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Review

# Medaka: a novel model for analyzing genome–environment interactions

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**Medaka is an established vertebrate model system for biological and biomedical research. It possesses unique features that make it particularly suitable for studying genome–environment interactions. Endemic to habitats spanning from 4 to 40°C and varying salinities, it combines broad ecological adaptability with experimental tractability. Its exceptional tolerance to inbreeding enabled the creation of the Medaka Inbred Kiyosu-Karlsruhe panel—80 near-isogenic, fully sequenced lines derived from a single wild population. More than 100 wild-derived, fully sequenced strains, collected throughout East Asia for more than 40 years, show relatively low intra-strain variation (inbreeding coefficient of >0.75) but high inter-strain variability (SNP rates >4%). Advanced quantification methods facilitate genome-wide association studies and quantitative trait locus mapping. The system's amenability to clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 editing and emerging epigenomic profiling enables causal validation and regulatory-mechanism discovery. Collectively, medaka offers an unparalleled vertebrate framework for integrating genetics, environment, and epigenetics—bridging evolutionary, biomedical, and population-level perspectives.**

## Highlights

As a temperate fish species, medaka tolerates fluctuating environments (e.g., seasonal changes and different water salinity levels).

Medaka are highly tolerant to inbreeding; a near-isogenic inbred panel named Medaka Inbred Kiyosu-Karlsruhe panel and several wild-derived inbred strains of medaka have been established.

These panels and strains allow the analysis of phenotype–genotype interactions under different environmental conditions and the modeling of human populations.

Advanced epigenomic approaches, including assay for transposase-accessible chromatin using sequencing, high-throughput chromosome conformation capture (Hi-C), and analysis of histone modifications and DNA methylation, extend genomic approaches in our understanding of gene–environment interactions.

## Medaka as a population genetics model for human biology

Natural populations are genetically polymorphic, with millions of genetic variants that are present mostly in the form of SNPs. This genetic diversity leads to variation of many traits. The analysis of the relationship between this natural genetic variation and the variance of quantitative traits is a very active field of research today [1]. Appropriate model organisms are crucial to analyze the underlying genetics of complex and quantitative traits. Amongst animal models, notably the fruit fly (*Drosophila melanogaster*) has proven very powerful for population genetic studies [2] but has limitations when studying vertebrate-specific traits. Mouse recombinant inbred lines were established as vertebrate laboratory genetic models to unravel phenotype–genotype relationships [3]. However, these mouse models originate from a limited parentage of a few inbred lines and thus encompass a restricted genetic variance. Therefore, their use to model outbred populations, such as humans, is restricted.

Several teleost genetic models have been established that offer many advantages, such as economical and easy breeding in captivity. In addition, extrauterine early development, combined with transparent embryonic and, to some extent, also adult tissue, permits unprecedented non-invasive observation of tissue and gene function. Zebrafish is one of the representative models [4]. The three-spined stickleback (*Gasterosteus aculeatus*) has been used to study complex quantitative traits, especially for adaptation to different ecological niches [5], and the African turquoise killifish (*Nothobranchius furzeri*) is an emerging model for studying aging and age-related diseases [6–8]. Cavefish research uncovers genetic and metabolic adaptations to extreme environments, providing models for understanding human metabolic adaptations to stress [9,10]. Platyfish (*Xiphophorus*) are key cancer models because the genetics of melanoma formation mirror

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human cancer pathways. Their hybridization-driven tumor formation allows researchers to study oncogene and tumor suppressor interactions in detail [11,12].

