

A Three-year-old Haploid Frog

By

Sumio MIYADA

Biological Institute, College of Liberal Arts, Kobe University, Kobe, Japan

(With 6 Text-figures and 1 Plate)

INTRODUCTION

It has been reported by several researchers that a small number of haploid amphibian embryos produced by various methods could take food and survive for a considerably long time (BALTZER, 1922; FISCHBERG, 1948; MIYADA, 1960; HAMILTON, 1963). Up to the present, metamorphosed haploids have been obtained in *Triton taeniatus* (BALTZER, 1922), *Triton alpestris* (FISCHBERG, 1948) and *Rana nigromaculata* (MIYADA, 1960). Besides them, haploid *Pleurodeles waltlii* were reared beyond the time of metamorphosis by means of parabiosis with diploids, and 12 of these haploids lived for 2 or 3 years, in the meantime attaining their sexual maturity (GALLIEN and BEETSCHEN, 1960; GALLIEN, 1963, '67). As for anurans, a 10-month-old haploid *Rana nigromaculata* obtained by the present author (1960) seems to have had the longest terrestrial life after metamorphosis.

In 1961 many viable haploid tadpoles were incidentally obtained in *Rana nigromaculata* by the gynogenetic experiments which were performed to examine the effect of environmental temperature on the early development of haploid frog embryos. One of them was very superior to the other haploids in viability and lived for 3 years. By a cytological observation, it was clearly found that this frog had diploid or nearly diploid cells in several small areas of the intestines as well as in the most parts of the pancreas, while no diploid cells were found in other organs. Therefore, this frog was identified to be nearly pure haploid. In this paper, the life history, characteristics and sex of this 3-year-old haploid frog will be reported.

MATERIALS AND METHODS

The gynogenetic haploid embryos of *Rana nigromaculata* HALLOWELL were produced by the same method as described in a previous paper of the present author (1960). The eggs obtained from the cloacae of the females whose ovulation occurred naturally were inseminated with spermatozoa treated with toluidine blue. Some of the eggs of each female were inseminated with untreated spermatozoa of the same male as the respective controls. Inseminated eggs were divided into three groups and reared at a comparatively low temperature (15~16°C), a comparatively high temperature (20~22°C) and room temperature (13

~22°C), respectively, until the feeding stage. Almost all the viable haploid tadpoles developed from embryos which were reared at the comparatively high temperature and room temperature. The ploidy of tadpoles was identified by the tail-tip method (*cf.* FANKHAUSER, 1945). Metamorphosed frogs were fed on flies and spiders. All the frogs were fixed in NAVASHIN'S fluid and preserved in 70% alcohol. Various organs of the preserved animals were cut into serial paraffin sections at 10 μ or 15 μ and stained with HEIDENHAIN'S iron hematoxylin. In order to compare histologically with the 3-year-old haploid frog, two control diploid frogs, a 2-year-old male and a 3-year-old female, were used.

OBSERVATION

I. Life history of the 3-year-old haploid frog

In the breeding season of the year 1961, seven metamorphosed haploid frogs were produced from the eggs of one and the same female. Five of them developed from among embryos reared at 20~22°C, as shown in Table 1. The 3-year-old haploid (No. CH-n5) was one of these five haploids.

TABLE 1
Life histories of haploid offspring produced from a female by gynogenesis in 1961

Exp. no. (Temperature)	No. of eggs	No. of cleaved eggs	Embryos which died before hatching	No. of hatched haploids		Haploids which reached the stage of metamorphosis	
				Invi- able	Viable	Died during metamor- phosis	Com- pleted metamor- phosis
DT1C-H (20~22°C)	362	339 (93.65%)	248 (68.51%)	14 (3.87%)	70 (19.34%)	2 (0.55%)	5* (1.38%)
DT1C-R (room temp., 13~22°C)	350	329 (94.00%)	290 (82.86%)	12 (3.43%)	24 (6.86%)	1 (0.29%)	2 (0.57%)
DT1C-L (15~16°C)	342	318 (92.98%)	295 (86.26%)	20 (5.85%)	3 (0.88%)	0 (0.0%)	0 (0.0%)

* The 3-year-old haploid frog was one of these 5 haploids.

