelson, 2006). This group includes other model organisms, such as zebrafish (*Danio rerio*), pufferfish (*Takifugu rebripes* and *Tetraodon nigroviridis*), and the three-spined stickleback (*Gasterosteus aculeatus*). Among these fishes, zebrafish (Cypriniformes) belong to the basal teleostean lineage (Ostariophysi) (Inoue et al., 2003). In contrast, the medaka (Beloniformes), pufferfish (Tetraodontiformes), and stickleback (Gasterosteiformes) are members of the higher teleosts (Percomorpha) (Miya et al., 2005; Figure 1-2). The approximate divergence times of the medaka are estimated to be 485 million years ago (mya) with mammals, 324 mya with zebrafish, and 191 mya with the lineage leading to pufferfish and the stickleback (Yamanoue et al., 2006).

The family Adrianichthyidae is a small group native to Asia, containing four genera, *Oryzias* with 20 species, *Adrianichthys* with two species, *Horaichthys* with one species, and *Xenopoecilus* with three species. Before the 1980s, this family was placed in the order Cyprinodontiformes. However, Rosen and Parenti (1981) indicated a monophyly of Adrianichthyidae within the order Beloniformes, based on characters of the gill arch skeleton hyoid apparatus. Nelson (1994, 2006) agreed with this relationship and placed Adrianichthyids within Beloniformes. A recent molecular phylogeny based on entire mitochondrial DNA sequences also supports a monophyly of the group containing the medaka and other beloniform fishes (Miya et al., 2005). Medaka is currently regarded as a member of Beloniformes.

Most fishes in family Adrianichthyidae are confined to freshwater, such as brooks, ponds, canals, paddy fields, and lakes, but some species are found in brackish- and seawater along the coast. Fishes in this family have a wide distribution, from India to Japan, and south along the Indo-Australian archipelago across Wallace's line to Timor, Sulawesi, and Luzon (Table 1-1). Above all, *Oryzias dancena, O. javanicus,* and

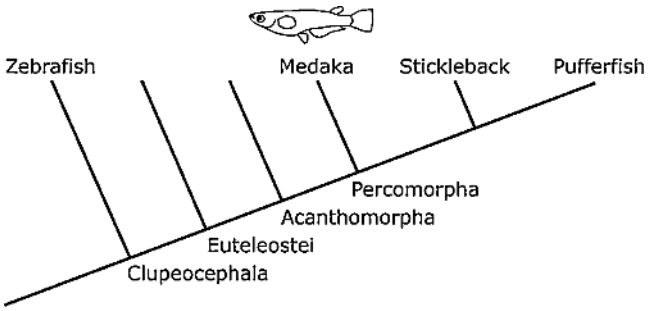

Figure 1-2. A simplified phylogenetic tree of Teleostei. The lineage leading to zebrafish is the most basally diverged group, while medaka, stickleback, and pufferfishes are members of Percomorpha.

Table 1-1. Geographic distribution of adrianichthyid fishes.

Species	Distribution	Reference
Adrianichthys kruyti Weber, 1913	Lake Poso in Sulawesi	Parenti and Soeroto (2004)
Adrianichthys roseni Parenti & Soeroto, 2004	Lake Poso in Sulawesi	Parenti and Soeroto (2004)
Horaichthys setnai Kulkarni, 1940	W India	Talwar and Jhingran (1991)
Oryzias carnaticus Jerdon, 1849	E India and Bangladesh	Roberts (1998)
Oryzias celebensis Weber, 1894	S Sulawesi	Parenti and Soeroto (2004)
Oryzias curvinotus Nichols & Pope, 1927	N Vietnam, Hainan, and S China	Uwa and Parenti (1988)
Oryzias dancena Hamilton, 1822	E India, a Bangladesh, Myanmar, and N Malaya	Roberts (1998)
Oryzias haugiangensis Roberts, 1998	S Vietnam	Roberts (1998)
Oryzias hubbsi Roberts, 1998	W Java	Roberts (1998)
Oryzias javanicus Bleeker, 1854	Java, Sumatra, Malaya, Borneo, Sulawesi, and Lombok	Kottelat et al. (1993) and Roberts (1998)
Oryzias latipes Temminck & Schlegel, 1846	Japan, Korea, Taiwan, and China	Uwa and Parenti (1988)
Oryzias luzonensis Herre & Ablan, 1934	N Luzon	Formacion and Uwa (1985)
Oryzias marmoratus Aurich, 1935	Lakes Towuti, Mahalona, and Wawontoa in Sulawesi	Kottelat (1990b)
Oryzias matanensis Aurich, 1935	Lake Matano in Sulawesi	Kottelat (1990b)
Oryzias mekongensis Uwa & Magtoon, 1986	NE Thailand	Roberts (1998)
Oryzias minutillus Smith, 1945	Thailand	Roberts (1998)
Oryzias nebulosus Parenti & Soeroto, 2004	Lake Poso in Sulawesi	Parenti and Soeroto (2004)
Oryzias nigrimas Kottelat, 1990a	Lake Poso in Sulawesi	Parenti and Soeroto (2004)
Oryzias orthognathus Kottelat, 1990a	Lake Poso in Sulawesi	Parenti and Soeroto (2004)
Oryzias pectoralis Roberts, 1998	Laos	Roberts (1998)
Oryzias profundicola Kottelat, 1990b	Lake Towuti in Sulawesi	Kottelat (1990b)
Oryzias timorensis Weber & de Beaufort, 1922	Timor	Uwa and Parenti (1988)
Oryzias uwai Roberts, 1998	Myanmar	Roberts (1998)
Xenopoecilus oophorus Kottelat, 1990a	Lake Poso in Sulawesi	Parenti and Soeroto (2004)
Xenopoecilus poptae Weber & de Beaufort, 1922	Lake Poso in Sulawesi	Parenti and Soeroto (2004)
Xenopoecilus sarasinorum Popta, 1905	Lake Lindu in Sulawesi	Parenti and Soeroto (2004)

P1: IFM/IFM P2: SFK/UKS QC: SFK/UKS T1: SFK
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Horaichthys setnai, which inhabit brackish- or seawater, are widely distributed in southwestern Asia or western India (Roberts, 1998; Talwar and Jhingran, 1991). On the other hand, all fishes in the genera *Adrianichthys* and *Xenopoecilus*, and six in *Oryzias*, are endemic to particular lakes in Sulawesi Island (Kottelat 1990a, 1990b; Kottelat et al., 1993; Parenti and Soeroto, 2004), suggesting high endemism of adrianichthyid fish on this island.

Phylogenetic information on *Oryzias* species was first provided by cytogenetic studies. Uwa and his colleagues collected 12 *Oryzias* species from throughout Asia, and studied their karyotypes (Uwa, 1986; Naruse, 1996). These studies showed a basic chromosome number in the genus of 2n, 48, without heteroploidy and polyploidy, and divided these species into the three groups (monoarmed, biarmed, and fused chromosome groups) based on their karyotypes. Furthermore, these three chromosomal groups were supported by some morphometric and meristic characters (Uwa and Parenti, 1988). The monoarmed chromosome group is characterized by 48 acrocentric and subtelocentric chromosomes. This group consists of four species, *O. hubbsi* (as *O. javanicus* from Jakarta), *O. javanicus, O. dancena* (as *O. melastigma*), and *O. minutillus* (Magtoon and Uwa, 1985; Magtoon et al., 1992; Uwa, 1986; Uwa and Iwata, 1981; Uwa et al., 1983). The biarmed chromosome group has meta- and submetacentric chromosomes. *Oryzias latipes, O. curvinotus, O. luzonensis,* and *O. mekongensis* are in this group (Formacion and Uwa, 1985; Uwa, 1991; Uwa and Ojima, 1981; Uwa and Magtoon, 1986; Uwa et al., 1982). The fused chromosome group possesses 1–4 pairs of large meta- or submetacentric chromosomes. These fused chromosomes are considered to arise from Robertsonian centric fusions. *Oryzias celebensis*, *O marmoratus*, *O. matanensis,* and *O. nigrimas* are in this group (Uwa et al., 1981; Naruse, 1996).

