

## Hybridization Experiments in Cyprinid Fishes. VIII. Two Kinds of Reciprocal Crosses, *Pseudogobio* *esocinus* × *Pseudorasbora parva* and *Biwia* *zezera* × *Pseudorasbora parva*\*

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Three species of *Pseudogobio esocinus*, *Pseudorasbora parva* and *Biwia zezera* belong to the same subfamily, Gobioninae and their habitats are confined to the same geographical area of river. However, there are some wide morphological and habitual differences between them. In order to elucidate whether or not the success of hybrid development is correlated with the supposed degree of taxonomic differences based on morphological traits, artificial hybridizations of *Pseudogobio esocinus* ♀ × *Pseudorasbora parva* ♂, *Pseudorasbora parva* ♀ × *Pseudogobio esocinus* ♂, *Biwia zezera* ♀ × *Pseudorasbora parva* ♂ and *Pseudorasbora parva* ♀ × *Biwia zezera* ♂ are carried out.

### Results

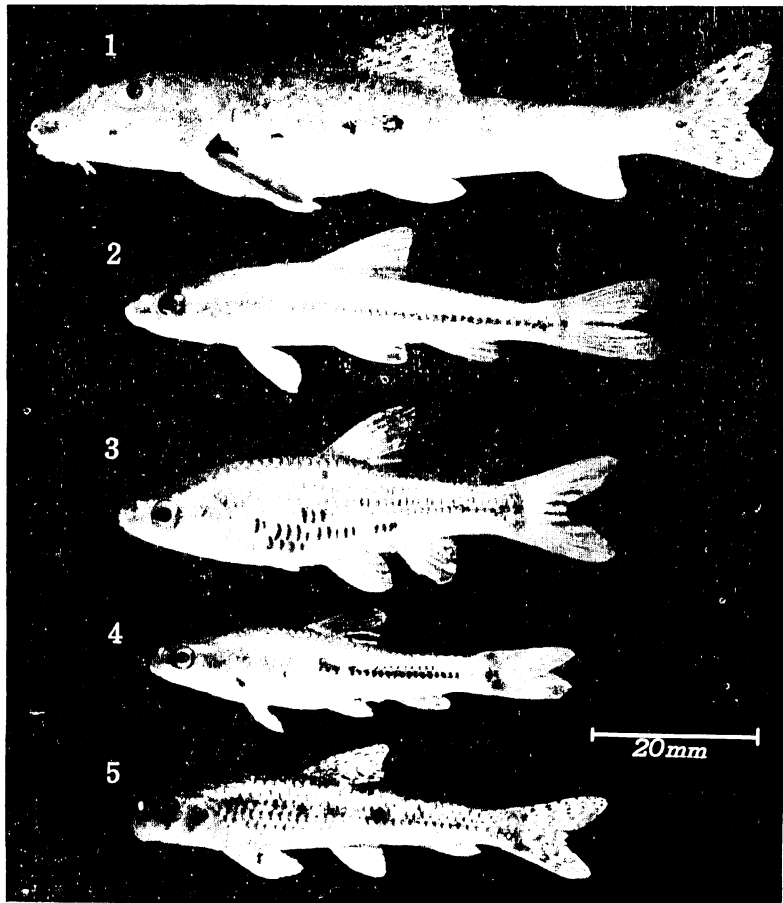
1) *Early development and viability* Eggs of *Pseudogobio* ♀ × *Pseudorasbora* ♂ proceeded through cleavage stage almost identical with that of straight-fertilized eggs and developed into embryos without any observable deformity. Their hatching time, however, was somewhat critical stage, mortality of the hybrid embryos being slightly higher than that of controls. The time interval needed for hatching of the hybrid embryos was nearly intermediate between that of respective parents under the same temperature (18° to 20°C). Namely, maternal *Pseudogobio* eggs hatched 5 to 6 days after fertilization, paternal *Pseudorasbora* 12 to 13 days and hybrids began to hatch after 7 days and finish after 11 days. Fifty-seven per cent of the embryos hatched out and resulted fry feed normally and reached the fourmonth old with a survival rate similar to that of controls. Two hybrids could be reared until the adult stage; two-year old, though the other all hybrids died of a technical mistake during five to seven months after hatching.