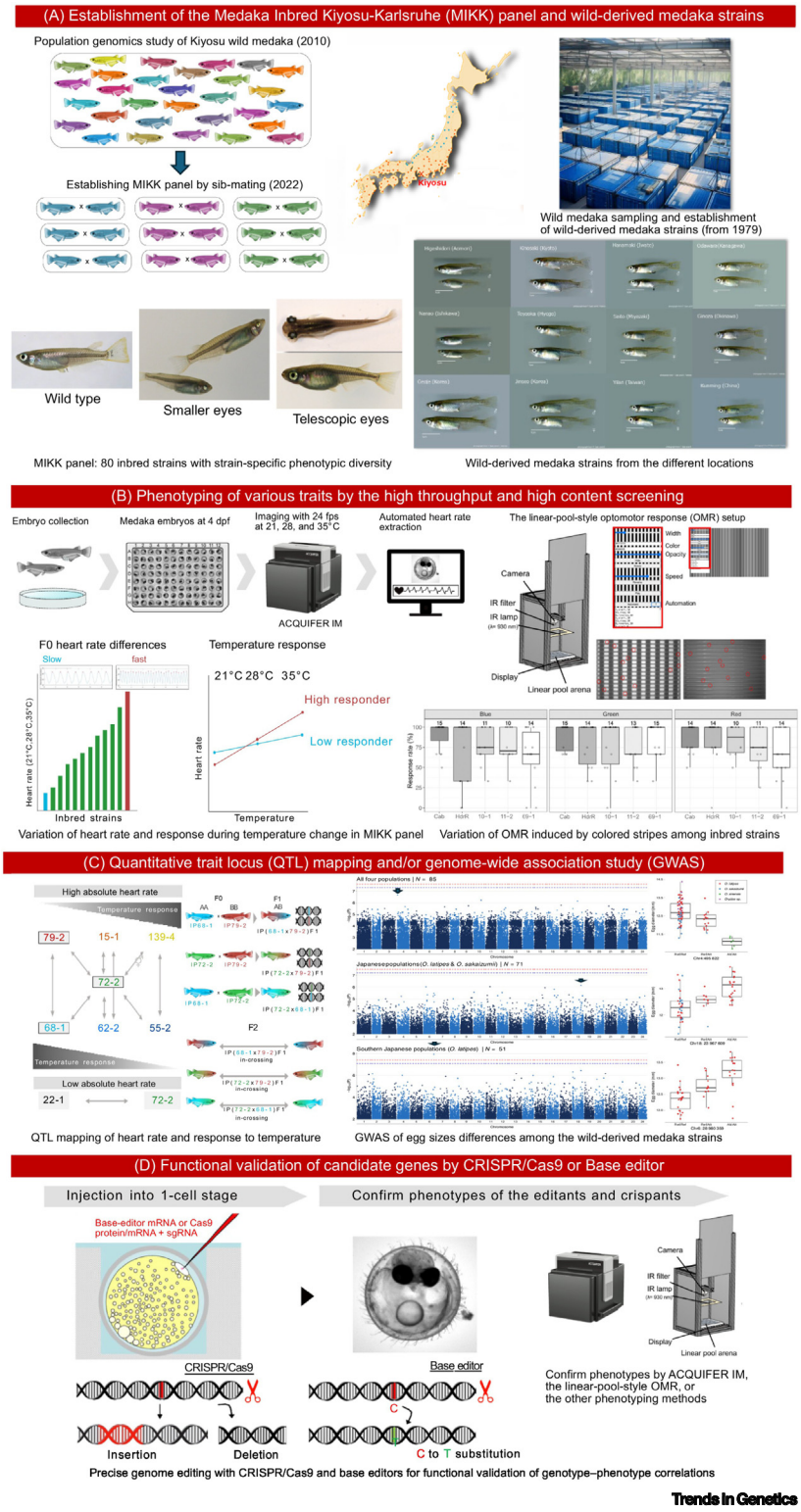
Medaka (*Oryzias latipes*), a small freshwater fish from East Asia, has emerged as an important vertebrate model organism for genetic studies [13–18]. Medaka are characterized by their small size (typically around 3–4 cm), rapid growth rate, and high fecundity and established molecular genetic tools, making them ideal for laboratory studies [18–20]. In addition to biological attributes such as a short generation time and transparent embryos, the availability of wild-derived stocks and closely related species from different localities makes medaka an excellent model for studying the evolution of sex determination [21,22], ecological strategies, the genetic basis of adaptations to different latitudes [23–26], and comparative genomic research between humans and medaka [27–29]. A major difference between medaka and other teleost models, such as zebrafish and African turquoise killifish, is their ability to adapt to a wide range of temperatures. Medaka are native to the temperate zone, so they need to be able to tolerate lower temperatures in winter, and in addition, medaka live in relatively shallow areas where the water temperature rises to 40°C in summer [16,20,30]. Medaka embryos are also tolerant of lower temperature. Embryos at the 2–4 cell stage are the most sensitive to chilling at 0°C, but 38% of them still survive after 40 min of chilling. There is no effect on the hatching rate of early gastrula embryos maintained at 0 or 5°C [31,32]. Medaka also have a high ability to adapt to seawater [33–35]. When acclimatized to freshwater, they can be transferred directly to 50% seawater, and after acclimatization for 1 week to 50% seawater, medaka can live and reproduce in seawater [33]. Spermatozoa of freshwater-reared medaka are only activated in fresh water, whereas those of 50% seawater-reared medaka are motile in both fresh and seawater [36]. This high adaptability to different environments makes medaka a unique model for studying the molecular basis of genome–environment interactions.

