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| Title            | No reduction of hatching rates among F1 hybrids of naturally hybridizing three Far Eastern daces, genus <i>Tribolodon</i> (Cypriniformes, Cyprinidae) |
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| Citation         | Ichthyological research, 65(1), 165-167<br><a href="https://doi.org/10.1007/s10228-017-0588-1">https://doi.org/10.1007/s10228-017-0588-1</a>          |
| Issue Date       | 2018-01                                                                                                                                               |
| Doc URL          | <a href="https://hdl.handle.net/2115/72247">https://hdl.handle.net/2115/72247</a>                                                                     |
| Rights           | The final publication is available at <a href="http://link.springer.com">link.springer.com</a>                                                        |
| Type             | journal article                                                                                                                                       |
| File Information | ICRE-D-17-00001_r.pdf                                                                                                                                 |



**No reduction of hatching rates among F1 hybrids of naturally hybridizing three Far Eastern daces, genus *Tribolodon* (Cypriniformes, Cyprinidae)**

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Running head: High hatching rate of hybrids among three Far Eastern daces

Type of paper: Short report

Number of text pages: 7; Number of figures: 0; Number of tables: 1

**Abstract** Egg hatching rates of F1 hybrids among three fishes, Japanese dace *Tribolodon hakonensis*, Pacific redbfin *T. brandtii*, and rosyface dace *T. sachalinensis* were compared with pure crosses. They are deeply diverged (10–20 million years ago) but naturally hybridizing. Although crosses involving female rosyface dace were not available, hatching success was high among all combinations of hybrids ( $76 \pm 23$  %) and comparable to pure crosses ( $86 \pm 8$  %), implying ecological or genetic isolation mechanisms maintaining each species in later life stages.

**Keywords** Genetic incompatibility · Intrinsic isolation · Hybrid inviability · Introgression

## Introduction

To understand the mechanisms of reproductive isolation and speciation, when and how hybrid fitness decreases should be uncovered. Such mechanisms can be either genetic or ecological factors, or both (Coyne and Orr 2004). Genetic or intrinsic isolation denotes that incompatibilities between parental genomes prevent normal development of hybrids, including hatching failure and adult sterility. Ecological or extrinsic isolation explains ecologically induced reduction of fitness due to their maladaptive phenotypes (Coyne and Orr 2004). In the process of speciation, it is generally assumed that fitness reduction of hybrid occurs first, often followed by premating (behavioral) isolation, and postmating (genetic) isolation will be strengthened (Coyne and Orr 2004). Divergence time required for the establishment of genetic incompatibilities in fishes varies from five to over 20 million years (myr) and generally 10 myr (reviewed in Russell 2003).

Despite the presumed old divergence (10–20 myr ago, Imoto et al. 2013), three species of Far Eastern daces *Tribolodon brandtii* (hereafter *TB*), *Tribolodon hakonensis* (*TH*), and *Tribolodon sachalinensis* (*TS*) can hybridize naturally, often with high frequencies (up to 20 %: Sakai and Hamada 1985). Hybrid swarms, however, have not been reported so far, indicating some isolation mechanisms. In these species, the mechanisms of reproductive isolation are largely unknown and strength of intrinsic isolation have not been investigated, whereas researchers have successfully reared *TB–TS* hybrids (e.g. Nakamura and Mochizuki 1953). Here, we investigated hatching rates of F1 hybrids among three *Tribolodon* species, comparing with that of pure crosses.

## Materials and methods

We caught three males and two females of *TB*, three males and one female of *TH*, and three males of *TS* in Abashiri River, eastern Hokkaido, Japan (43°82' N; 144°09' E), during their spawning season (6 July 2011). Three species sympatrically occurred and spawned below an artificial weir where daces were caught. At the time of sampling, breeding seasons of *TB* and *TH* were largely finished, whereas it was just before the beginning of the season for *TS*. Thus, females of *TS* were unavailable. All of them were involved in both pure and two hybrid crosses (Table 1), conducted at the Shibetsu Salmon Museum where water temperature was 16.8 °C. Eggs were fertilized artificially, and hatching rate in each pair was investigated 12–15 days later because *TB* and *TH* is known to hatch five days after fertilization at 11–17 °C (Nakamura and Mochizuki 1953). Hatching rates were compared between pure and hybrid crosses using Welch's t-test.

## Results

Hatching rates of F1 hybrids from all combination were generally high (>60–70 %) except for two crosses between male *TS* and female *TB* (15–16 %) (Table 1). Hatching rates did not significantly differ between pure ( $0.86 \pm 0.08$ ) and hybrid crosses ( $0.76 \pm 0.23$ ) ( $P > 0.05$ ).

## Discussion

The lack of fertility reduction among three diverged daces (10–20 million years ago) is notable compared to a similar experiment in cichlids that fertilization and two-week survival

rates of F1 hybrids decreased rapidly only after 1–4 myr divergence (Stelkens et al. 2009). As backcross hybrids of the daces exist in natural rivers (Sakai and Hamada 1985), F1 hybrids are not only viable but also fertile. Such low incompatibility might potentially lead genetic introgression among the three species.