Haploid tadpoles began to climb out of water 85~122 days after the insemination, while the control diploids did after 53~70 days. The haploid No. CH-n5 completed its metamorphosis 96 days after the insemination. A week after the completion of metamorphosis this haploid frog was 20.5 mm in body length, while ten control diploids were 22.0~25.5 mm. As four of the seven metamorphosed haploid frogs did not take food, they were killed and preserved about 10 days after metamorphosis, together with five control diploids. The remaining three haploid frogs as well as five control diploid frogs fed on flies and spiders. In spite of their good appetite, the haploids were so slow in activity that it was

hardly possible for them to take such actively moving food. So the spiders and flies were given to them after removing some of the legs of the former and the wings of the latter from their bodies. Three haploid frogs (Nos. CH-n1, CH-n4 and CH-n5) and five control diploid frogs (Nos. CH-2n3, CH-2n6, CH-2n8, CR-2n1 and CR-2n2) were made to hibernate in a box which was kept about 7°C. One of the haploids, No. CH-n1, died at the age of 7 months. Another haploid No. CH-n4 and a control diploid No. CR-2n1 were killed and preserved at the age of 8 months.

The remaining haploid frog No. CH-n5 and four control diploid frogs Nos. CH-2n3, CH-2n6, CH-2n8 and CR-2n2 marked their first birthday. At the age of just one year, the haploid was 25.5 mm in body length, while the four diploids were 32.3 mm, 31.0 mm, 30.5 mm and 28.6 mm, respectively. During the year 1962, the haploid frog was considerably delayed in growth as compared with the control diploids, although it vigorously took the food prepared by the same method as mentioned above. While two of the four control diploids, Nos. CH-2n6 and CR-2n2, died at the age of 19 months and 20 months, respectively, the haploid frog passed the second winter in good health. At the age of just two years, the haploid was 30.5 mm in body length, while the remaining two control diploids Nos. CH-2n3 and CH-2n8 were 46.3 mm and 45.0 mm, respectively. It was clear from their external appearances that one frog (No. CH-2n8) was a male and the other (No. CH-2n3) a female. In contrast with them, it was impossible to conjecture the sex of the haploid from its external characters. The diploid male died at the age of 2 years and 22 days.

In the third year, 1963, the haploid was remarkable in growth from summer to late autumn, although it was extremely inferior to the diploid female. This frog seemed to be a female, since it did not reveal any of the appearances characteristic of a male.

In the next year, soon after the end of the third hibernation, the haploid frog suddenly became inert and, moreover, somewhat edematous. As it was believed from these symptoms that the only haploid was in a critical condition, this frog was killed at the age of 3 years and 10 days, together with the control diploid female.

II. Evidence for the haploidy

1. Direct evidences

The haploidy of the 3-year-old haploid frog was already identified at the young tadpole stage by the counting of the chromosome number, $n=13$, in epidermal cells of the tail-tip. Moreover, the haploid number of chromosomes was exactly counted in two clear mitotic figures in the left ovary of this frog (Fig. 1). Approximately 13 chromosomes were also counted in epithelial cells of the intestines, cells of mesonephric tubules of the kidneys, and some other kinds of cells.