A recent phylogenetic analysis based on nuclear and mitochondrial DNA sequences shows a robust phylogeny of *Oryzias* (Figure 1-3; Takehana et al., 2005). In this study, the phylogenetic relationships among 13 *Oryzias* species were studied using nuclear *tyrosinase* and mitochondrial 12S and 16S rRNA genes. Based on the resultant phylogeny, *Oryzias* species have been divided into three monophyletic species groups: *latipes, javanicus,* and *celebensis* groups. These species groups are consistent with the previous biarmed, monoarmed, and fused chromosome groups, respectively, suggesting that each chromosome group has a monophyletic origin. Takehana et al. (2005) estimated the divergence time among the three species groups as about 30 mya, using a divergence rate of 0.11% transversions/million years for the 12S and 16S rRNA genes to the data (Bargelloni et al., 2000). Medaka is a member of the *latipes* group, and its closest relatives are *O. curvinotus* and *O. luzonensis*. The latter two species have a sister group relationship. In this species group, *O. mekongensis* is the most basal species, and subsequently *O. latipes* separates from the lineage leading to *O. curvinotus* and *O. luzonensis*.

Fishes in the *latipes* and *javanicus* group are distributed widely in eastern and western Asia, respectively, and the distribution range of O'latipes corresponds to the northern limit of that in the genus. On the other hand, all species in the *celebensis* group are endemic to Sulawesi Island. Historical variance between Sulawesi and the continental region has been explained by the high proportion of endemic fauna on the island. Sulawesi is a particularly rich area of endemism for atherinomorph freshwater fish (Parenti and Soeroto, 2004). In addition to the medaka-related fish, there are

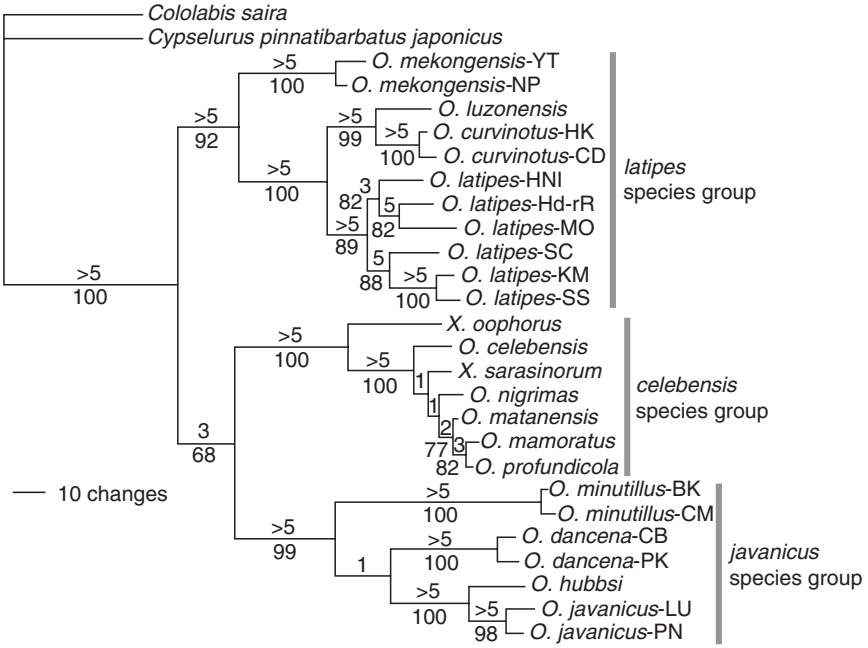

Figure 1-3. A Phylogenetic tree of medaka and its relatives, based on nuclear and mitochondrial DNA sequences (Takehana et al., 2005). Numbers above and below internal branches indicate bootstrap values and decay indices, respectively. Medaka fishes are divided into three monophyletic groups: *javanicus, latipes,* and *celebenesis* species. Each species group corresponds to the monoarmed, biarmed, and fused chromosome groups, respectively, as previously classified by karyological analysis.

10 endemic species of beloniform hemiramphids in the sister genera *Dermogenys* and *Nomorhamphus* (Meisner, 2001), and an estimated 17 species of atheriniform telmatherinins (Kottelat, 2002). Sulawesi Island is separated from the continental shelf by the Makassar Strait between Sulawesi and Borneo, which appears to have been a barrier to dispersal. A geological and tectonic study by Moss and Wilson (1998) suggests that the formation of the Makassar Strait occurred from the Eocene to the Oligocene (27–54 mya), and this isolation event may have contributed to the high degree of endemism in Sulawesi. The divergence time between the *celebensis* group and the other two groups, inferred from a molecular clock, roughly corresponds to the period when the Makassar Strait is presumed to have formed. Therefore, it is likely that the *celebensis* group has been isolated in Sulawesi Island for more than 30 million years.

The molecular phylogeny also revealed the phylogenetic positions of two species in *Xenopoecilus*, a genus closely related to *Oryzias*. The tree topologies obtained from the nuclear and mitochondrial data were consistent, indicating that *Xenopoecilus* is a polyphyletic genus nested within *Oryzias*. This result suggested the need for a systematic study and taxonomic revision of *Xenopoecilus*. However, the remaining *Xenopoecilus* species and fish in the genera *Adrianichthys* and *Horaichthys* were not available during the study. Further taxonomic sampling and subsequent analyses based

on molecular and morphological data should clarify these unexplored relationships in the family Adrianichthyidae.

In summary, medaka (*Oryzias latipes*) belongs to order Beloniformes and family Adrianichthyidae. The family contains four genera (*Oryzias, Xenopoecilus, Adrianichthys,* and *Horaichthys*), and is distributed widely in Asia. Recent molecular phylogenetic analysis shows a robust phylogeny of *Oryzias*, suggesting three monophyletic species groups (*latipes, javanicus,* and *celebensis* groups). These three groups correspond to the three chromosomal groups (biarmed, monoarmed, and fused chromosome groups) previously proposed from karyological analyses. Using a molecular clock calibration of mitochondrial DNA, the origins of each major group dates back to approximately 30 million years.

1.2.2 Genetic diversity of medaka

Primary freshwater fish are particularly suitable for biogeographic studies due to their limited dispersal capacity (Avise, 2000). It is generally supposed that land is a barrier to their dispersal and, thus, local populations are confined to their own watershed and isolated from one another, resulting in regional differentiation. In the last three decades, biogeographic studies have changed from earlier descriptions of phenotypic characters to phylogenetic approaches using various molecular markers. In particular, the phylogeographic approach that investigates the geographic distributions of genetic variations within species has become predominant. Such analyses of freshwater fish have shown their genetic differentiations, reflecting region-specific geographic distributions. In medaka, phylogeographic studies using allozymes and mitochondrial DNA (mtDNA) sequences for the last 25 years have revealed an extremely high genetic diversity in this species.

Medaka is widely distributed in Japan (except for Hokkaido Island), Korea, Taiwan, and China. So far, Sakaizumi and his collaborators have collected samples from the entire distribution range and studied genetic population structure in detail, based on allozymic variation (Sakaizumi, 1986; Sakaizumi et al., 1980, 1983; Sakaizumi and Jeon, 1987; Takehana et al., 2004a). These allozymic studies showed that wild populations of medaka were divided into four major regionally differentiated groups: the "Northern Japanese Population" from the Sea of Japan coast of eastern Japan, the "Southern Japanese Population" from the Pacific coast of eastern Japan and from western Japan, the "China–West Korean Population" from China and western Korea, and the "East Korean Population" from eastern and southern Korea (Figure 1-4). Nei's genetic distances among these groups are very large (0.35–0.88), indicating that the four groups have been isolated for a long time. However, it is possible to establish productive matings under laboratory conditions, and male and female progeny from hybrids among the groups are fully fertile (Sakaizumi et al., 1992).

