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Interspecific Hybrids between *Hynobius nigrescens* ♀ and *Hynobius tokyoensis* ♂

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(With 1 Text-figure)

Mature interspecific hybrids between males of *Hynobius nigrescens* Stejneger and females of some other *Hynobius* species have been reported in detail in a previous paper (Kawamura '53). As *Hynobius nigrescens* females containing full grown eggs in their ovaries or uteri can hardly be captured in the field, it is a difficult task to cross females of *Hynobius nigrescens* with males of other *Hynobius* species. Fortunately, however, the present author was able to catch a female of *Hynobius nigrescens* and to obtain two mature interspecific hybrids, using males of *Hynobius tokyoensis*. This paper will report on some characters of these hybrids.

The female of *Hynobius nigrescens* was caught on March 21st, 1953, at Yatsuo, Toyama Prefecture. As the female was found to have uterine eggs when captured, it was hurriedly carried to our laboratory in Hiroshima, which was about 700 km away from Yatsuo. About 30 hours after the collection a crossing experiment was made, using males of two local races of *H. nigrescens* (collected at Yatsuo, Toyama Prefecture and Yahiko, Niigata Prefecture), two local races of *H. tokyoensis* Tago (collected at Iwafune, Ibaraki Prefecture and Tahara, Aichi Prefecture), *H. nebulosus* Schlegel (collected at Tottori) and *H. dunni* Tago (Yamaga, Ooita Prefecture). Egg masses of the *nigrescens* female were taken out of the uteri after dissecting the uterine walls and they were cut into six pieces, each of which contained 9 to 16 eggs. Each piece of egg masses was artificially inseminated with the sperm of each male of the above mentioned kinds of *Hynobius*. Owing to the

Table 1. Inter- and intraspecific hybrids produced from a *Hynobius nigrescens* female

Father	Number of eggs	Number of normal cleavages	Number of swimming larvae
Toyama *nigr.*	12	0	0
Niigata *nigr.*	13	1	1
Ibaraki *tokyo.*	9	2	2
Aichi *tokyo.*	16	1	1
Tottori *nebul.*	13	0	0
Ooita *dunni*	13	1	1

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vibration during the transportation by train or by some other causes the jellies of egg masses in the uteri seemed to have been somewhat swelled and abnormally sticky and consequently to refuse excessively the penetration of spermatozoa into eggs. Only five eggs normally cleaved, as shown in Table 1. All the individuals grown from these eggs lived more than one month (Table 2). Two of them sur-

Table 2. Measurements of 5 hybrids produced from a *Hynobius nigrescens* female

Father	26-day-old larva	85-day-old larva		Age when died or was preserved
	Total length	Body length	Head breadth	
Niigata *nigr.*	17.3mm	26.1mm	8.5mm	14 months
Ibaraki *tokyo.*	16.6	25.0	8.2	33 months
Ibaraki *tokyo.*	15.5	17.9	7.5	78 days
Aich *tokyo.*	16.2	26.2	7.4	36 months
Ooita *dunni*	14.1	—	—	37 days

vived about three years and reached their complete maturity. The short life of the *nigrescens* ♀× *dunni* ♂ hybrid was recognized to be an intrinsic character, as it has been ascertained in other experiments performed by the present author that this kind of hybrids are always lethal.

The 3 *nigrescens* ♀× *tokyoensis* ♂ hybrids developed at nearly the same rate as the Toyama *nigrescens* ♀× Niigata *nigrescens* ♂ hybrid did and hatched about one month after fertilization. These larvae were narrower in head breadth than the *nigrescens* larva and showed a character of the paternal species, *H. tokyoensis*. The Aichi *tokyoensis* larva was particularly distinct in this respect. Of the Ibaraki *tokyoensis* hybrids one was somewhat deformed and ill-developed in the larval stage, and died 78 days after fertilization. The other normally developed and reached its maturity. However, as it was frequently affected with convulsions in the third winter, 33 months after fertilization, it was preserved for anatomical and histological observations. This individual was a female, 82 mm in total length, 48 mm in body length and 10.2 mm in head breadth. The dorsal color of the body was bluish dark brown, that is intermediate between the bluish brown black of *nigrescens* and the dark brown of *tokyoensis*. The ovaries were filled with large pigmented ova, which were about 2 mm in diameter (Fig. 1, A). The oviducts were well-developed.

