**Developmental potential of somatic and germ cells of hybrids between *Carassius auratus* females and *Hemigrammocypris rasborella* males**

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**Table S1** Species-specific PCR primers for goldfish *C*. *auratus* and golden venus chub *rag1* gene

|  |  |  |
| --- | --- | --- |
| Primer name | Primer sequence | Amplified length |
| KinRAG1\_1024F | 5′-CTGGACAAACAGCTGAGAAAGAAG-3′ | 332 bp |
| Cyp\_RAG1\_1355R | 5′-ACATGGGCCAGAGTCTTGTG-3′ |  |
| KawaRAG1\_330F | 5′-AGATGTCAGTGAGAAGCATGGAAC-3′ | 742 bp |
| KawaRAG1\_1071R | 5′-CCTCATCACAGGCTTGAGTTTCATT-3′ |  |

**Table S2** Fertilization, survival rates of control goldfish *C. auratus* and hybrid between female goldfish and male golden venus chub *H*. *rasborella* after heat shock (HS) treatment at 40oC starting 5 min post-fertilization

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Exp. no. | Experiment | Duration of HS (s) | Total eggs | Fertilized egg | | No. of survival eggs (%) | | | | | | | | | | | |
|  |  |  |  | No. | (%) | 1 dpf \*1 | | 3 dpf | | 5 dpf | | 7 dpf | | 10 dpf | | 15 dpf | |
| 1 | Control | – | 191 | 189 | 99.0% | 189 | (100) | 166 | (87.8) | 132 | (69.8) | 108 | (57.1) | – | – | – | – |
|  | Control | 50 | 133 | 129 | 97.0% | 124 | (96.1) | 16 | (12.4) | 12 | (9.3) | 9 | (7.0) | – | – | – | – |
|  | Control | 60 | 145 | 144 | 99.3% | 132 | (91.7) | 14 | (9.7) | 11 | (7.6) | 3 | (2.1) | – | – | – | – |
|  | Control | 75 | 82 | 81 | 98.8% | 75 | (92.6) | 4 | (4.9) | 4 | (4.9) | 4 | (4.9) | – | – | – | – |
|  | Hybrid | – | 125 | 117 | 93.6% | 75 | (64.1) | 42 | (35.9) | 33 | (28.2) | 26 | (22.2) | – | – | – | – |
|  | Hybrid | 50 | 219 | 211 | 96.3% | 191 | (90.5) | 41 | (19.4) | 29 | (13.7) | 19 | (9.0) | – | – | – | – |
|  | Hybrid | 60 | 219 | 211 | 96.3% | 177 | (83.9) | 39 | (18.5) | 25 | (11.8) | 16 | (7.6) | – | – | – | – |
|  | Hybrid | 75 | 228 | 220 | 96.5% | 182 | (82.7) | 19 | (8.6) | 10 | (4.5) | 6 | (2.7) | – | – | – | – |
| 2 | Control | – | 399 | – | – | 277 | (69.4) | 248 | (62.2) | 242 | (60.7) | 224 | (56.1) | 211 | (52.9) | 198 | (49.6) |
|  | Control | 50 | 136 | – | – | 68 | (50.0) | 58 | (42.6) | 56 | (41.2) | 53 | (39.0) | 51 | (37.5) | 41 | (30.1) |
|  | Control | 60 | 124 | – | – | 74 | (59.7) | 64 | (51.6) | 64 | (51.6) | 54 | (43.5) | 52 | (41.9) | 47 | (37.9) |
|  | Control | 75 | 125 | – | – | 68 | (54.4) | 51 | (40.8) | 49 | (39.2) | 44 | (35.2) | 44 | (35.2) | 40 | (32.0) |
|  | Hybrid | – | 259 | – | – | 134 | (51.7) | 114 | (44.0) | 106 | (40.9) | 101 | (39.0) | 85 | (39.0) | 13 | (5.0) |
|  | Hybrid | 50 | 394 | – | – | 180 | (45.7) | 126 | (32.0) | 117 | (29.7) | 84 | (21.3) | 63 | (21.3) | 19 | (4.8) |
|  | Hybrid | 60 | 359 | – | – | 157 | (43.7) | 107 | (29.8) | 96 | (26.7) | 79 | (22.0) | 41 | (22.0) | 15 | (4.2) |
|  | Hybrid | 75 | 280 | – | – | 175 | (62.5) | 119 | (42.5) | 108 | (38.6) | 77 | (27.5) | 61 | (27.5) | 18 | (6.4) |
| 3 | Control | – | 84 | – | – | 79 | (94.0) | 79 | (94.0) | 79 | (94.0) | 72 | (85.7) | 67 | (85.7) | 64 | (76.2) |
|  | Control | 50 | 96 | – | – | 88 | (91.7) | 84 | (87.5) | 83 | (86.5) | 70 | (72.9) | 58 | (72.9) | 43 | (44.8) |
|  | Hybrid | – | 197 | – | – | 154 | (78.2) | 149 | (75.6) | 135 | (68.5) | 110 | (55.8) | 21 | (55.8) | 3 | (1.5) |
|  | Hybrid | 50 | 185 | – | – | 139 | (75.1) | 118 | (63.8) | 108 | (58.4) | 103 | (55.7) | 63 | (55.7) | 16 | (8.6) |