Karyological studies have demonstrated that specimens belonging to the China–West Korean Population have 2n, 46 chromosomes, including a large metacentric pair (Uwa and Jeon, 1987; Uwa et al., 1988). In contrast, fish from the other three groups (Northern Japanese, Southern Japanese, and East Korean Populations) showed 2n, 48 chromosomes without the large chromosomes (Uwa and Ojima, 1981; Uwa, 1986; Uwa and Jeon, 1987; Uwa et al., 1988). The species closely related to medaka have 2n, 48

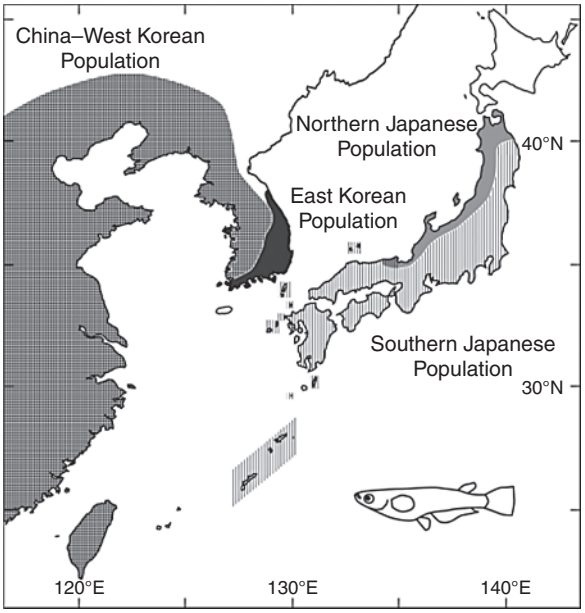

Figure 1-4. Distribution of medaka in China, Korea, and Japan. Medaka wild populations are divided into four distinct groups according to allozymic and mitochondrial cytochrome *b* gene-sequence variations.

chromosomes, suggesting that the large metacentric chromosomes arose from a centric fusion that occurred only in the China–West Korean Population. The geographic distributions of these two chromosomal forms in Korea are perfectly consistent with those of the two allozymically distinguished groups, namely, the 2n, 46 form in western Korea (also known as *O. latipes sinensis*), and the 2n, 48 form in eastern and southern Korea (Kim and Moon, 1987; Kim and Lee, 1992). This agreement suggests limited gene flow between the China–West Korean and East Korean Populations.

The boundaries separating the geographic distributions of the four major groups are well correlated with geographical barriers, such as mountains and sea, and gene flow between the groups has not been observed. However, introgressions between the groups have been found in limited areas within the boundaries. The Japanese wild populations with unique genotypes have been found around the western end of the boundary between the Northern and Southern Japanese Populations (Sakaizumi, 1984). This type of population is fixed with northern alleles at two allozymic loci, southern alleles at two loci and a unique allele at one locus. This unique genotype suggests that this 'boundary' population was formed by an introgression between the two groups followed by random drift at each locus. Another case of introgression has been observed in the western coast of Korea, in which two distinct genotypes (derived from the East Korean and China–West Korean Populations) at three loci were distributed in a mosaic fashion (Takehana et al., 2004a). However, each population was nearly fixed as either the eastern or western genotype at other six diagnostic loci, despite the geographic proximity among them. This could suggest the existence of reproductive isolation between groups in the wild.

Subsequent mtDNA analyses have also revealed regional differentiations that are consistent with those based on previous allozymic studies (Matsuda et al., 1997a, b). Recent phylogeographic analyses using mitochondrial cytochrome *b* sequences indicate a phylogenetic relationship among the groups, suggesting a monophyletic origin of the Japanese groups (Figure 1-5; Takehana et al., 2003, 2004b). In these studies, five major mitochondrial DNA lineages (A–E) have been observed. The geographic distribution patterns of lineages A, B, D, and E are identical to those of the Northern Japanese, Southern Japanese, China–West Korean, and East Korean Populations, respectively. The remaining lineage, C, is found only in the Kanto District of Japan, and has a sister group relationship with lineage B. Since previous allozymic analysis indicated that the populations from this region are part of the Southern Japanese Population (Sakaizumi et al., 1983), this lineage is considered to be a relic mtDNA, which diverged from the ancestral mtDNA with lineage B and remained in a limited region for a long time. The average sequence divergences are 11.3–11.8% among the three Japanese lineages, and 15.1% between the three Japanese and two continental lineages. Based on the faster molecular clock of the cytochrome *b* gene (2.8% per million years), the divergence times among the groups are estimated as approximately 4.0–4.2 mya between the Northern and Southern Japanese Population, and 5.4 mya between the Japanese and continental populations. This range in divergence time is similar to that between humans and chimpanzees (Patterson et al., 2006).

Phylogenetic analyses of mitochondrial gene sequences indicate that three of the four lineages comprise distinct phylogenetic subgroups showing strong geographic correlations (Figure 1-5). Lineage A (corresponding to the Northern Japanese Population) is subdivided into three subgroups (A-I to A-III), lineage B (the Southern Japanese Population) into 11 (B-I to B-XI), and lineage D (the China–West Korean Population) into three (D-I to D-III), with average sequence divergences of 3.0–4.1%, 1.3–5.8%, and 3.0–3.5%, respectively. The boundaries among the subgroups correlate with mountain barriers, suggesting that regional differentiations have been maintained by geographic isolation. According to molecular clock calibration, the approximate divergence times among subgroups are estimated at 1.1–1.6 mya for A-I to A-III, 0.5–2.3 mya for B-I to B-XI, and 1.1–1.3 mya for D-I to D-III.

Taken together, the wild populations of medaka consist of four genetically distinct groups (Northern Japanese, Southern Japanese, China–West Korean, and East Korean Populations), and the Japanese groups have a monophyletic origin. Each group is subdivided into several subgroups, with genetic differentiation reflecting their region-specific geographic distributions. Using a molecular clock calibration of mitochondrial DNA, the divergence times among groups and subgroups are estimated at approximately 4.0–5.4 and 0.5–2.3 mya, respectively.

1.3 Advantage of Medaka as a Model Fish

1.3.1 Advantageous features in general

Medaka has several advantageous features as an experimental animal. As it is an oviparous fish, embryonic development occurs externally and embryos, particularly the pigment-less mutants, are completely transparent throughout most of their embryonic