## Medaka Inbred Kiyosu-Karlsruhe panel

Genetic studies of complex traits, such as genome-wide association studies (GWAS), require large numbers of specimens to be analyzed in order to achieve statistical significance. Thus, an animal model should permit replication of studies with fixed genomes to control for variance of the genetic background. Medaka is highly tolerant to inbreeding, and several isogenic inbred strains have been established that have routinely been used for decades of successful laboratory studies [37,38]. Wild specimens can easily be obtained from diverse habitats, and importantly, these wild catches tolerate inbreeding without prior domestication [39,40]. To establish a population genetics resource, a panel of inbred lines was bred from a single wild founder population: the Medaka Inbred Kiyosu-Karlsruhe (MIKK) panel of Inbred lines (Figure 1A) [40]. A suitable unstructured population, without signs of introgression from aquarium medaka, such as the orange-red variety, was identified in the Kiyosu area near Toyohashi, Aichi Prefecture, Japan [41]. A full sibling-pair cross scheme was used as an efficient and economical inbreeding strategy. Wild fish were directly used for inbreeding to prevent prior genetic adaptation to husbandry conditions. From an initial 234 founder families, 80 lines reached nine generations of trait-unbiased inbreeding. The most frequent cause of extinction was all-cause mortality occurring during inbreeding generations three to five, indicating inbreeding depression was most severe during this period.

## MIKK genetic and methylation variances

Whole genome sequencing of the MIKK inbred lines revealed an increase in homozygosity across the entire genome within a line and a large number of genetic variants between different lines [40]. Whereas homozygosity of the founder Kiyosu wild population was in the range of 25%, and over 70% of the MIKK lines were found to be more than 70% homozygous. This level of homozygosity

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is comparable with that of the classical inbred strains such as iCab, Hd-rR, and HNI. Residual heterozygosity was mainly confined to the sex determination region on Chromosome 1, which is the medaka sex chromosome [51,52]. Compared with the Hd-rR reference genome [53,54], a total of 3 million variants, mostly SNPs and INDELs, were discovered in the MIKK panel. The vast majority were synonymous or intergenic variants. Among them, 600 000 were nonsynonymous and 80 000 were potential LoF (loss-of-function) variants. Comparison of human and medaka suggests that linkage disequilibrium (LD) may be lower in medaka. This indicates that when a causal variant is present in more than one MIKK panel line, the mapping resolution may be higher than in humans.

Apart from SNPs and INDELs, MIKK lines also harbor more intricate genetic variants, including copy number variants, translocation, and inversions that may also show nested arrangements [55]. It can thus be difficult to visualize the actual differences between MIKK genomic regions. Therefore, nonlinear reference alignment approaches (graph genomes) were applied to build improved representations of genetic variation in the MIKK panel [55]. This uncovered an additional variation that was masked when using standard reference alignment approaches.

## Trait variance across the MIKK panel

Wild Kiyosu fish exhibit variance of outer morphology [41]. The MIKK lines also show strain-specific craniofacial variation [40]. Relative eye size, distance between eyes, and female abdominal size parameters show line-specific variation. The broad-sense heritability ($H^2$) of these parameters is 0.68, 0.51, and 0.39, respectively. These heritability estimates are similar to those for human and mouse morphometric variance, suggesting a genetic basis for the line-specific morphometric variance in the MIKK panel. Also, at the molecular level, line-specific variation was detected within the MIKK panel. At the transcriptome level, investigation of adult liver identified more than 14 000 expression quantitative trait loci (eQTLs) in approximately 3800 transcripts. A detailed analysis of three eQTLs showed a strong correlation between the level of expression and genotype (aa, ab, and bb), indicating an expression difference as a direct consequence of the genomic sequence [40].

These findings indicate that the MIKK panel harbors a rich spectrum of genetically determined phenotypic variation as the starting point for ongoing and future phenotype–genotype association studies (Figure 1).

While variant detection represents a critical first step, establishing a causal link between a mapped polymorphism and its associated phenotype requires robust experimental validation. In this regard, medaka is a uniquely well-equipped model system. Although CRISPR/Cas9 has

Figure 1. Schematic representation of the MIKK panel and wild-derived medaka strains for analysis of gene–environment interaction and individuality using QTL mapping and GWAS. (A) The MIKK panel consists of 80 inbred lines established from a single unstructured wild population [41], Kiyosu Toyohashi, Japan, by brother-sister mating [40]. The wild medaka strains have been maintained as a closed colony since 1979. Intrastrain genetic diversity is relatively low [42], but inter-strain diversity is over 4%. As shown in Figure 1A, each inbred line of the MIKK panel has several strain-specific traits such as telescopic eye and smaller eye, as shown in Figure 1A. Wild-derived medaka strains also show several strain-specific traits. The mature size of Kunming and Yilan medaka is relatively small compared with medaka strains from Japan and East Korea [43]. (B) Examples of high-throughput phenotyping of heart rate [44] and OMR [45]. (C) Schematic of QTL mapping and genome-wide association mapping of hear rata phenotype and GWAS analysis of egg size differences among wild-derived medaka strains. Using GWAS, we identified significant peaks at chromosomes 4, 17, and 6 for egg size differences when we used the genomic data of a different population. For QTL mapping, over 1000 F2 individuals were genotyped and the median recombination block size was approximately 24 kbp [44]. (D) After identifying candidate genes with specific substitutions for the corresponding gene, base editor mRNA or Cas9 protein/mRNA + sgRNA was microinjected into 1-cell stage embryos [46–50] for functional validation of each polymorphism by phenotyping. GWAS: genome-wide association study; MIKK: Medaka Inbred Kiyosu-Karlsruhe; OMR: optomotor response; QTL: quantitative trait locus.

