論文の内容の要旨

論文題目：Study on intraspecific diversity and genetic background of startle response in medaka (*Oryzias latipes*)

（ニホンメダカの驚愕反応の種内多様性と遺伝的要因の解析）

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Genetic polymorphisms are thought to generate intraspecific behavioral diversities, both within and among populations. The mechanisms underlying genetic control of behavioral properties, however, remain unclear in wild type vertebrates, including humans. To explore this issue, I used diverse inbred strains of medaka fish (*Oryzias latipes*) established from the same and different local populations. In the process of inbreeding, the genetic variation within the species had led to differences among strains. The genetic background itself is thought to define strain-specific traits because each inbred strain is genetically homogeneous with its unique genotype.

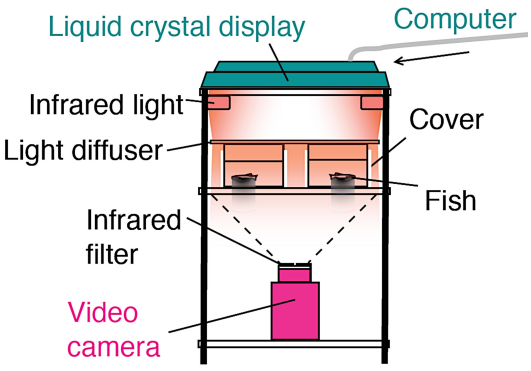

Figure 1　Experimental apparatus

Figure 2　Startle response of medaka

First, I established a behavioral paradigm to quantify visually-evoked behavioral characteristics of medaka using a liquid crystal display (Figure 1), and found that found that extinction of illumination robustly elicited a startle response in medaka. Medaka exhibited a startle response to the visual stimulus by rapidly bending their bodies (C-start) 20-ms after the stimulus presentation (Figure 2).

Then I measured the rates of the response to repeated stimuli (1-s interval, 40 times) among four inbred strains, HNI-I, HNI-II, HO5, and Hd-rR-II1, and quantified two properties of the startle response: sensitivity (response rate to the first

stimulus) and response stability index (halfway point of response reduction, which negatively correlates with degree of attenuation of the response rate). Among the four strains, the greatest differences in these properties were detected between HNI-II and Hd-rR-II1. HNI-II exhibited high sensitivity (approximately 80%) and no attenuation, while Hd-rR-II1 exhibited low sensitivity (approximately 50%) and almost complete attenuation after only five stimulus presentations (Figure 3). HNI-II and HNI-I, both of which had been generated from the same local-wild population, also showed a difference in the response stability index. These results suggested behavioral diversity of the startle response within a local-wild population as well as among different populations.

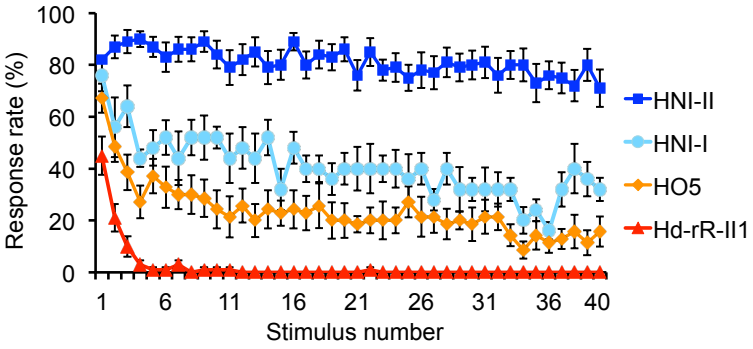

Figure 3 Transition of response rate of four inbred strains

Linkage analysis with F2 progeny between HNI-II and Hd-rR-II1 revealed different quantitative trait loci (QTL) for sensitivity and attenuation. For sensitivity, the QTL was detected on linkage group 3 from analysis with F2 progeny from Hd-rR-II1 female and HNI-II male. The QTL for attenuation was detected with a maximum logarithm of odds (LOD) score of 11.82 on linkage group 16 from analysis with the entire F2 population from reciprocal crosses (Figure 4).