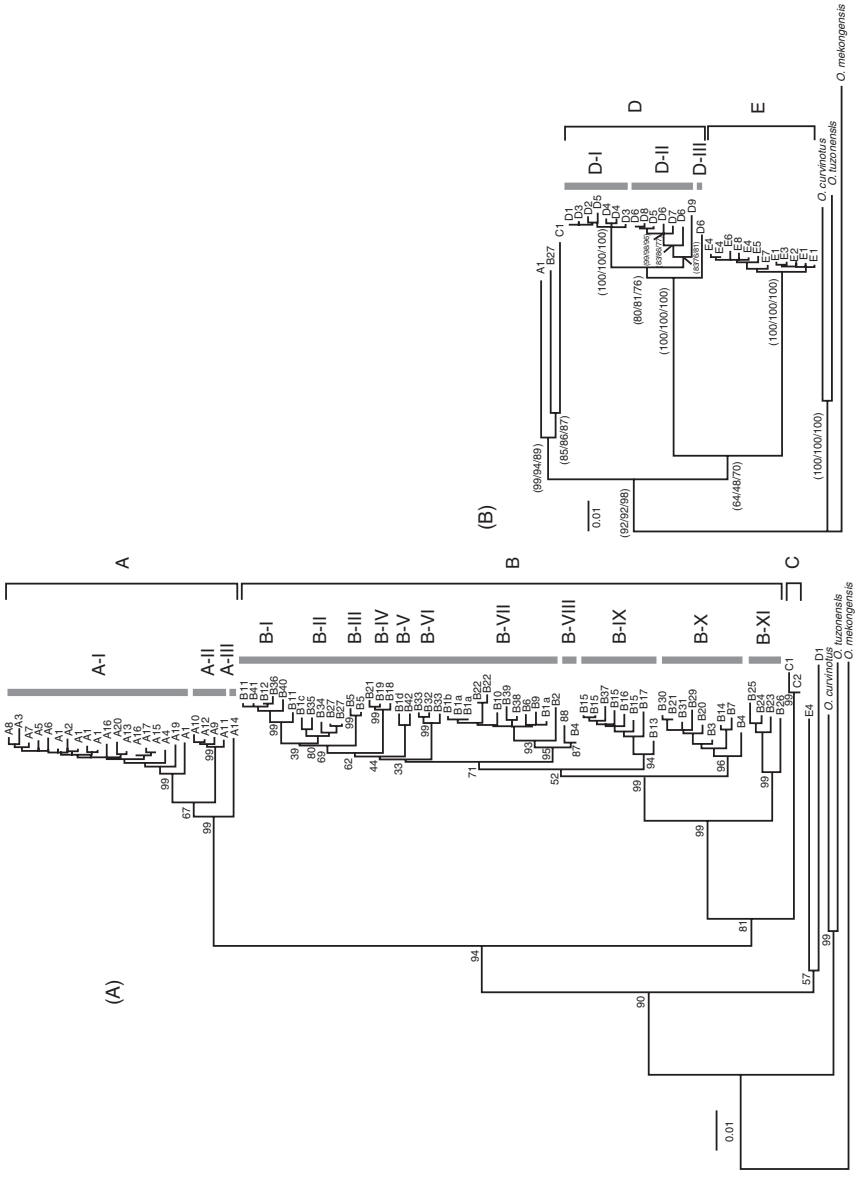

Figure 1-5. Phylogenetic trees based on mitochondrial cytochome *b* gene sequences from the Japanese wild populations (A) and continental wild populations (B) (modified from Takehana et al., 2003, 2004b). Numbers above internal branches indicate bootstrap values. Four major lineages, A, B, D, and E are recognized. Each lineage corresponds to the Northern Japanese Population, the Southern Japanese Population, the China–West Korean Population, and the East Korean Population, respectively. (Reproduced with permission of The Zoological Society of Japan; Copyright (2003, 2004) The Zoological Society of Japan.)

development. Even in the adult stage, some medaka strains, such as STIII (Figure 5-4; Wakamatsu et al., 2001) and Quintet, are transparent. As described in Section 1.3.2., medaka has four different pigment cells: melanophores, leucophores, xanthophores, and iridophores (detailed in Section 5.1.2). Vertebrates with leucophores are rare. Even some medaka-related species do not have leucophores. In addition, there are mutant lines available without chromatophores. Two loci that control pigment-cell development (xanthophores by the *r* locus and leucophores by the *lf* locus) are useful, because we can distinguish the sex of embryos with or without pigment cells (Aida, 1921; Wada et al., 1998).

Another advantage is medaka's natural tolerance to low temperature. Medaka is a temperate-zone fish and can survive at 40°C in summer and 4°C in winter without any thermostatic regulator. The rate of embryonic development can be controlled by environmental temperature. For example, the embryonic development is arrested at 10°C and resumes at 25°C (applicable until the blastula stage).

The large genetic divergence among regional populations is not found in other vertebrate models. Indeed, to our knowledge, medaka is the most genetically diverged vertebrate (3–4% sequence divergence among regional populations) (Takehana et al., 2003, 2004b; Kasahara et al., 2007). The estimated genome size of medaka is about 800 million base pairs (Mbp) and that of zebrafish is 1700 Mbp (Naruse et al., 2004b). This is also advantageous for isolating entire genomic regions of genes from a relatively small genomic DNA library, such as the Fosmid library. The existence of medaka-related species in South-East Asia to East Asia is also interesting (Takehana et al., 2005). Most genomic resources established in medaka are also applicable to their closely related species. These situations promote evolutionary studies using medaka-related species with several of the genetic/genomic tools used for medaka.

1.3.2 Color mutants

1.3.2.1 Introduction and history

Body color is a conspicuous feature of animals, and skin, hair, or eye color has been of considerable interest for humans. For instance, pets with a rare color are often traded at very high prices, and human skin colors sometimes play a major role in racial discrimination. In laboratory studies, scientists have described over 280 coat-color loci in mice (http://www.espcr.org/micemut/) and isolated ~100 body-color mutants in zebrafish (Kelsh et al., 1996; Odenthal et al., 1996).

About 50 spontaneous body-color mutants are known in medaka. It is surprising that this collection (together with an additional ~30 nonpigmented mutants) was achieved by a single researcher, the late Prof. Hideo Tomita of Nagoya University (Figure 1-6). He screened these mutants by extensive but simple backcrossing. A recent backcross also isolated a new body-color mutant (Yu et al., 2006). Considering that these spontaneous mutations are often caused by an insertion of transposon-like sequences (Koga et al., 1995; Kondo et al., 2001; Loosli et al., 2001; Iida et al., 2004), the medaka genome might contain significant numbers of genes disrupted by such unstable DNA fragments (Koga et al., 2006), and this might have enabled the successful isolation of mutants without using mutagens.

Figure 1-6. Portrait of the late Professor Dr. Hideo Tomita (1931–1998), provided by Dr. M. Sakaizumi of Niigata University.

The first scientific description of medaka mutants can be traced back to 1838, when orange-red and white medaka were drawn in a fish encyclopedia (Figure 1-7). These illustrations most likely correspond to the *b* and *b-r* double mutants, respectively. Mendelian inheritance of these phenotypes was described by Aida (1921), and the *b* gene was identified by the first positional cloning in medaka (Fukamachi et al. 2001).

1.3.2.2 Body color and chromatophores

In vertebrates, body colors and patterns are determined by pigment cells (chromatophores) in the skin. Chromatophores possess pigments (or light-reflecting structures) within organelles called chromatosomes, and are classified into about five types, depending on the color: black melanophores, white leucophores, red-yellow erythro-xanthophores, blue cyanophores, and silvery iridophores. Chromatosomes in cold-blooded vertebrates can bidirectionally move within a chromatophore to rapidly (physiologically) change the cell color (see Section 5.1.2. for details).

Figure 1-7. Medaka fishes illustrated by Motohisa Mouri (1798–1851) in his encyclopedia, *Baien-gyofu*. Three medaka are drawn on top: from left, shiro (white, *b-r*); hi (orange-red, *b*); and wild-type medaka. (Reproduced from http://www.ndl.go.jp/ with permission of National Diet Library, Japan.)

Medaka has four types of chromatophores: melanophores, leucophores, xanthophores, and iridophores. The color of wild-type medaka is somewhat brown, owing to the predominant distributions of melanophores and xanthophores. The ventral side, particularly the abdomen, is brighter than the dorsal side and looks iridescent due to increased iridophores and leucophores.

The Tomita mutants, screened by observing adult phenotypes, exhibit various skin colors depending on the chromatophore type(s) affected by the mutations. For example, colorless melanophores make the body (trunk region) orange (*b*), reduced xanthophores make the trunk gray (*r*), a lack of iridophores makes the abdomen black (*gu*), and colorless melanophores and xanthophores make the trunk white (*i* [albino]). Combinations of these mutations (double/triple mutants) produce even more colors (Figure 1-8). A systematic crossing of the Tomita and other mutants successfully established viable strains without any visible chromatophore (STIII, Wakamatsu et al., 2001; SK^2, Fukamachi et al., 2008). Their skin is transparent throughout their life and their internal organs are visible externally.

