

## Variation in the Number of Anal Fin-Rays in *Oryzias latipes* with Special Reference to Closely-Distributed Populations

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Variations in the number of anal fin-rays in natural populations of the Western Seto-Inland and the San-in varieties of medaka, *Oryzias latipes* were investigated. The number of anal fin-rays showed a Gaussian distribution with mean peak values ranging from 16.3 to 18.7. A single strain from the San-in district had significantly fewer fin-rays (16.3,  $p < 0.001$ ). The phenotype of the number of fin-rays is under polygenic control. Evolution of Japanese rice and medaka is discussed.

**Keywords:** Fin-rays; Gaussian distribution; evolution; medaka.

### Introduction

Killifish (*Oryzias latipes* Jordan and Snyder, 1906, medaka in Japanese) is endemic to all of the islands of Japan except Hokkaido. In the Yedo era (1603-1867) in Japan, medaka was a familiar fish and was depicted in the *ukiyo-e*, (woodblock print), “medaka Sukui” (Figure 1) by Harunobu Suzuki (1760). During the same era, the well-known scientist, P. F. B. von Siebold, imported medaka into the Netherlands from Japan where they have been maintained as an important species for study. The generic name of medaka, *Oryzias*, is derived from their primary habitat, which is rice “paddy fields”, and the specific name, *latipes*, which means “wide leg” and refers to the wide anal fin of the species.

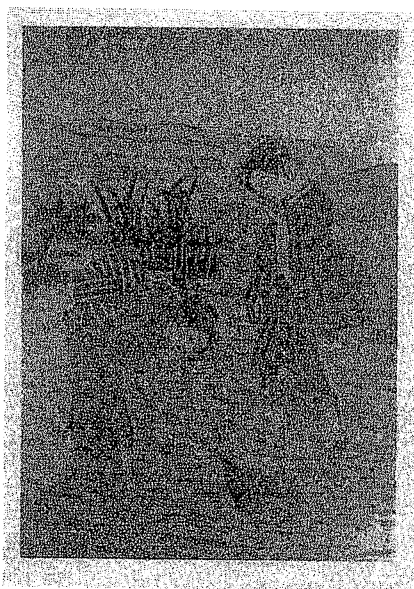


Fig. 1. “medaka Sukui” by Harunobu Suzuki (1760) in the Yedo era.

The number of anal fin-rays was first studied by Temminck and Schlegel (1842) who reported that specimens collected in Nagasaki Prefecture was 17 (cited in Egami, 1953). Egami (1953, 1954) and his colleagues and Egami and Yoshino (1958) then undertook a survey of the variation in the number of anal fin-rays in medaka. More recently, the Sakaizumi group at Niigata University have assayed the biodiversity of natural populations using allozymes and mtDNA variability. Genomic DNA Studies that include the Nago population from the island of Okinawa have also recently been undertaken (Kasahara *et al.*, 2007). Nonetheless, the biodiversity of closely distributed natural populations is unclear. In this article, therefore, we examined the variation in the number of anal fin-rays in the Western Seto-Inland and the San-in populations of *Oryzias latipes* (Sakaizumi, 1986, Figure 2).

## Materials and Methods

### Fish

A total of eight wild medaka strains and one mutant strain were used in this study. Alphabetical letters are representatives of the Northern population (T), the Southern population, the Western Seto-Inland population (K and K-11 are equal site but the different collected dates, K-17, I, and YG), and the San-in population (HK, HM, and HS) (Figure 2, 3). Individual strains were reared in 13 L containers measuring 33 cm (w) x 19 cm (d) x 21 cm (h) at 25 °C as reported previously by Kawata *et al.* (2006a, b) and Azeoka *et al.* (2008).

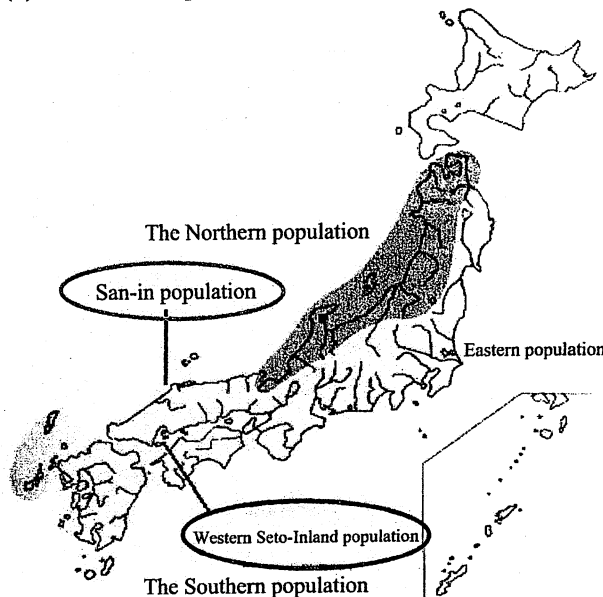


Fig. 2. The Western Seto-Inland and the San-in populations belonging to the Southern population of medaka cited by Sakaizumi (1986).