In the reverse combination, *Pseudorasbora* ♀ × *Pseudogobio* ♂, only about four per cent of eggs cleaved and developed up to early gastrula stage. All of them, however, never went beyond the closure of the blastopore.

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Eggs of *Pseudorasbora* ♀ × *Biwia* ♂ went through cleavage, developed into embryos without any observable deformity, and hatched with a survival rate similar to that of controls. Their hatching was faster than that of maternal control under the same temperature (18° to 21°C), i. e., nearly intermediate between those of respective parents. *Pseudorasbora* eggs hatched 12 to 13 days after fertilization, those of *Biwia* 4 to 5 days, and those of hybrids began to hatch after 8 days and finish after 10 days. Newly hatched fry grew normally and reached the four-month old. Only one hybrid could be reared until one-year age, while the other all hybrids died of a technical mistake during five to six months.

In the reverse cross, *Biwia* ♀ × *Pseudorasbora* ♂, about 91 per cent of eggs proceeded normally through early cleavage stage. This percentage was almost the same as in the cases of straight-fertilized eggs. About 56 per cent of embryos hatched



Figs. 1-5. 1. An adult specimen of female *Pseudogobio esocinus*, three-year old. 2. A neutral hybrid, *Pseudogobio esocinus* ♀ × *Pseudorasbora parva* ♂, two-year old. 3. An adult specimen of male *Pseudorasbora parva* ♂, two-year old. 4. A neutral hybrid, *Pseudorasbora parva* ♀ × *Biwia zezera* ♂, one-year old. 5. An adult specimen of male *Biwia zezera*, two-year old.

out normally, while a few of them developed into deformed larvae. Time interval needed for hatching was nearly intermediate between that of parental species. The majority of the hybrid fry died within 7 days after hatching. Although only about five per cent of hybrids took food, none of them could survive beyond 12 days after hatching.

Two adult hybrids from *Pseudogobio* ♀ × *Pseudorasbora* ♂ and one hybrid of one-year old from *Pseudorasbora* ♀ × *Biwia* ♂ provided the writer with an opportunity to study the inheritance of parental characters, which is described in the following paragraphs.

2) *External characters* *Pseudogobio* have inferior mouth being provided a pair of barbels longer than diameter of eye and their very thick lips are equipped with numerous nipplelike protuberances. The anus opens in the vicinity of the ventral fin insertion. The breast is callous, where no scale is present. Large pectoral fins are extend to the ventral fin base (Fig. 1). *Pseudorasbora* mouth is superior and has no barbel. There are many scales on the breast without callosity. The small pectoral fins reach the midway between the base of the pectoral fin and the insertion of the ventral fin (Fig. 3). The anus opens just in front of the anal fin insertion. *Biwia* have inferior mouth and neither the barbels nor the protuberances are observable in their jaws (Fig. 5). The anus opens in the vicinity of the ventral fin insertion. Their breast is callous, where no scale is present.

Hybrids indicate nicely intermediate in many external characters. Of hybrids from *Pseudogobio* ♀ × *Pseudorasbora* ♂ mouth is typically terminal and barbels are minute, the length being 1/3 to 1/5 diameter of eye. Nipplelike protuberances covering the lips are less conspicuous than those of *Pseudogobio* control. The anus opens midway between the base of ventral fin and the insertion of anal fin. The breast with few scales is slightly callous. The pectoral fin is not so long to reach the ventral fin (Fig. 2). A hybrid from *Pseudorasbora* ♀ × *Biwia* ♂ has terminal mouth without barbels (Fig. 4). The anus opens midway between the base of the ventral fins and the insertion of anal fin. The breast with few scales is slightly callous.

3) *Behavior* *Pseudogobio* always live on the sandy bottom of river and they actively dig out the bottom stirring up sands. Their breasts, therefore, become callosity without any scales. Sometimes, they burrow into the sands exposing only their protruded eye balls outside. A number of funnel-like hollows, therefore, are formed on the bottom, the diameter being about three times length of their head. In *Pseudorasbora*, however, such hollows are not observable, because they usually swim in the intermediate zone between the surface and the bottom.