Phenotypes of the color mutants appear in various aspects of chromatophores: pigment synthesis/storage (metabolic defects), chromatosome movement (physiological defects), and the number/shape of chromatophores (developmental defects), which are briefly summarized elsewhere (Tomita 1992; Iwamatsu 1997; see also Section 5.1.2.). Interestingly, however, these adult phenotypes are not always identical

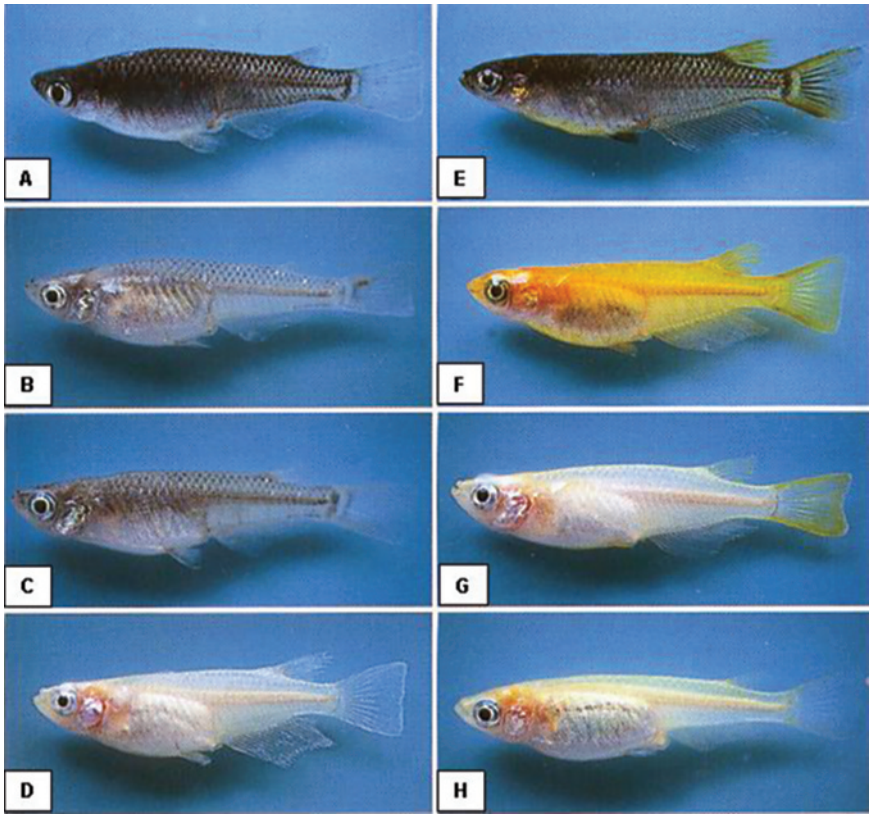

Figure 1-8. Examples of medaka color mutants. A, blue (*r*); B, light-blue (*r-ci*); C, gray (*ci*); D, milky (*b-r-ci*); E, wild-type; F, orange-red (*b*); G, cream (*b-ci*); and H, white (*b-r*). (Photo is taken from *The integrated book for the biology of the medaka* (Iwamatsu, 1997).)

to their embryonic/larval phenotypes (Kelsh et al., 2004). These observations could indicate that the genes have different functions between the embryonic/larval and adult stages.

The Tomita mutants, together with other color mutants, are available for all academic researchers through National Bioresource Project Medaka (http://www.shigen.nig.ac.jp/medaka).

1.3.2.3 Genes mutated in body-color mutants

The well-founded tools for molecular genetics in medaka (e.g., inbred strains, DNA markers, linkage maps, BAC libraries, and draft genome sequences) have facilitated the recent identification of the genes responsible for body-color anomalies by forward genetic approaches. So far, five color genes have been identified.

The first mutated gene identified in medaka was *tyrosinase*, which encodes a key enzyme for melanin biosynthesis (Koga et al., 1995). *Tyrosinase* is also mutated in human patients of oculocutaneous albinism type 1 (OCA1), which is characterized by white skin/hair and red eyes. The medaka *tyrosinase* (*i*-locus) mutants exhibit quite similar

phenotypes to OCA1; melanin deposition is severely suppressed in both the skin and eyes. Interestingly, xanthophores of the *i* mutant are also strongly hypopigmented, which could indicate an additional role of this enzyme in xanthophore differentiation in fish. Another interesting finding from this work is that a transposon (*Tol1/2*) is inserted in three of the four *i*-locus alleles (*i1, i4, ib*). *Tol2* is the first active transposon reported from vertebrates.

While the *i* gene was identified using the candidate approach, the other four genes were identified by positional cloning. The *b* locus encodes a putative transporter, *slc45a2* (Fukamachi et al., 2001). The *slc45a2* is mutated in human OCA4 patients, with polymorphisms possibly associated with population differences in skin color, and in mice is an *underwhite* mutant, in horses is a "cream" variant, and in chickens is a "silver" variant (Graf et al., 2007). Although the role of *slc45a2* in transporting a substance(s) or optimizing an intracellular condition necessary for melanin synthesis is proposed, its function remains unknown.

The *b*-locus mutants have colorless melanophores, as do the *i* mutants, but their xanthophores are less affected, which makes the trunk orange rather than white (Figure 1-8). Whereas most *b*-locus mutants with a mutation in the coding region of *slc45a2* show the typical OCA phenotype (hypopigmentation in both the eyes and skin), the original *b* mutant (which has the *b* allele at the *b* locus; Figure 1-7) shows skin-specific albinism. This is because the *b* mutation occurs in a promoter that is necessary for *slc45a2* transcription in the skin but not in the eyes (Fukamachi et al., 2008).

Another medaka OCA mutant is the *i-3*, which has a mutation at the *pink-eyed dilution* (*p*) gene that encodes a transporter called the p protein (Fukamachi et al., 2004b). Phenotypes of the *i* and *i-3* medaka are quite similar (i.e., both melanophores and xanthophores are hypopigmented), but can be biochemically distinguished as OCA1 and OCA2 in human patients by assessing their tyrosinase activity. The p protein seems to regulate pH in melanosomes, where melanin is synthesized from tyrosine (Brilliant, 2001). Taken together, three of the four types of human OCA exist in medaka. These medaka mutants reveal a conserved mechanism for melanin synthesis among vertebrates, and could be good models for investigating these human diseases.

The *pale gray eyes* (*pge*) mutant was recently isolated by backcrossing. All types of chromatophores are only faintly pigmented and the mutant dies ~1 week after hatching. Although the mutation has not been identified, the gene responsible for this phenotype is most likely *vps11*, whose expression is severely reduced in the *pge* mutant (Yu et al., 2006). Because *vps11* is necessary for vesicle docking/fusion to lysosome-like vacuoles in other organisms, intracellular trafficking of chromatosomes could be affected by the reduced expression of *vps11* in the *pge* mutant.

The *color interfere* (*ci*) locus encodes somatolactin (SL), a fish-specific and growth-hormone-like peptide hormone secreted from the pituitary (Fukamachi et al., 2004a). Differentiation and proliferation of leucophores are dramatically enhanced in the *ci* mutant, whereas those of xanthophores are oppositely suppressed, which makes the body color pale gray (Figure 1-8). As functions of SL other than in pigmentation have been proposed from studies of other fish, the *ci* medaka (though in good health) has increased lipid contents in its organs and decreased cortisol concentration in its plasma (Fukamachi et al., 2005). From an evolutionary perspective, it is intriguing that

land vertebrates have lost SL whereas it is widely conserved among fish. SL probably has a crucial function for fish to live in water. However, the link between body color and life in water is totally unknown to date.