The Aichi *tokyoensis* hybrid was also a female and reached its maturity about 3 years after fertilization (Fig. 1, B). It was 82.0 mm in total length, 43.5 mm in body length and 9.0 mm in head breadth. This hybrid was intermediate in coloration between *nigrescens* and Aichi *tokyoensis*. The dorsal body surface was greenish brown with many black spots. The upper margin of the tail fin was light brown, lighter than the surroundings. The black spots on the dorsal body surface and the lighter margin of the tail fin are usual characters of Aichi *tokyoensis*. On March 17th, 1956, 3 *Hynobius* pituitaries were transplanted under the

skin of the lower jaw of this female to accelerate ovulation and kept at 11°C. Five days after the transplantation egg masses were taken out from the uteri by dissecting the walls and used for back crossing.

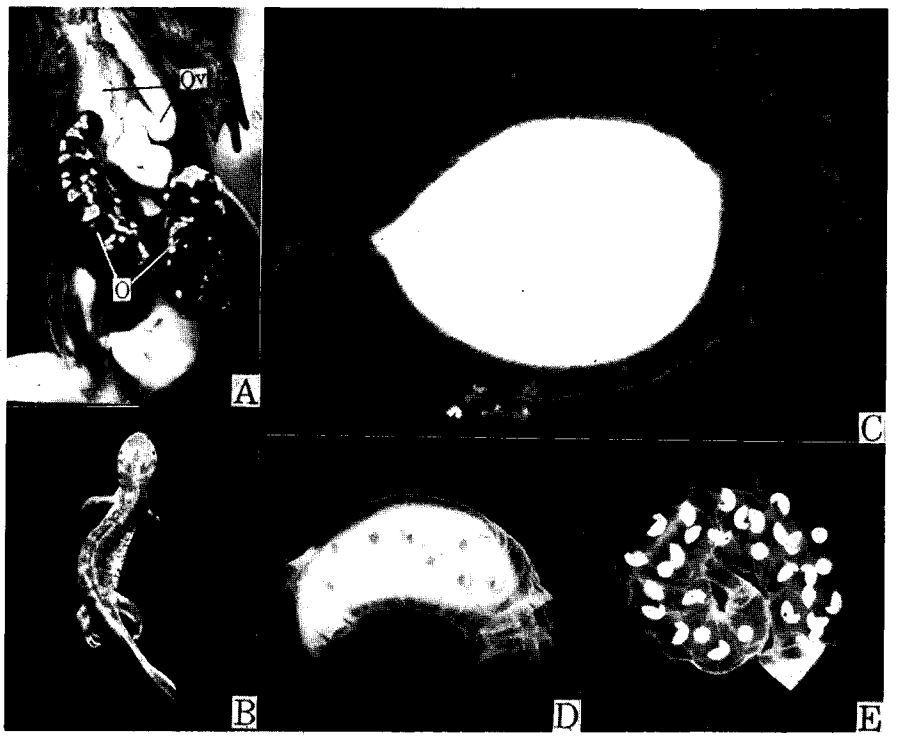

Fig. 1. Mature female hybrids, *Hynobius nigrescens* ♀ × *Hynobius tokyoensis* ♂, and egg capsules of 3 kinds of salamanders. A, Ovaries (O) and oviducts (Ov) of Toyama *nigrescens* ♀ × Ibaraki *tokyoensis* ♂ hybrid. ×2. B, Toyama *nigrescens* ♀ × Aichi *tokyoensis* ♂ hybrid. × 0.6. C, Egg capsule of Toyama *nigrescens*. ×0.7. D, Egg capsule of Toyama *nigrescens* ♀ × Aichi *tokyoensis* ♂ hybrid. × 0.7. E, Egg capsule of Aichi *tokyoensis*. × 0.7.