Hybrids from *Pseudogobio* ♀ × *Pseudorasbora* ♂ always creep on the bottom and sometimes they swim a few centimeters away from the bottom. Although they do not burrow as *Pseudogobio* do, they sometimes dig out the bed. Thereby, a few hollows are observed on the bottom, though they are smaller than those of *Pseudogobio*. On the other hand, the hybrid from *Pseudorasbora* ♀ × *Biwia* ♂ behaves also inter-

mediately between parental fishes. That is, paternal *Biwia* always live on the sandy bottom, sometimes creeping on it. Hybrid always creeps on the bottom as *Biwia* do, though sometimes they swim few centimeters away from the bottom.

4) *Sexuality* Anatomical investigation during the spawning season revealed that two adult hybrids from *Pseudogobio* ♀ × *Pseudorasbora* ♂ were neuters in which gonads are not found. One hybrid from *Pseudorasbora* ♀ × *Biwia* ♂ was also a neuter. On the other hand, controls of *Pseudorasbora* and *Biwia* had mature gonads.

### Discussion

SUZUKI (1959) reported that eggs of *Rhodeus ocellatus* are not fertilized by the spermatozoa of *Sarcocheilichthys variegatus* or *Cyprinus carpio*, but eggs of *Sarcocheilichthys* or *Cyprinus* are successfully fertilized by *Rhodeus* spermatozoa. He has supposed that spermatozoa of *Sarcocheilichthys* or *Cyprinus* cannot enter the *Rhodeus* eggs through the micropyle because of their size. In the present experiments, the cross fertilization of *Pseudogobio* eggs with *Pseudorasbora* spermatozoa were successful, but in the reverse crossing the fertilization did not succeed so well. Such low fertilization rate may be due to the same circumstances as in above cases. In the salmonid fishes, low fertilization rate in crosses has already been reported by DAY (1886), SVÄRDSON (1945) and ALM (1955). DAY (1886) firstly pointed out that when a little female fish, for example a small brown trout, is crossed with a large male, for instance a large salmon, the fertilization does not succeed so well. He has claimed that this is due to the eggs in the smaller female fish which generally are of a smaller size and consequently have a relatively small micropyle, through which the spermatozoon of a large male fish cannot enter for mechanical reasons.

As reported in an earlier paper of this series (SUZUKI, 1962), both hybrids from *Gnathopogon elongatus elongatus* ♀ × *Pseudorasbora parva* ♂ and the reverse cross were able to be brought to attainment of adulthood with a survival rate similar to that of controls. Present hybrids from *Biwia zezera* ♀ × *Pseudorasbora parva* ♂, however died during larval stage, while the reverse hybrids were reared to reach five month old. On the basis of these facts, it can be stated that the gap in phylogenetical relationship between *Biwia* and *Pseudorasbora* is far greater than that between *Gnathopogon* and *Pseudorasbora*.

The present hybrids are not only morphologically intermediate between the parental species but also have typically intermediate behavior. Furthermore, they take nearly intermediate time to hatch between that of the parental controls. Such intermediate hatching has been reported by many investigators in hybrid embryos between fishes which take wide difference of time until they hatch (NEWMAN, 1914, SUZUKI, 1953, 1956, 1962, 1963 and KOBAYASHI and YAMABAYASHI, 1957). Concerning this point, SUZUKI (1953) specially claimed that hybrid hatching may be affected by some genetical elements of parental species. On the other hand, HUBBS and STRAWN (1957) stated

that the darter hybrids developed at rates similar to that of their maternal controls, while the differences between the parental rates were minor. In cyprinodont hybrids, HUBBS and DREWRY (1959) reported that the first hybrids hatched typically at a time intermediate between that of the parental controls, and that the last hybrids hatched much later than either controls.

### Summary

The artificial crosses of *Pseudogobio esocinus* ♀ × *Pseudorasbora parva* ♂ and *Pseudorasbora parva* ♀ × *Biwia zezera* ♂ were successful and three hybrids were reared until they reach one or two-year old. All of them turned out to be neuters. They are not only intermediate in morphological characters but also have typically intermediate behavior between their parental forms. Time of hatching is also nearly intermediate.

In the cross of *Pseudorasbora parva* ♀ × *Pseudogobio esocinus* ♂, however, only about four per cent of eggs developed to gastrula and all of them never went beyond the closure of the blastopore.

In the cross of *Biwia zezera* ♀ × *Pseudorasbora parva* ♂, the fertilization were successful, while hybrids could not survive beyond 12 days after hatching.

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