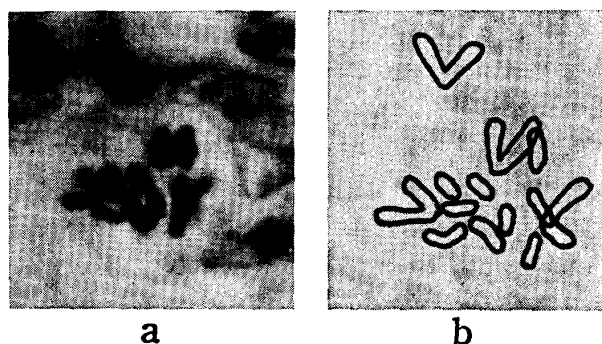


Fig. 1. a. Microphotograph of a haploid mitotic figure of an oogonium in the left ovary of frog No. CH-n5. $\times 1500$.
b. The same mitotic figure drawn by a camera lucida. $\times 2000$.

2. Indirect evidences

Interphase nuclei of various kinds of cells of the haploid frog were compared in size with those of the control diploid frogs. Fifty nuclei in each kind of tissues were sketched with the aid of a camera lucida, and then the longest and the shortest diameters of each nucleus were measured. The nuclear size was represented for the sake of comparison by the dimensions obtained from the product of the longest diameter multiplied by the shortest. Some of the results of measurements are presented in Table 2. In the nearly spherical nuclei of cells of mesonephric tubules, nerve cells and hepatic cells, the haploid-diploid ratios were 1: 1.42 to 1: 1.86 in dimensions. These were very near to the ratios, 1: 1.42 to 1: 1.96, which were reported in a previous paper (MIYADA, 1960).

TABLE 2
Comparison of nuclear sizes between the haploid frog and two controls

Kind of cells	Indiv. no. (Age)	CH-n5 (3 years)	CH-2n3 (3 years)	CH-2n8 (2 years)
	Ploid	n	2n	2n
Cells of mesonephric tubules	Mean dimensions of 50 nuclei (μ^2)	40.20 \pm 1.28	57.17 \pm 1.34	63.77 \pm 1.53
	Ratio	1	1.42	1.59
Nerve cells of cerebrum	Mean dimensions of 50 nuclei (μ^2)	48.25 \pm 2.15	86.55 \pm 1.33	78.36 \pm 2.51
	Ratio	1	1.79	1.62
Hepatic cells	Mean dimensions of 50 nuclei (μ^2)	29.68 \pm 0.10	55.16 \pm 0.91	49.10 \pm 1.21
	Ratio	1	1.86	1.65
Epithelial cells of small intestine	Mean dimensions of 50 nuclei (μ^2)	35.82 \pm 1.25	59.30 \pm 1.61	61.17 \pm 0.86
	Ratio	1	1.66	1.71

III. Morphological characters

The 3-year-old haploid frog was nearly the same in most of the morphological characters with the young haploid frogs which were previously described by the

author (MIYADA, 1960). Accordingly, in this paper several important characters of this haploid will be preponderantly described.

1. External characters

Measurements of some bodily parts of the haploid frog at the time of preservation are presented in Table 3, together with those of the control diploid frogs.

TABLE 3
Measurements of some bodily parts of the haploid frog and two controls

Indiv. no.	Ploid	Body length (mm)	Snout length (mm)	Diameter of eye (mm)		Diameter of tympanic membrane (mm)		Fore limb length (mm)		Hind limb length (mm)	
				Right	Left	Right	Left	Right	Left	Right	Left
CH-n5, 3 years	n	35.5	3.8 (0.107)	4.8 (0.135)	4.5 (0.127)	2.8 (0.079)	2.9 (0.082)	12.5 (0.352)	13.0 (0.366)	41.7 (1.175)	42.0 (1.183)
CH-2n3, 3 years	2n	55.5	8.0 (0.144)	7.0 (0.126)	6.5 (0.117)	5.0 (0.090)	4.6 (0.083)	23.0 (0.414)	22.0 (0.396)	84.0 (1.514)	83.0 (1.495)
CH-2n8, 2 years	2n	45.0	6.2 (0.138)	5.8 (0.129)	5.6 (0.124)	4.0 (0.089)	3.8 (0.084)	18.0 (0.400)	18.7 (0.416)	66.0 (1.467)	64.5 (1.433)

Parentheses present the ratio of the length or diameter of each bodily part to the body length.