proven to be broadly effective across diverse organisms [46], medaka's biological features, such as smaller genome size and slower embryonic cell division with an early extension of the G2 phase, make it particularly amenable to precise genome editing via homology-directed repair, enabling efficient replacement of sequences ranging from single nucleotides to entire genes [47]. Recent advances—such as enhanced Cas9 variants [48] and the deployment of highly efficient base-editing tools [49]—have further expanded the genome engineering toolkit. Notably, the strategic use of neutral protospacer adjacent motif (PAM) sites now enables stepwise edits at previously inaccessible loci [50], opening new avenues for the precisely targeted functional interrogation of genetic variation.

## Wild-derived medaka strains

Wild-derived medaka strains have been established from medaka collected throughout East Asia over a period of more than 40 years (Figures 1A and 2) and preserved under outdoor conditions (Figure 1A) [56–58], representing a globally unique biological resource. The gradual disappearance of wild medaka [59] and the genetic introgression caused by the artificial introduction of ornamental medaka strains [60–62] make it impossible to create a similar resource at the present time.

Mitochondrial DNA analyses divide medaka into four populations: Northern Japanese, Southern Japanese, China–West Korean, and East Korean [57,58]. The Northern Japanese population was later described as *Oryzias sakaizumii* [63], and the China–West Korean population was described as *Oryzias sinensis* [64,65]. Nuclear DNA analysis identified five groups, including the Tajima–Tango group [42,66], and these groups form the *O. latipes* species complex. While no

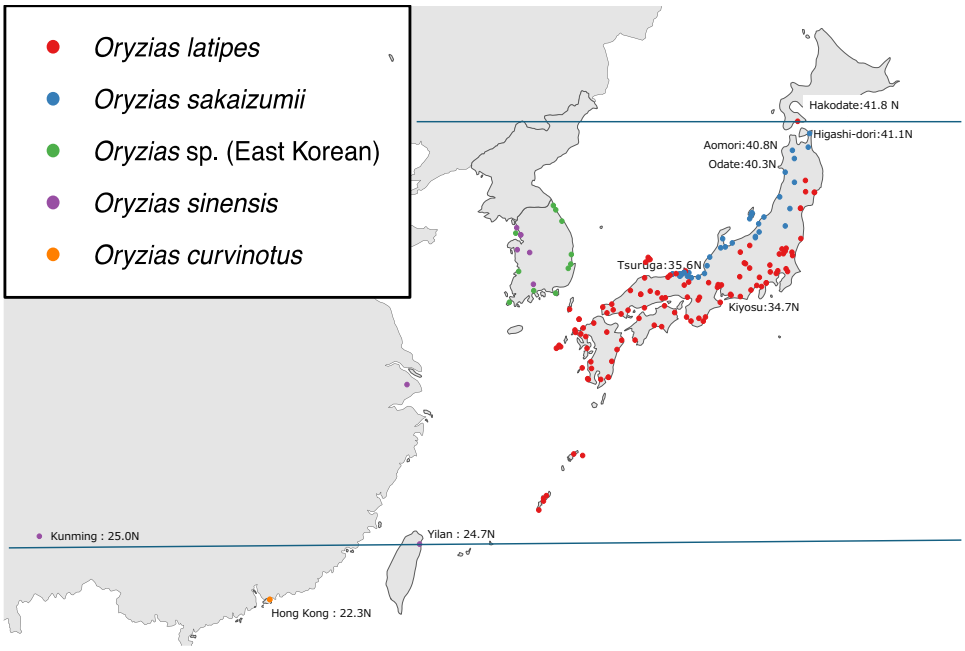


Figure 2. Original sampling sites of wild-derived medaka strains in Japan Wild medaka have been sampled from Japan, Korea, and China until 1979. There is no natural distribution of medaka in Hokkaido, but a sampling site in Hakodate in Hokkaido is believed to be an artificial introduction. This is because the medaka in the Hakodate area are *O. latipes* instead of *O. sakaizumii*. The northernmost limit of strains preserved in NBRP Medaka is Hakodate at 41.8°N, and the southernmost limit is Yilan at 24.7°N. The north latitudes of each place mentioned in the text are shown in the figure. *Oryzias curvinotus* in Hong Kong is a sister species of the *O. latipes* species complex. NBRP Medaka: National BioResource Project Medaka.

significant hybridization occurs between Southern and Northern Japanese groups, mitochondrial introgression and hybridization were detected in the Yura River population (Figure 2) [67], caused by a past river channel shift about 0.08–0.2 million years ago [68].