1.3.2.4 Future use of body-color mutants

Over 110 coat-color genes cloned in mice have made a great contribution to the basic/clinical studies of human skin/hair-color disorders (Spritz et al., 2003). However, because mammals have lost other types of chromatophores during the long nocturnal lifestyle of their ancestors, studies using mammals are insufficient for understanding colors and color patterns in lower vertebrates, which are often more colorful and complicated than those in mammals. Medaka whole-genome sequences (Kasahara et al., 2007) will no doubt accelerate the forward-genetic studies of the Tomita mutants, which will shed fresh light on the regulation and differentiation of chromatophores. Functional comparisons of such body-color genes with those of other vertebrates will help our understanding of the development and evolution of animal body colors, which are often conspicuously divergent, even between closely related species.

1.3.3 Wild strains

Wild populations are a rich repository of variations and mutations. Many inbred strains and color mutants of medaka were originally collected and established from wild populations (see Sections 1.3.2 and 1.3.4). The National Bioresource Project Medaka (http://shigen.lab.nig.ac.jp/medaka/) has maintained more than 60 wild populations of medaka and closely related species, as well as various inbred strains and mutants. Current stocks in the resource center include 66 wild populations of medaka (55 from Japan, nine from Korea, one from China, and one from Taiwan) and 12 related congeneric species (Figure 1-9). These wild stocks are an important genetic resource, containing various levels of genetic diversity.

Since wild populations of medaka potentially have phenotypic and genetic variations, these stocks can provide important insight into intraspecific variations and spontaneous mutations in particular genes. For example, phylogeographic studies using mitochondrial cytochrome *b* gene sequences have shown a detailed genetic population structure and extremely high genetic diversity in the medaka (Takehana et al., 2003, 2004b). Furthermore, a broad survey of the *DMY* gene has revealed that this gene is the common sex-determining gene in wild populations, and isolated several sex-reversed mutants (XY females and XX males) in these wild stocks (Shinomiya et al., 2004). As for these XY sex-reversed females, mutations in the amino acid coding sequence of *DMY* or reduced *DMY* expression were observed (Otake et al., 2006), suggesting that spontaneous mutations of the *DMY* gene have been preserved in wild populations. Further comparative analyses of various genes and genomic regions should demonstrate the distribution patterns of specific alleles and the genetic background in individual differences. In future, these wild stocks of medaka will become a good model system for analyzing natural selection and local adaptation.

Comparative study is an essential approach to identifying the conserved and species-specific molecular mechanisms that underlie development and evolution. In particular,

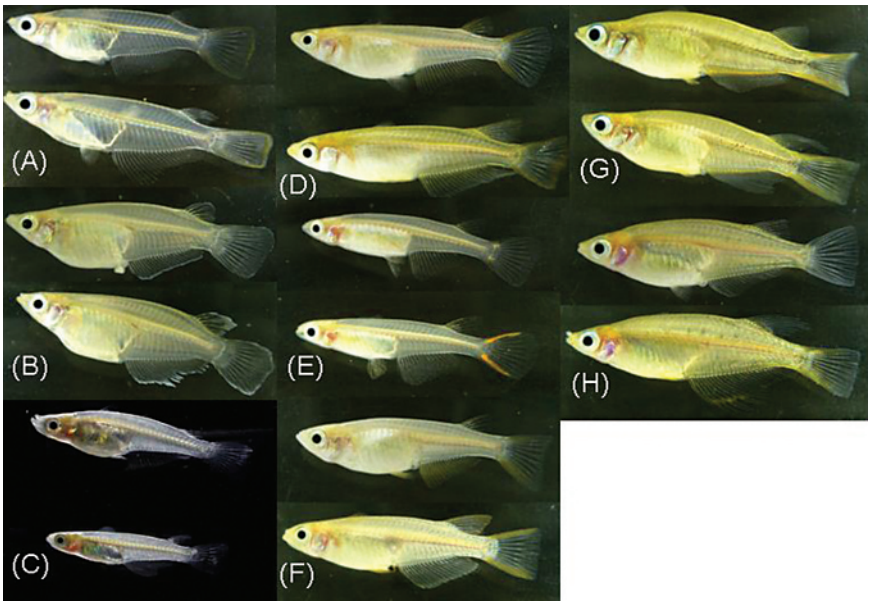

Figure 1-9. Examples of medaka fishes in *Oryzias*. (A) *O. javanicus*, (B) *O. dancena* (*O. melastigma*), (C) *O. minutillus*, (D) *O. curvinotus*, (E) *O. mekongenesis*, (F) *O. luzonensis*, (G) *O. celebensis*, (H) *O. marmoratus*. The upper is female and lower is male in each panel. The first three species are included in the *javanicus* species group, corresponding to the monoarmed chromosome group. The next three species are included in the *latipes* species group, corresponding to the biarmed chromosome group. The last two species are included in the *celebensis* species group, corresponding to the fused chromosome group.

comparisons among closely related species are expected to provide meaningful insights that are not easily obtained from distantly related species. In *Oryzias*, the advantages of medaka as a model organism are also present in closely related species, and a reliable phylogeny of the genus is available (Takehana et al., 2005). Such a situation in *Oryzias* species enables comparisons of phenotypes among closely related species in a phylogenetic context, providing ideal conditions for evolutionary study. *Oryzias* exhibit different degrees of adaptability to seawater (Inoue and Takei, 2002, 2003), and have a variety of sex-determination systems and sex chromosomes (Matsuda et al., 2002, 2003; Hamaguchi et al., 2004; Takehana et al., 2007a, b). Therefore, comparative studies among these fishes are expected to become an excellent model system for understanding the evolutionary mechanisms for osmotic adaptation and sex determination. Future comparative studies of *Oryzias* species should provide important insights into a wide range of biological disciplines, including genetics, developmental biology, physiology, and evolution.

Oryzias species are also useful for studying reproductive isolation mechanisms, because interspecific hybrids exhibit various abnormalities in development, reproduction, and sex determination. For example, all hybrid embryos failed to develop and died before hatching in interspecific hybridization between *O. latipes* and *O. hubbsi* (Iwamatsu et al., 1994, 2003a; Sakai et al., 2007). On the other hand, male sterility and

the production of nonreductional 2n eggs by females were observed in interspecific hybrids between *O. latipes* and *O. curvinotus* (Sakaizumi et al., 1992; Hamaguchi and Sakaizumi, 1992; Shimizu et al., 1997, 2000). Furthermore, sex-reversed XY females have been observed in hybrids between *O. latipes* and *O. curvinotus* (Shinomiya et al., 2006). Accordingly, these hybrid fish provide an experimental approach for investigating the molecular mechanisms underlying postzygotic reproductive isolation and sex determination/differentiation.

1.3.4 Inbred strains

1.3.4.1 History for establishing inbred lines

Efforts to create a standard inbred strain of medaka for laboratory use were started in 1974 by Hyodo-Taguchi, and several inbred strains have since been successfully established in the National Institute of Radiological Sciences (NIRS). In 1980, the first paper on inbred strains of medaka was published by Hyodo-Taguchi; two pedigrees of the orange-red variety (HO4 and HO5) and three pedigrees of wild-type fish (HB11, HB12, and HB32) were established. Following this, two families from different populations, a stock (d-rR) at Nagoya University and another (NI) at the University of Tokyo, have been inbred by sib-mating since 1980 and 1982, respectively, and two inbred strains of medaka, Hd-rR and HNI, were established in 1988 and 1989, respectively (Hyodo-Taguchi and Sakaizumi, 1993; Figure 1-10). In addition, two