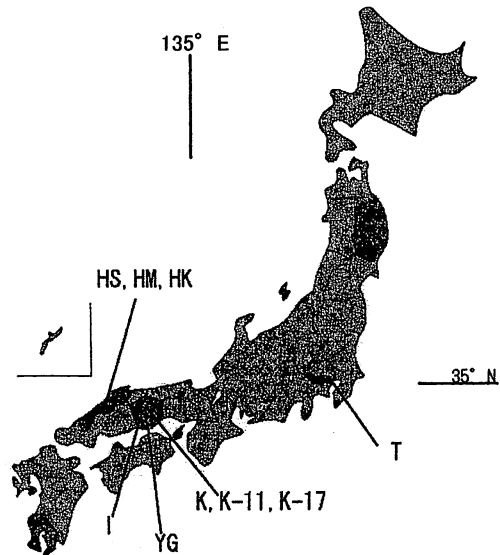


Fig. 3. Collection sites of medaka natural populations. Alphabetical letters show in Materials and Methods.

### Calculation of the Number of Anal Fin-Rays

Anal fin-rays were counted using the method of Kawata *et al.* (2006a) (Figure 4). Individual medaka were placed in a small volume of water (10 mL) and examined under a microscope (15 x magnification).

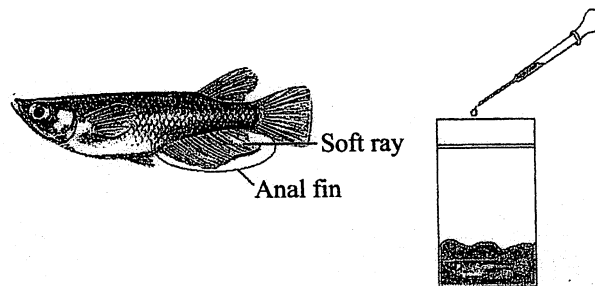


Fig. 4. Measurement of the number of anal fin-rays.

## Statistics

Statistical significance was determined as in Sokal and Rohlf (1969).

## Results and Discussion

### Intraspecific Variation

Since no statistical difference in the number of anal fin-rays was observed between male and female medaka, mean values (mean  $\pm$  standard deviation) were pooled for both sexes. Table 1 summarizes the mean number of anal fin-rays in eight strains in addition two strains cited in Egami (1953); a total of nine (YG strain) to 89 (K-17 strain) specimens of each strain were examined. Values were the same in 10 strains, except for the HS strain of the San-in population, in which the mean value was significantly smaller ( $16.3 \pm 0.7$ ,  $p < 0.001$ ,  $n = 27$ ). In addition, statistically significant genetic differences were observed in the three strains (HK, HM, and HS) of the San-in population collected from fields in close geographic proximity with no geographic barriers as islands and the water system. These findings suggest that genetic differentiation is not due to migration around the domestication alongside a land and/or river, but rather that it is due to random genetic drift in the San-in population.

Table 1. Number of the anal fin-rays.

Population	Strain	N	Mean $\pm$ SD
Western Seto-Inland population	K,K-11	87	$18.7 \pm 1.1$
	K-17	89	$17.5 \pm 1.0$
	I	12	$18.3 \pm 0.8$
	YG	9	$17.3 \pm 0.5$
San-in population	HK	17	$17.6 \pm 0.8$
	HM	18	$17.3 \pm 0.8$
	HS	27	$16.3 \pm 0.7$ ***
Eastern population	T	19	$18.6 \pm 1.2$

\*\*\*  $p < 0.001$

### Fin-Ray Distribution and Variance

An examination of the frequency distribution of fin-ray number showed that fin-rays on the left and right sides were equally distributed with a Gaussian distribution (Figure 5). These results indicate that the number of fin-rays is a typical quantitative genetic trait and also that it is under polygenic control and does not exhibit Mendelian inheritance.