A genome sequencing project of about 132 wild-derived strains and related species revealed an inbreeding coefficient within the wild-derived strains of 0.75 or higher [42], indicating a high genome homogeneity and, consequently, low phenotypic variation. Today, about 180 strains are maintained at Utsunomiya University, and 100 strains have been made available from the National BioResource Project Medaka (NBRP Medaka) (https://shigen.nig.ac.jp/medaka/) (Figure 1A) [69]. The LoF variants detected by SnpEff are accessible in the MedakaBase (https://medakabase.nbrp.jp/viewer/Hd-rR/) [70]. This represents a valuable resource for phenotype/genotype association of complex traits in the wild. In addition to the wild strains, medaka-related species from Southeast Asia are available from the NBRP Medaka (https://shigen.nig.ac.jp/medaka/strain/strainTop.jsp) (see details Box 1). There are 19 species/strains available from different locations. The genome sequences of seven species [*Oryzias celebensis* (Ujung_pandang), *Oryzias javanicus* (Penang), *O. latipes* (HSOK), *O. latipes* (Hd-rR), *Oryzias luzonensis* (Solsona), *Oryzias mekongensis* (Kalasin), and *O. sakaizumii* (HNI)] have been determined [80–82] and are accessible on the MedakaBase [70]. Genome sequence information for *Oryzias dancena* (also known as *Oryzias melastigma*) and *O. sinensis* is also available at Ensembl and National Center for Biotechnology Information (NCBI) (Kim, 2018, #152; Dong, 2024, #153). Recently, medaka T2T-level genome assembly for three inbred lines (Hd-rR, HNI, and HSOK) has become publicly available on the NCBI BioProject website (https://www.ncbi.nlm.nih.gov/bioproject/PRJDB19938/). These are powerful resources for genetic and genome research.

## Trait variances of wild-derived medaka strains

The northernmost limit of wild medaka is Higashi-dori (Aomori Prefecture) in Japan, at 41.1° northern latitude, and the southern-most limit is Yilan in Taiwan, at 24.7° northern latitude (Figure 2, see a map for wild medaka sampling points in NBRP Medaka). The different latitudes mean different environmental conditions, such as day/night length, temperatures, and rain/snowfall. It has been reported that the critical day length and critical temperature for medaka oocyte maturation depend on the latitude of the habitat [24,26]. Furthermore, wild medaka living in Aomori (40.8°N), a high-latitude region, have a higher growth rate than wild medaka living in Tsuruga (35.6°N), a low-latitude region [83].

Between *O. latipes*, a southern population, and *O. sakaizumii*, a northern population, differences in egg size [43], body size, fecundity [84], vertebral number [85], and the degree of aggressive behavior between dominant and subordinate males [86] have been reported. The seasonal

### Box 1. Medaka classification and the phylogeny of *Oryzias* and relatives

The first description of medaka dates back to 1846, when Temminck and Schlegel described it as *Poecilia latipes* in Philipp Franz von Siebold's 'Fauna Japonica.' Jordan and Snyder (1906) proposed a new genus, *Oryzias*, after the Latin word for rice (*Oryza*). The order of the genus *Oryzias was largely revised by* Rosen [71] and Rosen and Parenti [72], in 1964 and 1981, respectively, mainly on the basis of morphological features such as the structure of the gill arch skeleton and hyoid apparatus, and the genus *Oryzias* as a whole was transferred to the order Beloniformes [72]. Today, the family Adrianichthyidae, to which medaka belong, consists of two described genera, *Oryzias* and *Adrianichthys*, with about 40 species in the two genera [73–75] (see Figure 3). Twenty species are now found on Sulawesi island, Indonesia, which is also the location with the highest number of species in the family Adrianichthyidae [17,74–77]. These species were previously divided into three monophyletic groups (*latipes*, *celebensis*, and *javanicus* groups, respectively) based on mitochondrial DNA and nuclear tyrosinase gene sequences [78], but in 2021, *Oryzias setnai* was found to be sister to all other groups (Figure 3) [74,79]. It is now clear that the medaka and its relatives comprise four monophyletic groups, and it is assumed that their common ancestor originated on the Indian subcontinent around 70 million years ago [74].

change in gut length—longer in summer and shorter in winter—occurs in southern medaka, but not in the northern population. This plasticity is regulated by CpG methylation upstream of the *Plxnb3* gene, while genetic variation in the promoter region of *Ppp3r1* makes the gut stably longer in northern medaka [87]. Wild medaka from Odate (40.3°N) display a regular heart beat at both 15°C and 25°C, whereas the southern Hd-rR strain develops irregular heartbeats at 15°C [88].