The haploid was a distinct dwarf, being about two-thirds of the control diploid No. CH-2n3 in body length (Plate I, 1, 2). The snout and the hind limbs of the haploid were apparently short for the body length, as compared with those of the control diploids. The ratio of the snout length to the body length of the haploid frog and that of the control diploid No. CH-2n3 were 0.107:1 and 0.144:1, respectively. On the other hand, the ratio of the hind limb length to

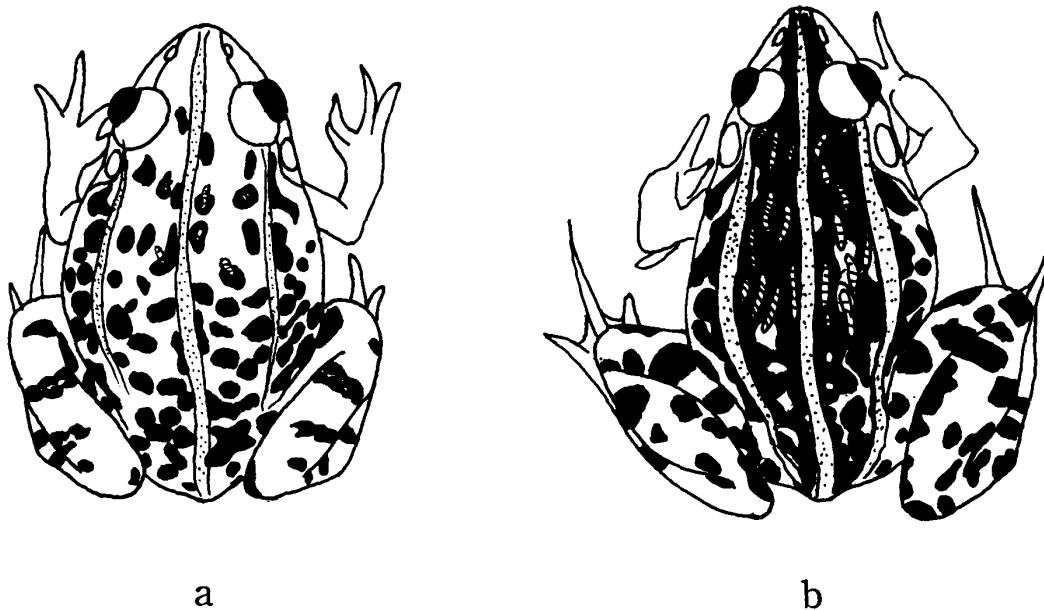


Fig. 2. Diagrammatic drawings showing differences in appearance between the haploid and the control diploid frogs.

- a. Three-year-old haploid frog No. CH-n5. $\times 1.5$
b. Control diploid female No. CH-2n3 at the same age. $\times 1$.

the body length of the haploid and that of the diploid were 1.175:1 and 1.514:1, respectively. The tip of the snout of the haploid frog was more obtuse than those of the control diploids. Accordingly, the haploid frog looked pudgy.

The black spots on the dorsal and lateral surfaces, the dorsal ridges and dorso-lateral folds of the haploid frog were compared with those of the control diploid female No. CH-2n3 by means of the diagrammatic drawings, as shown in Fig. 2, in which the body size of the haploid was magnified nearly to that of the control diploid. Main differences between them were as follows:

- a. The haploid frog had numerous, small and roundish black spots on the dorsal and lateral surfaces, differing from the diploid, which revealed the sexual color and pattern characteristic of a mature female. The haploid did not reveal the sexual color and pattern in spite of the age of three years.
- b. The haploid frog had a very few small dermal ridges on the back, differing from the diploid which had many distinct rod-shaped ridges arranged nearly in parallel.
- c. The dorso-lateral folds of the haploid were less prominent than those of the diploid.

2. Internal characters

a. Anatomical characters

The liver, especially its middle lobe, of the haploid frog was small, as compared with those of the control diploid frogs (Plate I, 3, 4).

