ANNOTATIONES ZOOLOGICAE JAPONENSES
Volume 32, No. 1—March 1959

Published by the Zoological Society of Japan
Zoological Institute, Tokyo University

The Hatching Glands of *Rana nigromaculata brevipoda* and of the Reciprocal Hybrids between *Rana nigromaculata brevipoda* and *Rana nigromaculata nigromaculata**

With 1 Text-figure

Takaichi YANAI

Biological Institute, Faculty of Liberal Arts and Science, Shizuoka University

(*Communicated by* K. TAKEWAKI)

In a previous paper (Yanai, 1951), the present writer has already reported the development and structure of the hatching glands of *Rana nigromaculata nigromaculata*. The present paper deals with the results of observations on the hatching glands of another subspecies, *R. n. brevipoda*, and of the reciprocal hybrids between *nigromaculata* and *brevipoda*. The results confirm the author's previous conclusion that the morphology of the hatching glands of amphibians is specific to each species and subspecies, and show that in certain characteristics of the hatching glands of the hybrids the effects of the paternal as well as maternal subspecies are manifested.

MATERIALS

Eggs obtained from the frogs, *brevipoda* and *nigromaculata*, collected in the City of Shizuoka, were inseminated artificially in this laboratory. Four kinds of male-female combinations were made:

brevipoda ♀ × *brevipoda* ♂
nigromaculata ♀ × *nigromaculata* ♂
brevipoda ♀ × *nigromaculata* ♂
nigromaculata ♀ × *brevipoda* ♂

In the combinations using *brevipoda* eggs, embryonic development was slightly delayed in comparison with the combinations with *nigromaculata* eggs. Especially in the *brevipoda* ♀ × *nigromaculata* ♂ combination, the development was occasionally interfered with, resulting in the death of embryos.

R. n. brevipoda used in this investigation was "the Nagoya *brevipoda* race" of Moriya (1954).

* Supported by a grant-in-aid from the Ministry of Education.

OBSERVATIONS

Morphology of hatching glands of Rana nigromaculata brevipoda.

The hatching glands of this subspecies closely resembled those of *R. n. nigromaculata*, but were different from the latter in the amount of melanin content, the distribution pattern on the body surface, the total number and the height of the gland cells.

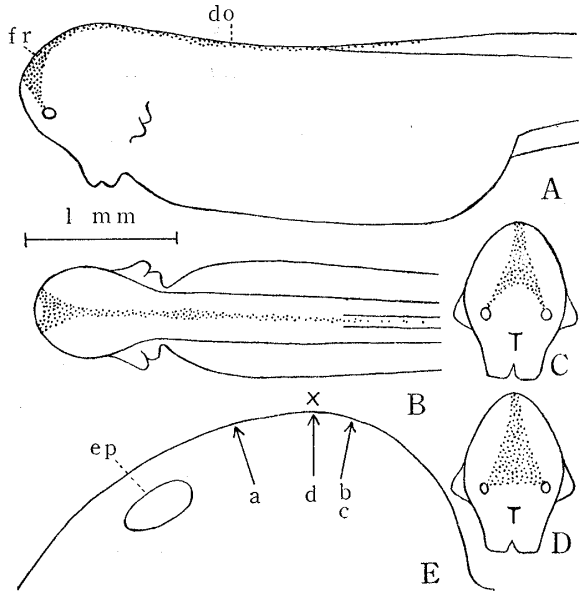

Fig. 1. Distribution of hatching gland cells on body surface of newly hatched larvae. A, B, C, *Rana nigromaculata brevipoda*. D, *R. n. nigromaculata*. Dots correspond to gland cells. fr, frontal group; do, dorsal group. E, anterior limits of distribution of gland cells in median sagittal section of frontal head of *brevipoda* (a), *nigromaculata* (b), *brevipoda* ♀ × *nigromaculata* ♂ (c) and *nigromaculata* ♀ × *brevipoda* ♂ (d). e, epiphysis; ×, anterior tip of front.

Since the amount of melanin in the gland cells was much smaller than that in *nigromaculata* larvae, the glands were hardly distinguishable from the surrounding ordinary epidermal cells, especially in the dorsal region, when examined from the outside. The exact distribution of the gland cells could only be determined by histological study.