## Genomic and epigenomic resources for understanding genome–environment interactions

Recent advances in genomic and epigenomic methodologies have significantly expanded the resources available for medaka research, providing crucial insights into the regulatory mechanisms underlying quantitative trait variation. This is particularly relevant given that many quantitative trait variations arise from functional changes in *cis*-regulatory sequences or alterations in 3D chromatin architecture rather than from changes in the coding sequence alone.

Analysis assay for transposase-accessible chromatin using sequencing (ATAC-seq) in medaka provides a resource for chromatin accessibility and identified over 149 000 accessible chromatin regions at nine developmental stages, 90% of which displayed dynamic changes during development [89]. Combining ATAC-seq and transcriptomic data [89] revealed *cis*-regulatory elements that drive isoform switching for the developmental key genes, deepening our understanding of developmental gene regulation [89].

The characterization of the 3D chromatin architecture by Hi-C analysis in medaka has revealed mammalian-like 3D chromatin structures, including A/B compartments and CCCTC-binding factor (CTCF)-mediated loops [90,91]. Although CTCF binding starts early in development, chromatin loop formation starts mainly during gastrulation, aligning with key developmental transitions [90]. According to recent research, developmental genes and housekeeping genes are differentially regulated by histone acetylation during zygotic genome activation, which is also intriguing to epigenetic researchers [92].

Advanced epigenome editing systems [93] have allowed direct testing of histone mark functions, establishing medaka as a key teleost model for epigenetics research. Finally, medaka exhibits histone mark dynamics that are conserved across teleosts and is therefore a key model for both mechanistic and environmental epigenetics research [94]. Studies on histone modification in medaka revealed that H3K27ac, H3K27me3, and H3K9me3 marks are retained after fertilization, while H3K4 methylation is erased during cleavage stages. Retained H3K27ac enables gene activation, and H3K9me3 at telomeres ensures genome stability [95]. At the level of DNA modification, CpG islands in developmental genes remain hypomethylated, which helps to preserve their transcriptional activity during development [96].

## Future use of the MIKK panel and the wild-derived medaka strains

Using classical inbred medaka strains such as HNI and Hd-rR for quantitative trait locus (QTL) mapping has enabled the analysis of complex traits, including craniofacial morphology [97] and startle response behavior [98]. Hundreds of morphological features with variation between different strains have been identified. F2 association mapping identified QTLs that account for a substantial phenotypic variance, confirming a genetic contribution to these complex traits in medaka [97].

High-resolution mapping of genetic variants regulating complex traits has recently been achieved in three studies addressing heart rate [44,99] and the variance of the embryonic somitic clock driving axis segmentation [100].

The somitic clock regulates antero-posterior axis segmentation and shows temporal and spatial variation between classical inbred strains [100]. A classical F2 association study of 600 F2 embryos was employed to map QTLs. Functional validation by CRISPR/Cas9 showed that two of eleven associated QTLs affected the period of the somitic clock, while two of four candidates influenced presomitic mesoderm size [100].

Loci contributing to heart rate and function were mapped by a GWAS using two classical isogenic inbred strains (Hd-rR and HO5), which exhibit a difference in heart rate and function. Functional validation of the top 12 candidate genes by CRISPR/Cas9 genome editing verified their causal role in heart rate, heart development, ventricle size, and arrhythmia [44].

A study exploiting the MIKK panel as a population genetics resource used 76 inbred strains to fine map heart rate plasticity between inbred lines (G × G, gene by gene) and across temperatures (G × E, gene by environment). This GWAS identified 16 heart rate QTLs. Subsequent validation confirmed functional relevance for eight of these QTLs. Four of the five candidate genes (*atg7*, *ryr2b*, *ccdc141*, *ppp3cca*, and *sptbn1*) were found to have temperature-sensitive effects on heart function using gene editing [99].