Although it was very difficult to take an accurate measurement on a fixed frog, the intestines of the haploid frog were distinctly shorter and less coiled than those of the control diploids (Fig. 4). The intestines of the haploid were about 28 mm in length, while those of the diploid male No. CH-2n8 and female No. CH-2n3 were about 48 mm and 61 mm, respectively.

The spleen of the haploid frog was extremely small in size; the dimensions of the largest cross section was $810 \mu \times 580 \mu$, while those of the diploids Nos. CH-2n8 and CH-2n3 were $2750 \mu \times 2500 \mu$ and $4400 \mu \times 3560 \mu$, respectively.

b. Histological characters

i) Intestines

In the mucous membrane of the intestines of the haploid frog, there were several small areas which mainly consisted of diploid cells* (Fig. 3, a). The diploid areas were so narrow that in a cross section of the small intestine, for example, even the largest area occupied only one of 16 folds of the mucous membrane. Each of the diploid areas was usually spindle-shaped. Along the intestines, there were about ten areas; two in the duodenum, five in the small intestine and three in the large intestine. These diploid areas were scattered along the whole length of the intestines, as shown by a diagrammatic drawing in Fig. 4. In this diagram, each of the ten circular outlines, representing the diploid areas, does not show

* The diploid cells were identified not by the number of chromosomes but by the comparison in size between their interphase nuclei and those of the control diploid cells.

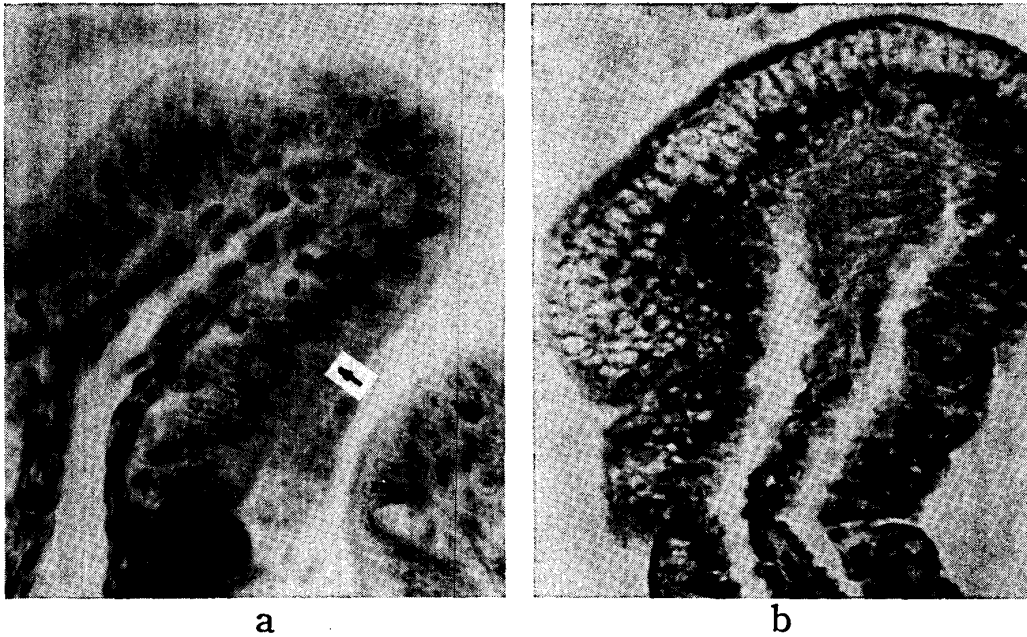


Fig. 3. Microphotographs of cross-sections of the intestine of the 3-year-old haploid frog. $\times 460$.

- a. A diploid area in a fold of the mucous membrane of the small intestine. An arrow indicates this area.
- b. Abnormal epithelium of the duodenum.