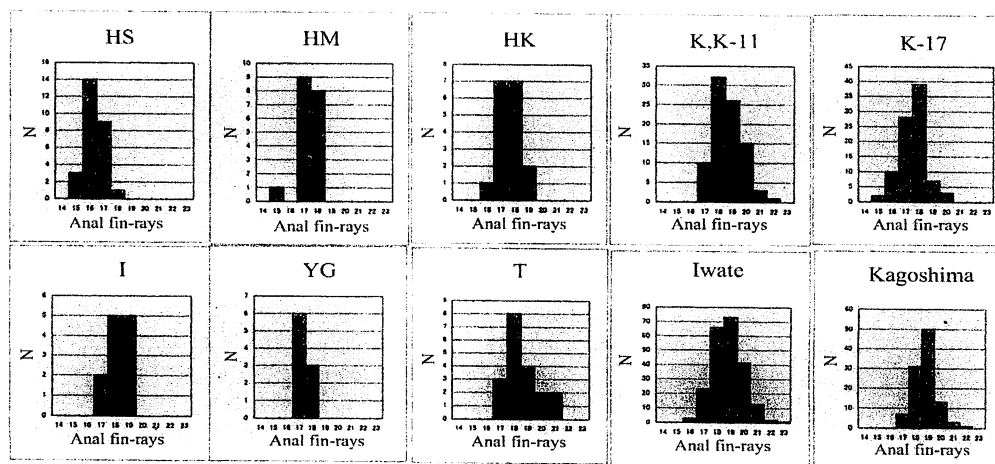


Fig. 5. Gaussian distribution of the number of anal fin-rays.

### Cumulative Analysis

The frequency distribution of mean number of anal fin-rays shown in Figure 5, each number of anal fin-rays was added and the cumulative percentage was plotted (Figure 6). These findings illustrate that the means of samples are themselves Gaussian distributed regardless of sample size ( $n$ ). Thus, the mean number of anal fin-rays in specimens of a Gaussian distributed of the data. Medaka has a Gaussian distribution whether  $n$  is nine or 222.

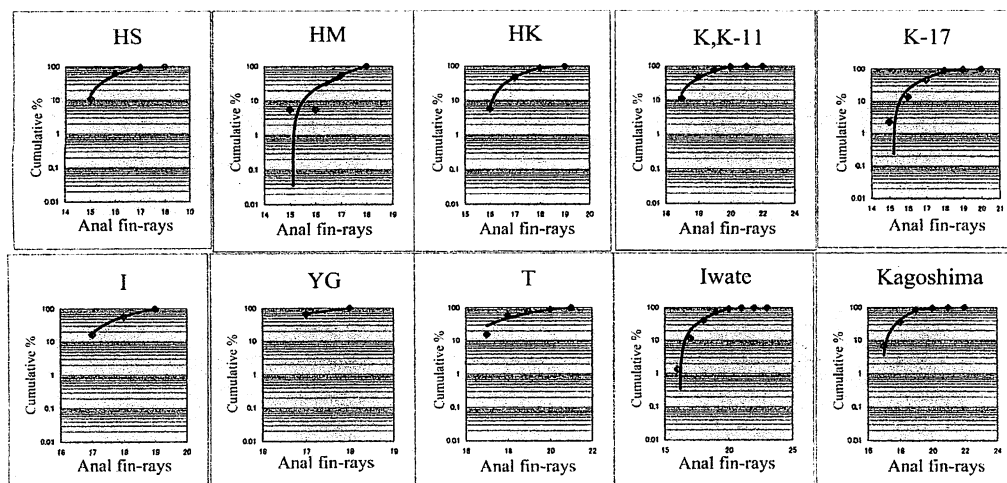


Fig. 6. Cumulative percentage of distribution of the number of anal fin-rays.

### Theoretical Considerations

As shown above, the number of anal fin-rays is controlled by polygenes and has a Gaussian distribution, which can be represented using the following model equation:

$$P = G + E$$

where  $P$ ,  $G$ , and  $E$  refer to the Phenotypic component, Genotypic component, and Environmental variation, respectively. In other words,  $P$ ,  $G$ , and  $E$  refer to the number of anal fin-rays, polygenes, and natural conditions at the site where the medaka were captured, respectively. If the genotype-environmental covariance of  $G$  and  $E$  is greater than 0, ( $2 \times V_{GE}$ ), then variance in the sample population as:

$$V_P = V_G + V_E + 2 \times Cov_{GE}$$

where  $V_P$ ,  $V_G$ ,  $V_E$ , and  $Cov_{GE}$  refer to Phenotypic, Genotypic, Environmental variability, and Covariance between Genotype variability and Environmental variability, respectively. In the three populations from the San-in district (HK, HM, and HS), no geographic barriers have acted to isolate the natural populations. This relationship can be written as:

$$V_P = V_G + V_E \quad (V_E \doteq 0),$$

suggesting that the number of anal fin-rays is not controlled by environmental factors, but rather by polygenes and genetic variability.