The identification of CCDC141 and RYR2 as temperature-responsive genes in medaka that correspond to human cardiac GWAS signals represents a fundamental discovery: core cardiac rate regulators are evolutionarily conserved across vertebrates. CCDC141 has been identified in multiple human GWAS studies as a locus associated with resting heart rate and cardiac conduction phenotypes [101], while RYR2 mutations are clinically associated with arrhythmogenic diseases, including catecholaminergic polymorphic ventricular tachycardia [102]. Despite the ecological differences between medaka thermal adaptation and human cardiac physiology, these findings demonstrate that the molecular mechanisms governing heart rate determination are remarkably consistent throughout vertebrate evolution. Beyond individual genes, medaka temperature-dependent heart rate responses involving PPP3CC, SPTBN1, and ATG7 reveal a striking concordance with human GWAS signals for cardiovascular stress adaptation, myocardial remodeling, and vascular resilience. The largest recent meta-analysis of resting heart rate identified 493 genetic variants across 352 loci in up to 835 465 individuals, demonstrating that genetic signals affecting heart rate commonly correlate with blood pressure traits and multiple cardiovascular disease outcomes [102]. This correspondence indicates that the molecular machinery for physiologic stress sensing and response is deeply conserved across species—a critical observation for understanding how organisms maintain cardiac homeostasis across diverse environmental conditions.

Medaka offers a powerful experimental system for functionally validating human GWAS loci affecting cardiac traits. A systematic validation study comparing 40 human heart phenotype-associated GWAS candidates with medaka embryonic models found that 57% of candidates assigned to human heart rate in GWAS also affected heart rate in fish embryos, successfully identifying 16 genes with diagnostic and predictive power for human cardiovascular disease [103]. The medaka model integrates three essential components—genomic variation, environmental perturbation (temperature), and molecular phenotyping—that directly mirror the fundamental challenges in human cardiovascular genetics: elucidating how genetic and environmental factors interact to determine heart rate and cardiac function across vertebrates [99,102]. The convergence of medaka and human GWAS findings extends beyond individual genes to encompass shared biological pathways: calcineurin signaling, cytoskeletal organization, and autophagy. This pathway-level perspective is more informative than single-gene approaches for two critical reasons: it reveals the functional networks underlying complex trait variation, and it provides a

more robust framework for identifying therapeutic targets that address root causes rather than isolated molecular players.

Together, these findings establish medaka as an invaluable model for understanding the conserved molecular basis of vertebrate cardiac rate control and stress responses, while demonstrating that pathway-level integration—not single-gene analysis—is essential for translating GWAS findings into mechanistic insights and clinical applications.

Already several assays for high-throughput quantitative phenotyping in medaka have been published. For example, the morphometric micro-computed tomography (CT) analysis of medaka fish *in toto* [104], the resting native heart rate analysis [105], a NMR-based metabolome fingerprinting [106], and the optomotor response assay [45] have been shown to detect quantitative strain-specific variance and are scalable to reach statistical significance for QTL mapping [44].

Integrating genomic, transcriptomic, and epigenomic datasets in medaka enables comprehensive gene regulatory network analyses for complex traits. ATAC-seq combined with RNA-seq identifies stage-specific regulatory elements and targets [89,107], while Hi-C data provide spatial context for regulatory interactions [90,91]. This multiomics approach helps to clarify how genetic variants impact gene regulation via chromatin accessibility, 3D chromatin contacts, histone modification, and DNA methylation [108]. The importance of noncoding and structural variants is increasingly recognized in human genetics, and similar research in medaka reveals their contribution to quantitative trait variation. Extensive epigenomic data for the MIKK panel [55] and wild strains [87] will provide valuable resources to study genome–environment interactions in vertebrates. Thus, with the integration of multiomics approaches, the stage is set for medaka as a vertebrate model for high-resolution GWAS and QTL mapping of complex traits linking genome and environment.

## Concluding remarks

The medaka MIKK panel and wild-derived strains provide a unique resource for dissecting the complex interplay between genotype, environment, and phenotype. Their high adaptability and genetic diversity make medaka an exceptional vertebrate model for gene–environment interaction and individuality studies. Integrating genomics, multiomics, and advanced genome editing has greatly expanded the capacities for deep functional analyses. Comparative approaches, including comparisons with human GWAS findings, highlight medaka's role in modeling human population traits. Ongoing research continues to leverage the MIKK panel and wild strains for high-resolution mapping of complex traits (see Outstanding questions). With scalable quantitative assays and accessible genetic resources, medaka research is poised for rapid progress. Their broad utility extends to questions in population biology, epigenetics, adaptation, and evolutionary genomics. Future integration of new molecular tools and high-throughput phenotyping will further enhance the relevance of medaka as a model. Medaka prominently complements other vertebrate models, bridging environmental, molecular, and quantitative genetic research for the next generation of biological discovery.

### Acknowledgments

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## Outstanding questions

How does phenotypic plasticity in medaka populations facilitate adaptive evolution, and how does its contribution compare with that of genetic variation in shaping evolutionary trajectories?