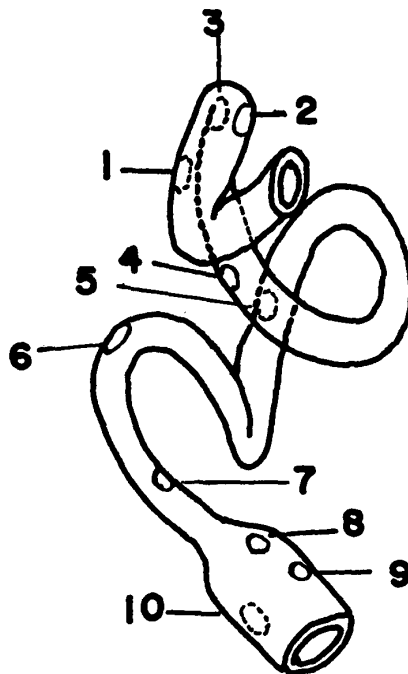


Fig. 4. Diagrammatic drawing showing distribution of diploid areas in the intestine of the haploid frog. \times ca. 5.

its real size. For convenience' sake, the size of each diploid area was represented by the product of the width of the largest cross section and the length of the area

TABLE 4
The size of each diploid area in the mucous membrane of the
intestine of the haploid frog

	Area no.*	Largest width (μ)	Length (μ)	Largest width \times Length (μ^2)
Duodenum	1	330	180	59400
	2	280	150	42000
Small intestine	3	355	350	124250
	4	250	220	55000
	5	225	100	22500
	6	215	280	60200
	7	155	170	26350
Large intestine	8	370	190	70300
	9	460	180	82800
	10	595	250	148750

* Each figure is the same as shown in Fig. 4.

which was calculated from the number of cross sections cut at 10μ . Such sizes are presented in Table 4.

Apart from the existence of the diploid areas, the small and large intestines were normal in histological structure. However, in the duodenum, especially near the opening of the bile duct, there were abnormal or morbid parts, in which the epithelial cells mostly contained a distinct vesicle. Their nuclei seemed to lose their basophilic nature or to become pycnotic (Fig. 3, b).