\*1 dpf: days post-fertilization.

**Table S****3** Survival of chimeric embryos transplanted with goldfish × HR blastomeres (donor) into goldfish × goldfish (host) at blastula stage

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Eggs | Egg no. | Fertilized egg | | Survival rate (%) | | | | | | | |
|  | No. | (%) | 1 dpf | | 3 dpf | | 7 dpf | | 10 dpf | |
| Host (goldfish × goldfish) | 63 | 46 | (73.0) | 37 | (80.4) | 32 | (69.6) | 32 | (69.6) | 32 | (69.6) |
| Donor (goldfish × HR) | 67 | 47 | (70.1) | 0 | (0) | 0 | (0) | 0 | (0) | 0 | (0) |
| Chimera | 53 | – | – | 53 | (100) | 52 | (98.1) | 50 | (94.3) | 26 | (49.1) |

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**Figure S1** Schematic illustration of blastoderm transplantation. Transplantation was performed between goldfish *C*. *auratus* and hybrid female goldfish × male golden venus chub *H*. *rasborella* (HR). (*A*) The entire blastoderm was cut off from yolk cells of a hybrid blastula and transplanted onto the animal part of a goldfish blastula. (*B*) The lower part of the blastoderm was cut off a hybrid blastula and transplanted into the middle of a goldfish blastula. (*C*) The upper blastoderm from both hybrid and goldfish blastulae was cut off by glass needle and reciprocally exchanged.

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**Figure S2** Relative DNA contents of hybrid between female goldfish *C*. *auratus* and male golden venus chub *H*. *rasborella* and parent species. (*A*) *H*. *rasborella*. (*B*) *C*. *auratus*. (*C*, *D*) Hybrids.

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**Figure S3** Backlit (*A*) and GFP fluorescence (B) view of hybrid between female goldfish *C*. *auratus ×* male golden venus chub *H*. *rasborella* at 1 day after fertilization. The embryo is seemingly dead, but the GFP-positive cells are alive, suggesting that the primordial germ cell (PGC) differentiation occurs in seemingly dead embryos. Yellow arrows indicate PGCs.

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**Figure S4** GFP fluorescence of a goldfish embryo transplanted with hybrid PGCs. (*A*) 1 dpf, (*B*) 2 dpf, and (*C*) 5 dpf post-transplantation with blastomeres from hybrid female goldfish *C*. *auratus* × male golden venus chub *H*. *rasborella*. GFP-positive cellsfrom the hybridare located around the host genital ridge, suggesting that hybrid primordial germ cells (PGCs) have migratory ability. Scale bars indicate 1 mm. Yellow arrow indicates a PGC from the hybrid near the host genital ridge.