### Evolution of Medaka

Given the prevalence of medaka in rice field paddies, and the similarity in the taxonomic names of medaka and Japanese rice -“*Oryzias latipes*” and “*Oryza sativa*”, respectively- it has long been thought that medaka immigrated to Japan “with” Japanese rice. However, medaka has been distributed from the continental clade to Japan (5.4-6.0 million years ago, in the Paleolithic era, Takehana *et al.*, 2003) and associated with Japanese rice since about 0.3 million years ago (the Jomon period, approximately 0.6-0.3 million years ago). Consequently, in this article we propose that medaka were not introduced into Japan with rice.

The phylogenetic relationships, migration history of the species to Japan and estimated divergence of the Western Seto-Inland and the San-in medaka populations are unclear. Takehana *et al.* (2003) proposed that a mitotype of the San-in subcrade distributed in the region approximately 0.5-2.3 million years ago, and that these populations of the San-in subcrade were primarily distributed the northern Kyushu district of western Japan based on mtDNA (*cytochrome b*) analysis. In addition to medaka, the “Jomon-people”, Shiba dog (San-in, Tanabe, 2006), and Tsushima wild cat (web letter, 2008) all came from Central Asia to Japan via the Korea

Peninsula. Genetic divergence in the closely distributed natural populations of the San-in district “may” indicate that random genetic drift came from the Korean Peninsula to the San-in district and that this was genetic differentiation has subsequently been maintained.

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### References

- Azeoka, H., Kasugaya, S., Higashi, M., Takeuchi, T., and Asada, N. (2008)  
Genetic variation of the number of anal fin-rays in medaka, *Oryzias latipes*. Proceedings of the Chugoku-Shikoku branch of the Zoological Society of Japan, 60: 19. (Japanese).
- Egami, N. (1953)  
Studies on the variation of the number of the anal fin-rays in *Oryzias latipes*. I. Geographical variation in wild populations. Japanese Journal of Ichthyology, 3: 33-35. (Japanese).
- Egami, N. (1954)  
Studies on the variation of the number of the anal fin-rays in *Oryzias latipes*. II. Cross experiments. Japanese Journal of Ichthyology, 3: 171-178. (Japanese).
- Egami, N., and Yoshino, M. (1958)  
Studies on the variation of the number of the anal fin-rays in *Oryzias latipes*. III. Supplementary note on the geographical variation in the wild populations. Japanese Journal of Ichthyology, 7: 83-88. (Japanese).
- Kasahara, M. *et al.* (2007)  
The medaka draft genome and insights into vertebrate genome evolution. Nature, 447: 714-719.
- Kawata, N., Yamaguchi, Y., Tanigawa, S., Takeuchi, T., and Asada, N. (2006 a)  
Genetic variation of natural population in the experimental species, *Oryzias latipes*. Proceedings of Okayama Association for Laboratory Animal Science, 23: 47. (Japanese).
- Kawata, N., Yamaguchi, Y., Moriyama, A., Takeuchi, T., and Asada, N. (2006 b)  
Genetic variation in the southern population of medaka, *Oryzias latipes*. Proceedings of the Chugoku-Shikoku branch of the Zoological Society of Japan, 58: 21. (Japanese).
- Sakaizumi, M. (1986)  
Genetic diversity in wild populations of medaka, *Oryzias latipes* (Pisces: Oryziatidae) from Japan and China. Genetica, 69: 119-125.
- Sokal, R. R., and Rohlf, F. J. (1969)  
Biometry, The principles and practice of statistics in biological research.  
In: W. H. Freeman and Company, San Francisco, CA.
- Takehana, Y., Nagai, N., Matsuda, M., Tsuchiya, K., and Sakaizumi, M. (2003)  
Geographic variation and diversity of the *cytochrome b* gene in Japanese wild populations of medaka, *Oryzias latipes*. Zoological Science, 20: 1279-1291.
- Tanabe, Y. (2006)  
Phylogenetic studies of dogs with emphasis on Japanese and Asian breeds. Proceedings of Japanese Academy, 82: 375-387.
- Yamaneko <http://www.tokyo-zoo.net/> (Inokashira Park Zoo, Musashino, Tokyo).