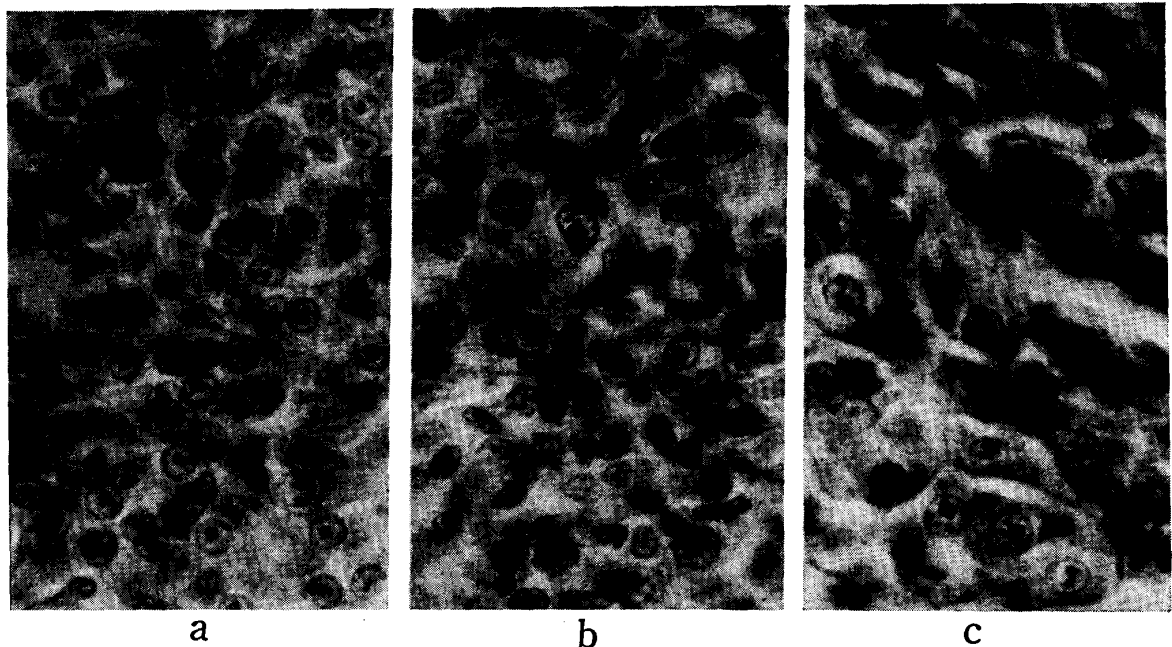


Fig. 5. Microphotographs of sections of pancreases. $\times 700$.
a. An almost pure haploid area in the haploid frog.
b. A haploid-diploid area in the haploid frog.
c. The pancreas of the control diploid frog No. CH-2n3.

ii) Pancreas

The pancreas was mostly composed of a mixture of haploid and diploid cells.* Almost pure haploid areas occupied only narrow place in the neighborhood of the center of the pancreas. Moreover, it was worthy to note that no tubular gland structure characteristic of the pancreas was found in the haploid-diploid areas, while in the haploid areas there was a somewhat normal tubular structure was found (Fig. 5, a, b).

iii) Spleen

As stated above, the spleen of the haploid frog was remarkably small. However, it seemed normal in histological structure.

iv) Sense organs

The heads of the haploid frog and the two control diploids Nos. CH-2n3 and CH-2n8, were cut into serial sections after removing the lenses from their eyes.

The ears and the nose of the haploid were approximately normal in size in proportion to the body size; they were quite normal in structure.

The eyes of the haploid frog were normal in structure, except for the reduced thickness of the retinae. Measurements of some parts of the eyes are presented in Table 5. The retinae of the 3-year-old haploid frog were about two-thirds of

TABLE 5
Measurements of the eyes of the haploid frog and two controls

Indiv. no. (Age)	Ploid	Lateral and dorso-ventral diameters of lens (μ)	Thickness of cornea (μ)	Thickness of retina (μ)
CH-n5 (3 years)	n	R, 2750×2910 L, 2640×2850	54.5 48.8	130 140
CH-2n3 (3 years)	2n	R, 2950×3250 L, 2800×3300	46.0 50.5	207 215
CH-2n8 (2 years)	2n	R, 2850×3100 L, 2900×3250	47.5 45.6	210 235

R, right eye. L, left eye.

those of the control diploids in thickness, as presented in Table 5. The same fact was already reported in nine haploid frogs by the author (1960).

The other haploid organs such as tongue, stomach, liver, heart, brains, lungs, skin, muscle, thymus gland and kidneys were normal or nearly normal in anatomical and histological structures. These organs as well as the above described spleen and sense organs had no diploid areas.

IV. Sex

1. Gonads

The 3-year-old haploid frog was a female. This frog had ovaries which were

* See footnotes in p. 218.

the best in development among haploid *Rana nigromaculata* obtained hitherto by the present author. The right and left ovaries were about 7.8 mm and 8.6 mm in length and composed of 8 and 11 lobes, respectively. They were almost normal in structure, although they were much delayed in development as compared with those of the control diploid female No. CH-2n3 (Plate I, 5, 6). The haploid ovaries were filled with a large number of growing auxocytes which contained no yolk granules and a considerable number of degenerating auxocytes (Fig. 6, a). It was noticeable that the degenerating auxocytes occupied one-fourth to one-third of the total number of auxocytes in each of the ovaries. On the other hand, the largest auxocyte in the ovaries of the haploid frog was about 330 μ in diameter, while that in the diploid female No. CH-2n3 was about 1100 μ . Large auxocytes of the diploid were compactly laden with yolk granules (Fig. 6, b).