How can functional insights from the Medaka Inbred Kiyosu-Karlsruhe panel and wild-derived medaka strains be leveraged to interpret human genome-wide association studies findings for complex traits and gene-by-environment interactions?

To what extent do genetic variants in the Medaka Inbred Kiyosu-Karlsruhe panel and wild-derived strains shape genome-wide epigenetic modifications (DNA methylation, histone marks), and how do these genotype-dependent epigenetic differences modulate phenotypic variation and plasticity?

What are the molecular sensory and signaling mechanisms that allow medaka to detect and respond to seasonal environmental cues (temperature and photoperiod), and how do these mechanisms translate environmental signals into adaptive physiological and behavioral responses?

What molecular and genetic mechanisms underlie phenotypic plasticity in medaka, and do these mechanisms differ across distinct plastic traits—such as salinity-dependent sperm motility versus seasonally regulated gut length variation?

Why do some natural alleles exhibit temperature-dependent phenotypic effects, while others do not? Are these effects primarily mediated by altered protein thermostability (as in classical temperature-sensitive mutations), or do alternative mechanisms—such as temperature-sensitive gene regulation—also contribute?

Can amino acid substitutions in specific genes alter protein thermostability to mediate temperature-dependent phenotypic effects observed in medaka? Alternatively, are there temperature-sensitive phenotypic effects mediated by nonprotein mechanisms—such as temperature-dependent changes in regulatory element activity or RNA secondary structures?

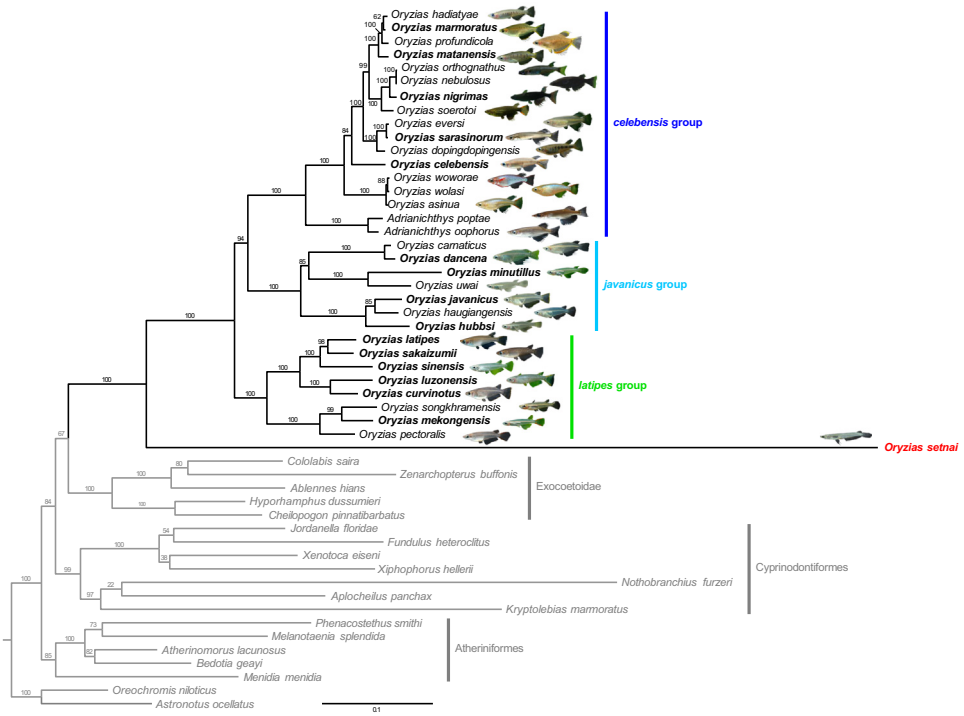


Figure 3. Maximum likelihood phylogeny of Adrianichthyid taxa based on the concatenated sequences of mitochondrial (11 233 bp) and nuclear (4204 bp) DNA sequences. Two species of Cichlidae, *Oreochromis niloticus* and *Astronotus ocellatus*, are outgroups. Numbers on branches are maximum likelihood bootstrap values. The family Adrianichthyidae consisted of four monophyletic groups (*celebensis*, *javanicus*, and *latipes* groups and *O. setnai*). The Adrianichthyidae and Exocoetoidae have a sister relationship and are both members of the order Beloniformes [71,72]. The order Beloniformes and Cyprinodontiformes have a sister relationship. Modified from Supplementary Figure 2 of Yamahira *et al*. [74]. Species shown in bold type are available from NBRP Medaka (https://shigen.nig.ac.jp/medaka/). NBRP Medaka: National BioResource Project Medaka.

## Declaration of interests

The authors declare no competing interests.

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