



Review of USB FFPW PhD thesis

First name(s), surname, titles of the PhD student: Tomáš Tichopád, Mgr.	First name(s), surname, titles of supervisor: Assoc. Prof. Dipl.-Ing. Martin Pšenička, Ph.D.
Title of PhD thesis: The effect of polyploidization and hybridization on reproductive physiology in fish	

REVIEWER:

Surname: Arai	Institution: - Hokkaido University, Japan
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Please describe your professional relationship to the PhD student: International reviewer	Please describe your field of expertise: Genetics and Developmental Biology of Fishes

QUESTIONNAIRE

Originality, scientific importance, perspectives and impacts of results presented in the PhD thesis for basic and/or applied research

Evaluate competitiveness of the PhD thesis in the international context and compare its level with the current state of the art in the field (extent ¼ – ½ page):

Both sterility and asexual reproduction are generally considered consequences of certain incompatibility between nonhomologous genomes brought by hybridization and polyploidization, which frequently give rise to fluctuation in meiosis and gametogenesis. The present Ph.D studies provided new insights into well understanding of inter-relations among hybridity, polyploidy and asexuality and all the results obtained contribute the profound comprehension on the most important biological event, i.e., meiosis. Especially, approaches using unique materials including hybrid and polyploid fishes which asexually reproduce in nature were evaluated to be highly original. Experimental procedures used in the Ph.D works, combining germ cell transplantation, transcriptomics, artificial hybridization, induced triploidization, immunofluorescence analysis on meiotic configurations and other conventional methods were well organized to disclose or strongly suggest putative mechanisms underlying the initiation of asexual mode of reproduction, aberrant meiosis and sterility, sex determination and asexuality in animals with incompatible nonhomologous genomes. Obtained results shown in Chapter 2 to 5 were highly evaluated because they contributed to our knowledge on aberrant and asexual reproductive phenomena frequently found in hybrid and polyploid fishes (and other lower vertebrates). In addition, provided results including induction of sterile hybrid and triploid (Chapter 3 and 5) and surrogate production using germ line chimeras (Chapter 2 and 5) are widely applicable for aquaculture-oriented implementation in other commercially important fishes. Nowadays, induced sterility is important technique to diminish a risk against genetic contamination of indigenous wild populations by introducing exotic aquaculture species as well as genetically manipulated strains. Germ cell transplantation is also now widely applied both in basic and applied aquaculture-related studies. Consequently, this Ph.D work is evaluated to be an internationally recognized one, which has various impacts to related studies and industry beyond national borders.



Elaboration of the PhD thesis, objectives of the work and deliverables

Evaluate the overall level of elaboration of the PhD thesis (structuring of the main text, comprehensibility, logicity of the chapters and their ordering) and the originality of the selected approaches to solve the objectives; evaluate publications and whether the results described correspond to objectives of the PhD thesis (extent ¼ – ½ page):

The Ph.D thesis is well elaborated by active introducing newly developed biotechniques such as germ cell transplantation, immunofluorescent microscopy on meiotic configurations, transcriptomics, together with conventional hybridization, triploidization, morphology and reproductive physiology. General introduction which mentioned back-grounds of the thesis is well described based on comprehensive reviews on enough numbers of previous related papers. Since materials from different taxa were used in the thesis, consistency among chapters seems to be a little bit poor as written in the following section on overall commentary. Inter-relationship among chapters should be further explained based on the common rationale underlying asexuality and sterility in hybrids and polyploids. However, the overall quality of the thesis is highly evaluated because the candidate actively challenged to disclose new insights into studies on asexuality and sterility as well as to provide biological techniques for near future aquaculture-oriented implementation of surrogate production, gene banking and induced sterilization. All the results obtained in each chapter were well corresponding to the aims which were listed up in the first introductory chapter 1. And thus, the purpose of the Ph.D thesis was evaluated to be successfully achieved. This thesis had enough quality and quantity as the Ph.D thesis. Publications were all peer-reviewed and published on internationally recognized journals with enough impact factors (1.173 – 6.684). These publications clearly certified implementation capability of the candidate of Ph.D degree. Active presentation experiences also indicated his implementation capability in scientific researches.