On the head, the glands cells were distributed between the right and left nasal pits, the whole frontal group being roughly Y-shaped. Unlike in *nigromaculata* larvae in which the anterior margin of the frontal group was more or less straight, the whole group being triangular in shape, in the present subspecies the anterior margin of the group was concave, considerably depressed rearward in the center (Fig. 1 A, B, C, D). Consequently, in the median sagittal section of the head, the anterior limit of the distribution of the gland cells failed to reach the anterior tip of the front (Fig. 1 E). In the trunk, the posterior end of the dorsal group extended a little more posteriorly in *brevipoda* than in *nigromaculata* (Fig. 1 A, B).

The total number of the gland cells per larva was only about 260, being smaller than that of *nigromaculata* (650) (Table 1). In the frontal group, gland cells were lower in height than in *nigromaculata* (Table 2). However, there was no difference in the structure of each gland cell between the two subspecies.

Morphology of hatching glands of reciprocal hybrids.

Concerning the appearence of the glands from the outside and the amount of melanin in each gland cell, the hybrid *brevipoda* ♀ × *nigromaculata* ♂ was in

agreement with *brevipoda*, and *nigromaculata* ♀×*brevipoda* ♂ with *nigromaculata*, respectively. On the contrary, with respect to the total number, the distribution pattern and the height of the gland cells, the hybrids bore some resemblance to the paternal subspecies; especially, in *nigromaculata* ♀×*brevipoda* ♂, the total number of the gland cells was approximately the same as that in *brevipoda*. In *brevipoda* ♀×*nigromaculata* ♂, the number was intermediate between those of the parental subspecies, more closely resembling *nigromaculata* (Table 1).

Table 1

Comparison of total numbers of hatching gland cells

Subspecies and hybrid	No of individuals	Mean with S. E.	Range of variation	*t*-test (*P*)	
Rana nigromaculata brevipoda	9	258.3± 8.2	226–303		
R. n. nigromaculata	13	649.2±15.5	560–758	<0.005	0.7–0.8
brevipoda ♀×*nigromaculata* ♂	16	508.0±17.7	389–618		
nigromaculata ♀×*brevipoda* ♂	21	273.2± 7.1	214–333		

Table 2

Comparison of heights of hatching gland cells in frontal region

Subspecies and hybrid	No. of individuals	Mean with S. E. (μ)	Range of variation (μ)	*t*-test (*P*)	
Rana nigromaculata brevipoda	9	24.8±0.92	21–30	0.02–0.05	
R. n. nigromaculata	13	27.6±0.66	23–32	<0.005	0.1–0.2
brevipoda ♀×*nigromaculata* ♂	17	32.5±1.11	26–40	<0.005	
nigromaculata ♀×*brevipoda* ♂	27	23.2±0.51	20–32		

As regards the anterior limit of distribution of the gland cells in the median sagittal section of the head, *brevipoda* ♀×*nigromaculata* ♂ agreed well with *nigromaculata*, and *nigromaculata* ♀×*brevipoda* ♂ was situated between the two subspecies, the anterior margin reaching the anterior tip of the front (Fig. 1 E). The same was the case with the posterior limit of the distribution of the gland cells on the dorsal trunk, i.e., in both hybrids, it was more like the paternal subspecies than the maternal. The glands cells of the frontal group of *brevipoda* ♀×*nigromaculata* ♂ were very high, even higher than those of *nigromaculata*, while those of *nigromaculata* ♀×*brevipoda* ♂ were approximately as high as those of *brevipoda* (Table 2).

These results appear to show that, in the hybrids, the accumulation of melanin in the gland cells is largely controlled by a maternal factor (or factors), while the distribution pattern and the total number of the gland cells as well as the height of the cells are determined by a paternal factor (or factors). Whether

the controls are brought about by the nucleus or by the cytoplasm is yet to be determined.

SUMMARY

1. The hatching glands of *Rana nigromaculata brevipoda* closely resembled those of *R. n. nigromaculata*, but the amount of melanin in each gland cell in *brevipoda* was much smaller than in *nigromaculata* and the total number of the gland cells was much larger in *nigromaculata* (650) than that in *brevipoda* (260). The distribution pattern of the gland cells and the height of the gland cells in the frontal region were also different between the two subspecies.

2. In the hybrids between the two subspecies, the amount of melanin in the hatching glands more closely resembled the maternal subspecies than the paternal, whereas the distribution pattern and the total number of the gland cells as well as the height of the cells were more like the paternal subspecies than the maternal.

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