The egg-capsules were intermediate in shape, structure and color between those of the parental species (Fig. 1, C, D, E). The full swollen egg-capsule of *nigrescens* is usually a thick spindle in shape. The two gelatinous common envelopes are very characteristic. The outer envelope is very thick and flabby and there are innumerable fine stripes on the surface, which are apt to be overlooked. The inner envelope is white and opaque and contains many eggs, which can not be seen from outside. Each egg is covered with its proper gelatinous coat. In

contrast with the egg-capsule of *nigrescens*, that of Aichi *tokyoensis* is rather a circlewise coiled cord. The outer envelope is very thin and comparatively tough. The inner one is transparent. While the animal half of the *nigrescens* egg is light brown, that of the Aichi *tokyoensis* egg is dark brown. The egg-capsule of the *nigrescens* ♀× Aichi *tokyoensis* hybrid was a crescent-shaped spindle, about 70 mm in length, 22 mm in diameter of the middle portion and 17 mm in diameter of the inner envelope. The outer envelope was thick and tender and had innumerable fine stripes on the surface. Although the inner envelope was white, the position of eggs was able to be seen through the envelope. Both egg-capsules contained 19 and 26 eggs, respectively, which were about 2.5 mm in diameter. The animal half of each egg was brown, intermediate in color between those of the *nigrescens* and Aichi *tokyoensis* eggs.

One of the egg-capsules of the hybrid was used for the back-crossing with Toyama *nigrescens* and the other was done with Aichi *tokyoensis*. Of the 26 eggs inseminated with the sperm of a *nigrescens* male only 3 were fertilized and irregularly cleaved and the other 23 eggs were not fertilized. One of the 3 eggs made partial cleavages and died after several divisions. Another egg died at the gastrula stage before closing the blastopore. The third egg became an extremely abnormal neurula with an incomplete neural tube. In this embryo the formation of head portion scarcely occurred. Of the 19 eggs inseminated with the sperm of two *tokyoensis* males 10 were fertilized and made irregular cleavages. Two of them partially cleaved only several times, 4 died before completion of gastrulation and the remaining 4 became abnormal neurulae which were similar to those of the abovementioned hybrids back-crossed with the *nigrescens* sperm.

The present author previously ('53) reported on the hybrids between Aichi *tokyoensis* ♀ and Toyama *nigrescens* ♂. All the 6 hybrids which lived more than three years were males. One of them had a lot of spermatozoa of normal appearance in the seminal vesicles. However, the spermatozoa were almost immotile and produced only a few offspring from among many eggs by crossing with Aichi *tokyoensis* and Tottori and Okayama *nebulosus* females. The fact that the Toyama *nigrescens* ♀× Aichi *tokyoensis* ♂ hybrid produced eggs, which were not fertilized by artificial insemination or quite lethal in the early developmental stages after fertilization, seems to indicate a similar situation in hybrid sterility to the abovementioned male reciprocal hybird.

In the Okayama *nebulosus* ♀× Toyama *nigrescens* ♂ hybrids there were males and females. The females produced egg capsules, which were intermediate in shape, structure and color between those of Okayama *nebulosus* and Toyama *nigrescens* (Kawamura '53, Fig. 4). As the egg-capsules of Aichi *tokyoensis* are very similar in appearance to those of Okayama *nebulosus*, it is an interesting problem to compare the egg-capsules of the Toyama *nigrescens* ♀ × Aichi *tokyoensis* ♂ with those of the Okayama *nebulosus* ♀× Toyama *nigrescens* ♂ hybrids. Although both kinds of egg-capsules were intermediate in appearance between those

of the parental species, it was very clear that they were much closer to those of their maternal species than to those of the paternal one in the shape of the capsule and the structure of the outer envelope. This situation seems to indicate that the inheritance of characteristics of egg-capsules is matroclinous to some extent. Such an inheritance will be ascertained by future investigations on more abundant materials, including females of Aichi *tokyoensis* ♀× Toyama *nigrescens* ♂ hybrids.

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Literature cited

Kawamura, T. 1953. Studies on hybridization in amphibians. V. Physiological isolation among four *Hynobius* species. Jour. Sci. Hiroshima Univ. Ser. B, Div. 1, 14 : 73-116.