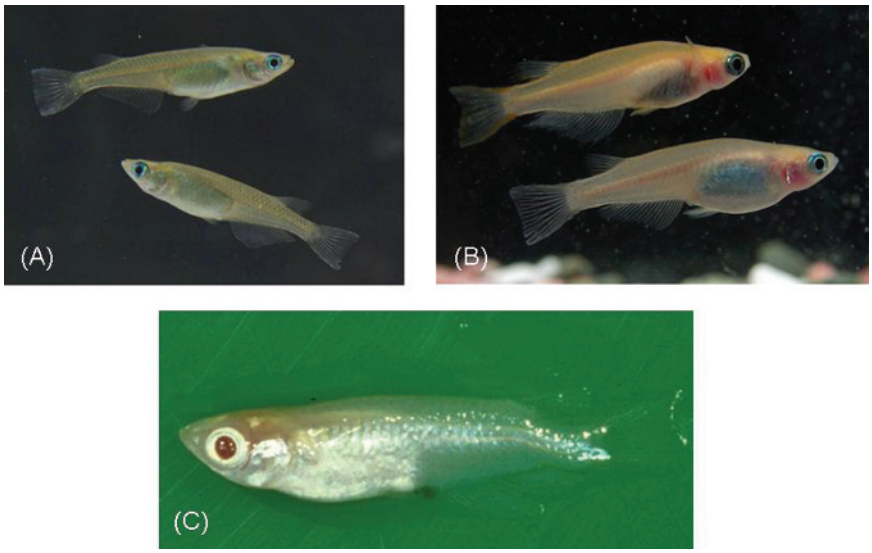

Figure 1-10. Examples of medaka inbred strains. (A) The HNI-II strain. Derived from a wild stock from Niigata. HNI-II belongs to the Northern Japanese Population. (B) The Hd-rR strain. Derived from the closed colony of d-rR strain, established by Yamamoto (1953, 1975). Hd-rR belongs to the Southern Japanese Population. (C) The Hi3 strain. Derived from the offspring of fish obtained from a stock (i3) mutant at Nagoya University. Hi3 also belongs to the Southern Japanese Population.

more inbred strains were established as HSOK and Hd-rr in 1995 from stocks at the University of Tokyo (SOK) and Nagoya University (d-rr), respectively.

1.3.4.2 Characteristics of medaka inbred strains

Thirteen medaka inbred strains are established in several laboratories in Japan and Germany. Some strains die out due to low fecundity. The following is a list of the inbred strains available (as of December 2006).

Inbred lines from wild type fish

HB32D. Derived from offspring of fish obtained from a stock at Chiba University, collected near Chiba city around 1970; inbred for 81 generations. This strain belongs to the Southern Japanese Population.

HB11A. Derived from an offspring pair of the original pair from which HB12A was derived; inbred for 92 generations. The eggs are characterized by an unusual pattern of oil globule fusion after fertilization. This trait is under single-locus control. The symbol for the gene, *of* (oil globule fusion delay), has been proposed, and the gene is recessive and autosomal (Hyodo-Taguchi, 1980). HB11A is derived from a different pair in the same stock from which HB32D was derived. This strain also belongs to the Southern Japanese Population.

HNI-II. Derived from offspring of fish obtained from the NI stock at the University of Tokyo, collected from Niigata city in 1980 (Sakaizumi et al., 1980, 1983); inbred for 57 generations (Figure 1-10 (A)). This strain was used for the genome sequencing project to identify genetic variations such as single nucleotide polymorphism (SNP) and insertion/deletion polymorphism. This strain belongs to the Northern Japanese Population.

HNI-I. Derived from a different pair in the same stock as *HNI-II*; inbred for 64 generations. HNI has different alleles to those of HO4, HB32, and HB12 at many protein loci. This strain belongs to the Northern Japanese Population.

Kaga. Derived from the Northern Japanese Population, established in EMBL (European Molecular Biology Laboratory, Heidelberg) from the closed colony of Kaga stock at the University of Tokyo (Wittbrodt et al., 2002). Kaga is the place name in Ishikawa prefecture, Japan.

HSOK. Derived from the offspring of fish from one of the Korean medaka collected in Sokcho city (Sakaizumi and Jeon, 1987); inbred for 39 generations. This strain is belongs to the East-Korean Population.

Inbred lines from the orange-red variety

HO4C. Derived from a stock in NIRS, originally obtained from a dealer in Chiba prefecture; inbred for 100 generations. Inbreeding of sub-strains HO4A, HO4B, and

HO4C3 did not succeed, due to low fecundity at around 50–60 generations. This strain belongs to the Southern Japanese Population.

HO5. Derived from a different pair in the same stock from which HO4C was derived; inbred for 93 generations. This strain belongs to the Southern Japanese Population.

Inbred lines from the d-rR strain

Hd-rR. Derived from offspring of fish obtained from a stock (d-rR) at Nagoya University. The females are white (bb, X^rX^r) and males are orange strain (bb, X^rY^R); inbred for 68 generations (Figure 1-10 (B)). As shown in body-color dimorphism, the region around the sex-determination gene, *DMY*, and the *r* locus is not homozygous (Kasahara et al., 2007). The d-rR strain has been used in experiments on sex differentiation (Yamamoto, 1953, 1975). This strain was utilized for the medaka genome-sequencing project. It belongs to the Southern Japanese Population.

Inbred line from other mutant lines

AA2. The AA2 strain is a multiple recessive tester strain with three marker loci (*b*/*b, lf*/*lf, gu*/*gu*), whereas the T5 strain is a derivative tester strain with two additional specific marker loci (i^b/ i^b, *wl*/*wl*), each of which lies on a different chromosome. This strain belongs to the Southern Japanese Population.

Hi3. Derived from offspring of fish obtained from a stock (i3) mutant at Nagoya University. An albino strain; inbred for 33 generations (Hyodo-Taguchi et al., 1997; Figure 1-10 (C)). This strain belongs to the Southern Japanese Population.

NCMH. Derived from a cultivated variegated stock in North Carolina; inbred for 35 and 47 generations. This strain belongs to the Southern Japanese Population.

Cab. This strain was originally obtained from Carolina Biological Supply, North Carolina (https://www2.carolina.com/webapp/wcs/stores/servlet/StoreCatalogDisplay?storeId=10151&catalogId=10101&langId=-1) and established as an inbred strain in Germany. It has a variegated pigmentation phenotype because it carries the *B'* allele at the *b* locus. It is noteworthy that although the original Cab strain is inbred, some laboratory lines, the so-called 'Cab', are not inbred. We observed some genetic variation within the 'Cab' strain that is widely used in many laboratories.

Column 1.1 For those who cannot decide which medaka to use

Standard strains repeatedly used among medaka researchers are the orange-red variety (himedaka), d-rR, Hd-rR, Cab, HNI, and Kaga. Generally, they are suitable for analyses of biological phenomena and are easily maintained and readily reproduce in the laboratory. It would be wise to choose one of these medaka strains for the first trial. The use of transparent strains may be another option for those who wish to study internal processes *in vivo*. The development of transgenic skills accelerates research that enables the observation of specific types of cells and molecules in living medaka. Several tranparent medaka have been developed with a variety

of uses for studying body-color alleles. The following tips may be useful as basic knowledge for choosing strains.

Medaka derived from the Southern population (Cab and Hd-rR) show polymorphic differences to those established from the Northern population (HNI and Kaga). The whole genome sequence was determined using BAC libraries constructed from Hd-rR.

Hd-rR and HNI are maintained as inbred strains. Although a little polymorphic variation has been reported in Cab, most of the artificial mutants were generated in Cab and the positional cloning was successfully performed utilizing larger polymorphism between Cab and Kaga. This may indicate that there are no substantial problems in using Cab for most phenotypic analyses.

The orange-red variety is well known as himedaka in Japanese society and can be purchased from aquarium shops. This variety was commonly employed in the lab before the strains mentioned above were readily available for researchers, but it is still used in research and education due to its easy maintenance and good reproduction under laboratory conditions.

For specific comments and details of strains, see Sections 1.2.2 (genetic diversity of medaka), 1.3.4.2 (medaka inbred strain), 1.3.4.3 (polymorphic variation), column 1.2 (variation among strains), column 3.1 (interstrain variation in reproductive performance), and 5.1.2.5 (transparent medaka).