OVERALL COMMENTARY ON THE PhD THESIS

Please write in the box specific comments concerning the PhD thesis in extent of 1-2 pages:

In the first chapter of the Ph.D thesis, the aims were clearly listed up after description on back grounds of the study based on fully comprehensive reviews of previous and recent studies which related to asexual reproduction, sterility or fertility, induced triploidy, germ cell transplantation, meiosis, sex determination, and others. All these scientific aims were almost achieved by results obtained in Chapter 2 to 5.

In Chapter 2, triggering mechanisms to initiate unreduced gametogenesis for asexual mode of reproduction was experimentally investigated by transplanting spermatogonial stem cells (SSC) to artificial sterilized morphant hosts. Although successful cases of germ line chimeras were smaller than expectation (but, almost enough), the Ph.D candidate could disclose that hybrid *Cobitis* SSC underwent unreduced oogenesis by doubling genomes even in gonadal environment of bisexually reproducing females, while chimeras became sterile in males. Diploid *Cobitis* SSC differentiated into regular gametes when transplanted to hybrid gonads. Such approaches were new to science and gave various important findings. This work is expected to stimulate the progress of studies on asexual reproduction by providing new method; germ cell transplantation and related techniques.

In Chapter 2 and 4, immunofluorescence staining with antibodies for SYCP3 and SYCP1 proteins which had important functions in synapsis was successfully applied to analyze meiotic configurations of pachytene oocytes. This state-of-the-art approach successfully identified pairing between sister chromosomes duplicated from each original chromosome by premeiotic endomitosis or endoreduplication for unreduced oogenesis in *Cobitis* hybrids. The same approach also identified that unreduced oogenesis was underwent by apomixis in Amazon molly *Poecilia formosa*. The absence of signals of SYCP1 clearly indicated that bivalent formation was not present in asexual Amazon molly and thus apomixis produced isogenic eggs. Molecular cytogenetics using antibodies against synaptonemal complexes successfully identified presence or absence of pairing (synapsis) were high-end techniques and may become popular soon in studies in gametogenesis and meiosis.



In Chapter 4, transcriptome analysis also disclosed the importance of down regulation of *spo11* gene in asexual reproduction. Integration of molecular cytogenetics and transcriptomics in this work gave important conclusion in a related scientific area. These new techniques and subsequent findings should dramatically proceed the studies on asexual reproduction and thus this Ph. D work was highly evaluated to contribute the progress in studies of atypical mode of reproduction.

This Ph.D work also showed biological influence of inter-generic hybridization on spermatogenesis in Chapter 3 as well as optimum triploid induction as an surrogate host in Chapter 5. Both approaches are now conventional, but obtained results were important to recognize significance of hybridization and triploidization on reproductive success (fertility/ sterility) and sex determination system. Both chapter's results may contribute aquaculture-oriented studies. In Chapter 5, shift to sex ratio to males was observed in induced triploid zebrafish. Unfortunately, discussion on the reason or the mechanisms of all-male occurrence seems to be relatively poor. As I understand the difficulties in sex determination system of zebrafish, I would like to hear an aggressive hypothesis to explain all-male occurrence in triploids based on sex determination and sex differentiation system in zebrafish.

In Chapter 6, general discussion was provided. Overall, each chapter of the thesis was well prepared and reasonably written. However, topics of each chapter have not been organized to a powerful one-convincing-story thesis. However, inter-relationship among topics is relatively weak. What is the common rationale underlying the superficially independent phenomena of (1) asexual reproduction by premeiotic genome doubling in germ cells (chapter 2), (2) gametic sterility (spermatozoa dysfunction) in inter-generic hybrid (chapter 3), (3) asexual reproduction by failure of bivalent formation (chapter 4), and (4) induced triploids as an ideal host for surrogate reproduction? If inter-relation or common biological rationale underlying independent topics was much more clarified by deeper considerations focusing on common mechanisms among aberrant meiosis, unusual gametogenesis, asexuality, and interspecific incompatibility due to genomic merging, this Ph.D thesis should become much more outstanding one. However, as an original version, the Ph.D thesis is evaluated as an excellent output from powerful studies at the international level of reproductive biology of fishes.

FINAL RECOMMENDATION



- ☒ PhD thesis can be recommended for defence
- ☐ PhD thesis can be recommended for defence with reservations
- ☐ PhD thesis cannot be recommended for defence

July, 6, 2022

Sapporo, Hokkaido, Japan

on July 6, 2022 in Sapporo

Date and place

Katsutoshi Arai

Name and signature