The levels of introgression among the three daces remain unknown, but it seems that individual species are maintained despite hybridization (Sakai and Hamada 1985). Since hybrids are more or less viable in early life stages, extrinsic isolation should exist in later life stages. One of potential isolation mechanisms is the fitness reduction of hybrids during migration from freshwater to the ocean. The three species have different migratory tendency: all *TB* migrate to the ocean, all *TS* are reportedly stream resident in Japan, and *TH* is partially migratory (some migrate to ocean and some reside) (Sakai 1995). Hybrids involving *TS* might be maladaptive in terms of ocean migration. In addition, it is also known that fitness of later generation hybrids decreases dramatically due to deleterious interactions among parental alleles (i.e. hybrid breakdown: Stelkens et al. 2015), which may be also the case for the daces (Sakai 1995).

Another intriguing isolation mechanism is premating behavioral isolation. Despite the similar appearance during non-breeding season, the three daces develop marked species specific nuptial coloration even in females (Sakai 1995; Atsumi and Koizumi, in revision). This mutual ornamentation might enhance their species recognition. In addition, breeding season and spawning site are partially segregated, which may also prevent hybridization (Sakai 1995). Further research should address these factors for understanding how the three species have been maintained over 10 million years with weak intrinsic isolation.

**Acknowledgments** We appreciate two anonymous reviewers for helpful comments and C. Ayer for English check. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. We have no conflict of interest.

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**Table 1** Hatching rates of F1 hybrids among three Far Eastern daces

| parents                 |                        | hatched<br>eggs | total<br>eggs | hatching<br>rate |
|-------------------------|------------------------|-----------------|---------------|------------------|
| pure crosses            |                        |                 |               |                  |
| <i>TB<sub>m</sub>1</i>  | <i>TB<sub>f</sub>1</i> | 153             | 220           | 0.70             |
| <i>TB<sub>m</sub>2</i>  | <i>TB<sub>f</sub>1</i> | 326             | 380           | 0.86             |
| <i>TB<sub>m</sub>3</i>  | <i>TB<sub>f</sub>1</i> | 216             | 241           | 0.90             |
| <i>TB<sub>m</sub>1</i>  | <i>TB<sub>f</sub>2</i> | 317             | 369           | 0.86             |
| <i>TB<sub>m</sub>2</i>  | <i>TB<sub>f</sub>2</i> | 407             | 493           | 0.83             |
| <i>TB<sub>m</sub>3</i>  | <i>TB<sub>f</sub>2</i> | 386             | 429           | 0.90             |
| <i>TH<sub>m</sub>1</i>  | <i>TH<sub>f</sub>1</i> | 456             | 534           | 0.85             |
| <i>TH<sub>m</sub>2</i>  | <i>TH<sub>f</sub>1</i> | 442             | 477           | 0.93             |
| <i>TH<sub>m</sub>3</i>  | <i>TH<sub>f</sub>1</i> | 339             | 352           | 0.96             |
| Average $0.86 \pm 0.08$ |                        |                 |               |                  |
| hybrid crosses          |                        |                 |               |                  |
| <i>TB<sub>m</sub>1</i>  | <i>TH<sub>f</sub>1</i> | 61              | 105           | 0.58             |
| <i>TB<sub>m</sub>2</i>  | <i>TH<sub>f</sub>1</i> | 365             | 411           | 0.89             |
| <i>TB<sub>m</sub>3</i>  | <i>TH<sub>f</sub>1</i> | 144             | 211           | 0.68             |
| <i>TH<sub>m</sub>1</i>  | <i>TB<sub>f</sub>1</i> | 245             | 293           | 0.84             |
| <i>TH<sub>m</sub>2</i>  | <i>TB<sub>f</sub>1</i> | 245             | 323           | 0.76             |
| <i>TH<sub>m</sub>3</i>  | <i>TB<sub>f</sub>1</i> | 325             | 408           | 0.80             |
| <i>TH<sub>m</sub>1</i>  | <i>TB<sub>f</sub>2</i> | 284             | 313           | 0.91             |
| <i>TH<sub>m</sub>2</i>  | <i>TB<sub>f</sub>2</i> | 253             | 267           | 0.95             |
| <i>TH<sub>m</sub>3</i>  | <i>TB<sub>f</sub>2</i> | 289             | 324           | 0.89             |

|         |         |     |     |      |
|---------|---------|-----|-----|------|
| $TS_m1$ | $TB_f1$ | 204 | 293 | 0.70 |
| $TS_m2$ | $TB_f1$ | 38  | 203 | 0.19 |
| $TS_m3$ | $TB_f1$ | 58  | 284 | 0.20 |
| $TS_m1$ | $TB_f2$ | 277 | 327 | 0.85 |
| $TS_m2$ | $TB_f2$ | 315 | 344 | 0.92 |
| $TS_m3$ | $TB_f2$ | 180 | 248 | 0.73 |
| $TS_m1$ | $TH_f1$ | 355 | 383 | 0.93 |
| $TS_m2$ | $TH_f1$ | 319 | 361 | 0.88 |
| $TS_m3$ | $TH_f1$ | 235 | 248 | 0.95 |

Average  $0.76 \pm 0.23$

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$_m$  Male,  $_f$  female