1.3.4.3 Polymorphic variation among inbred strains

Polymorphism in several protein loci has been detected among medaka inbred strains, as revealed by electrophoresis (Hyodo-Taguchi and Sakaizumi, 1993). Polymorphic variants in the inbred strains are useful for studies of genetics and embryology. In the inbred strain HNI, derived from the Northern Japan Population, protein polymorphism differs quite markedly from those of the other inbred strains belonging to the Southern Japanese Population (Sakaizumi, 1986). The restriction fragment length polymorphisms (RFLPs) between HNI and other inbred strains are frequently observed (Naruse et al., 2000, 2004).

Polymorphism of a few enzymes has even been observed among inbred strains of the Southern Japanese Population. Three types of LDH (lactate dehydrogenase) isozymes (types I, II, and III) were found in the muscle of adult medaka from a commercial (outbred) stock. The LDH patterns of types I and III were found to have been fixed in HO4C and HB12, respectively. This difference was useful for studies of the regulatory mechanisms of expression for LDH subunits of isozymes in ontogeny (Ohyama et al., 1986) and for purification and characterization of these subunits (Sasaki et al., 1989).

1.3.4.4 To generate and maintain medaka inbred strains

It is difficult to generate new inbred strains because it takes a long time (>5 years), is labor-intensive, and care and patience are needed. One careless contamination can cause a fatal blunder. However, the basic method for generating a new inbred strain is very simple:

1. One male and female full-sibling pair are selected and mated in each generation.
2. The method of feeding larvae and adult medaka are described in Chapter 2. Under these conditions, larvae develop to sexual maturity within 2–3 months, and four to five generations can be obtained in 1 year.
3. Steps 1 and 2 are repeated 20 times.

(Comments)

During inbreeding, the reproductive potential becomes reduced or the mortality of fish becomes high in many pedigrees. Also, we have encountered a situation where all offspring were female. To avoid extinction, we have prepared 3 pairs from 1 pair as compensation. We have also prepared an exclusive room for medaka inbred strains to avoid contamination and disease.

Column 1.2 Variation among strains

Inbred strains are highly variable in morphology, physiology, and ethological properties. Such variations are derived from polymorphic genomic constructions among the original strains and biased selection during brother–sister mating. Following are some brief introductions to some examples of the remarkable characteristics of HNI and Hd-rR (Figure 1-10).

1. Morphology
   Morphological traits of inbred strains are largely dependent on their origin of wild populations. Interestingly, the traits change little due to brother–sister mating processes. HNI still strongly resembles the original Niigata medaka of the Northern Japanese Population, while Hd-rR still resembles the original d-rR strain that is derived from the Southern Japanese Population via fish farmers.

   Fins. Fins of HNI in both sexes are relatively small compared to those of Hd-rR.

   Trunk. HNI is waistless.

   Head. HNI is characterized by a smaller mouth and a more oval-face compared to Hd-rR (Figure 1-10).

2. Growth
   Growth rate. Hd-rR grows very fast after hatching and becomes an adult in 1 month at 27°C with 14 hour-light:10 hour-dark cycles. It is fascinating that growth inhibition is not clearly seen in this strain, even under high-density culture conditions.

   Maturation. It takes more than 3 months for HNI to fully mature. Another northern-type inbred strain, Kaga, can mature in a month or so. Accordingly, maturation retardation in HNI is evident and may be strain specific.

3. Behavior
   Habitat preference. HNI swims near the surface of the water. In contrast, Hd-rR prefers the bottom. When we shade the surface of the water with our hands, HNI jumps out of the water, possibly trying to get away, while Hd-rR hides in the algae on the bottom. The F1 progeny of a HNI and Hd-rR cross appear to have an intermediate habitat preference.

   Character. HNI seems to be gentle and insensitive, and is difficult to tame. In contrast, Hd-rR seems to be cautious and sensitive, but once it becomes accustomed to people it approaches them with apparent enthusiam. It is possible that they have a high learning ability to get food.

1.3.5 Differences from zebrafish

The major features of the medaka were compared with the zebrafish and are shown in Table 1-2. There are more established inbred lines of medaka than of zebrafish. There are currently only three inbred zebrafish lines, sjA, sjC, and sjD (http://zfish.wustl.edu/), whereas standard inbred lines of medaka for laboratory use were started in 1974 by Hyodo-Taguchi, and there are now thirteen such lines (Hyodo-Taguchi, 1996; Wittbrodt et al., 2002; NBRP http://www.shigen.nig.ac.jp/medaka/indexEn.html). Different inbred lines are useful for genome mapping. In fact, the

Table 1-2. Comparison of features of medaka and zebrafish.
Both animals have a lot of advantages as a laboratory animal. The remarkable features of medaka were shown in red.

Character	Zebrafish	Medaka
Generation time	8–12 weeks	8–12 weeks
Sex determination	no major gene	XX-XY (DMY gene in male)
Egg envelope	soft	hard
Period of incubation	2–3 days	7–10 days
Fecundity	100–200 eggs/week	10–30 eggs/day
Chromosome numbers	25 pairs	24 pairs
Genome size	1700 Mbp	800 Mbp
Number of inbred lines	3	13
SNP rate among strains	1%	4%
High-density genetic map	available	available
Genome analysis	in progress	finish at the draft level
Transgenesis technology	well established	well established
Active transposon in genome	not yet found	found
ES-like cells	not available	available
Gynogenetic production of embryos	available	available
Sperm cryopreservation	available	well established
Gene and/or enhancer trap method	well established	available
Oocyte culture method	not established	established
Temperature sensitive mutants	rare	many

sex-determining gene DMY was identified using the Hd-rR and HNI lines (Matsuda et al., 2002; Nanda et al., 2002). The identification of DMY has made medaka a good model for studying the mechanisms of sexual differentiation.

Mutants of the medaka have been collected for a long time in Japan. About 40 natural-color mutants of medaka are maintained in the Laboratory of Freshwater Fish Stocks, Bioscience Center, Nagoya University (Nagoya, Japan). In particular, transparent medaka are useful for noninvasive studies of morphological and molecular events that occur in internal organs. (This line is not inbred.) Because most pigments are genetically removed in fish, this fish is transparent not only at the embryonic stage but also in the later stage of life (Wakamatsu et al., 2001; Iwamatsu et al., 2003b). As with zebrafish, large-scale mutagenesis screening of mutations affecting development have been successfully performed using medaka (Furutani-Seiki et al., 2004). But as for the results of small-scale pilot screenings for mutations affecting the eye and nervous system development (Loosli et al., 2000; Ishikawa, 2000), mutants of medaka show a significantly different spectrum of mutant phenotypes from those of zebrafish. In addition, when the mutants are maintained, those fish can be preserved by cryopreservation of the sperm in the medaka. In physiological and behavioral studies, medaka exhibit superb visual acuity, optomotor tracking capabilities, and a high response of vestibuloocular reflexes (Carvalho et al., 2002; Beck et al., 2004). Medaka also exhibits sophisticated social behaviors, exemplified by schooling (Yamamoto, 1975; Egami et al., 1990; Iwamatsu, 2006). This highlights their potential as an experimental model for the analysis of various levels of behavioral response.

The tolerance of medaka to micro-electrode recording is relatively high compared to zebrafish (Suwa personal communication). This feature is also attractive for simultaneous recordings of behavior and electrophysiological response.

As described in Chapter 1, medaka has several excellent features for an experimental model in vertebrate. One prominent trait of medaka for biological material is a connection to the natural populations or natural environments. Medaka is living and evolving here. We can learn many aspects of the living organisms not only for development but also for the evolution and adaptation to the environment. A phylogenetic relationship between medaka, zebrafish, and pufferfish is also fruitful. We can understand the generality and specialty of the biological evidence found in our study with comparison of these three species. We believe these fish will shed light on understanding the new aspects of the living organisms.

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