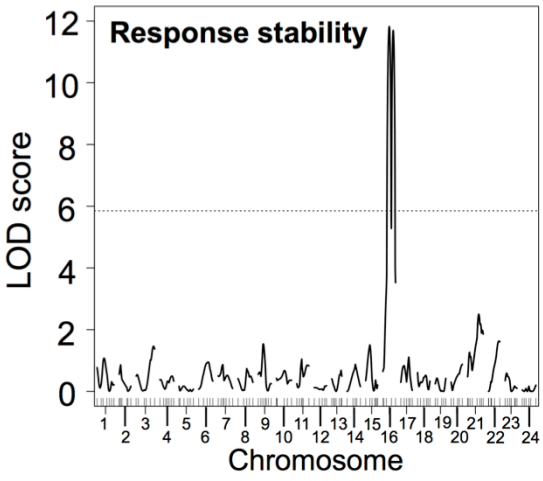

Figure 4 QTL analysis for response stability index (Dashed line: p = 0.05)

To narrow down the candidate region, and to examine interaction with other genomic regions, I established three congenic lines, each of which has genomic background of Hd-rR-II1 and has only limited region on chromosome 16 replaced by genome of HNI-II (Figure 5). One of the congenic lines showed significantly lower degree of attenuation than Hd-rR-II1, suggesting that the certain genomic region can alter the trait without interaction between other loci (Figure 6). The degree of attenuation of the congenic line was still higher than HNI-II (Figure 6), suggesting additive effects of other loci and/or interactive effect with other loci to this trait.

The findings in this study are the first to suggest that a single genomic region might be sufficient to generate a portion of individual differences in startle behavior between wild type animal strains. Further identification of genetic polymorphisms that define the behavioral trait will contribute to our understanding of the neural mechanisms underlying behavioral diversity, allowing us to investigate the adaptive significance of intraspecific behavioral polymorphisms of the startle response.

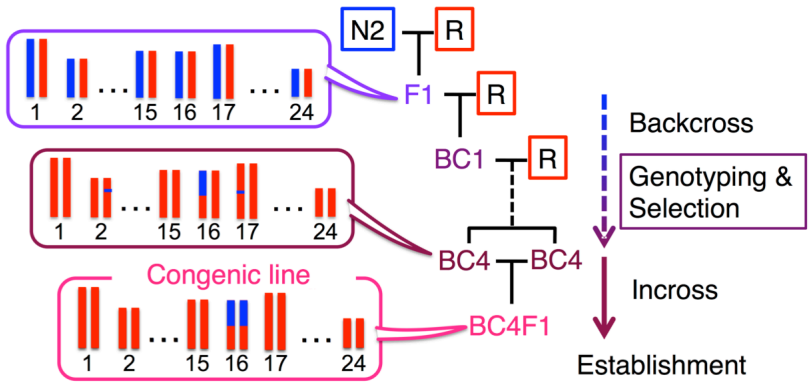

Figure 5 Establishment of a congenic line by speed congenic method

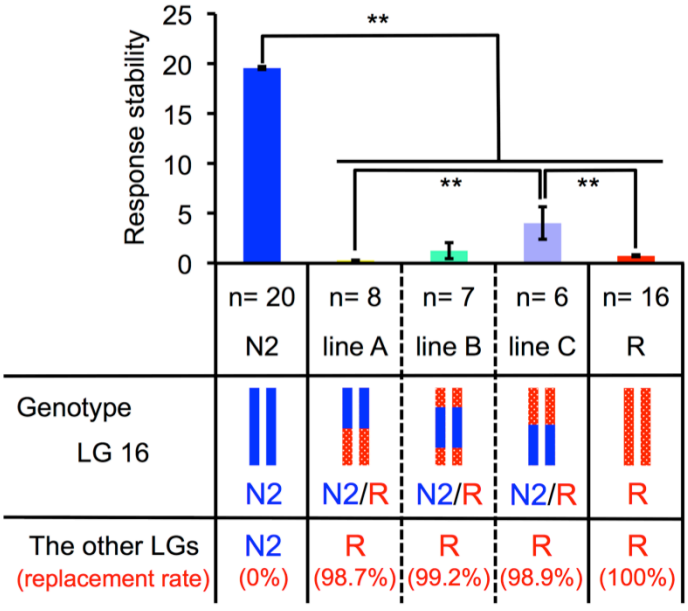

Figure 6 Genotypes and values of response stability index of the congenic lines

(N2, HNI-II; R, Hd-rR-II1; LG, linkage group. Bars = SEM. **: $p<0.01$ by Scheffe's F test)