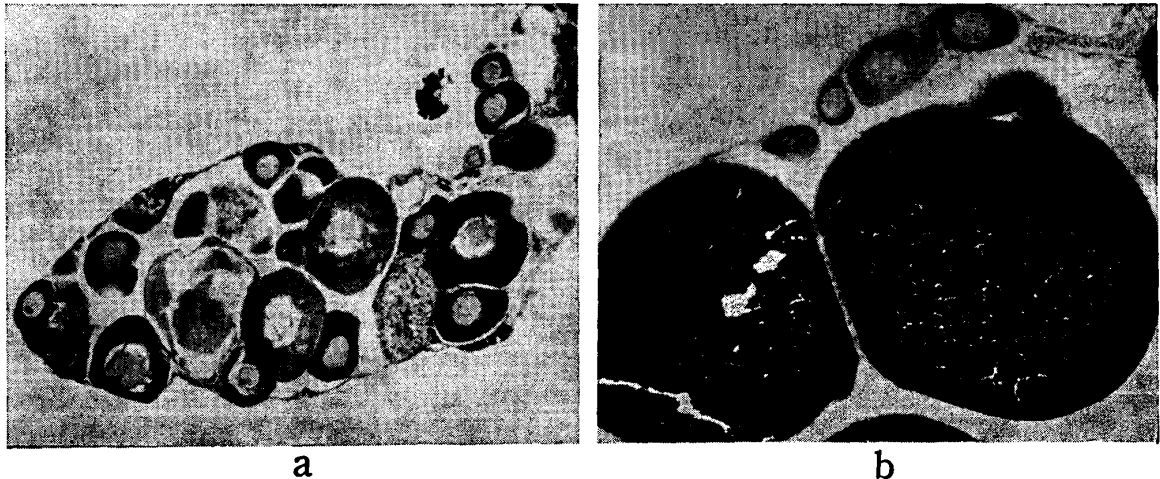


Fig. 6. Microphotographs of cross-sections of the ovaries of the haploid and the control frogs. $\times 32$.

- a. The left ovary of the haploid frog.
- b. The right ovary of the control diploid frog No. CH-2n3.

The facts described above seemed to show that in the ovaries of the haploid frog all the auxocytes degenerate, before yolk deposition begins to occur in them.

2. Oviducts

The oviducts of the haploid frog were fairly well-developed and convoluted, although very inferior to those of the diploid female No. CH-2n3 (Plate I, 7). They were quite normal in histological structure, and were very similar to those of diploid frogs in the non-breeding season, that is, there was no hypertrophy of the walls attributable to the seasonal development of the mucous membrane.

DISCUSSION

1. Haploidy of the 3-year-old haploid frog

As described above, the 3-year-old haploid frog was identified to be almost pure haploid individual, having a small number of diploid cells in the intestines and pancreas.

As for amphibians, it seems that all the individuals included hitherto under the name of "haploid" are not always pure haploids in a strict sense of the word. PARMENTER (1933) has described that there are a small number of larvae containing a few diploid cells besides numerous haploid ones among haploid *Rana pipiens* and *Rana palustris* produced parthenogenetically. Similar observations have also been reported in parthenogenetic *Rana nigromaculata* by KAWAMURA (1939), in androgenetic *Rana pipiens* by PORTER (1939), BRIGGS (1946) and SUBTELNY (1958). According to these investigators, some regulation to diploidy is expected in the tissues of haploid embryos, especially in older haploids. Moreover, it must be pointed out that the haploidy of most of the haploid embryos used as the material for various experiments is usually identified by the number of chromosomes and the nuclear size of the epidermal cells in their tail-fins, together with their external appearances, etc. (BRIGGS, 1946, '49; MIYADA, 1953; HAMILTON, 1963, '65, '66). Concerning the purity of metamorphosed haploid amphibians, FANKHAUSER (1938) has reported that a haploid *Triton taeniatus* obtained by BALTZER (1922) was uniformly haploid in various organs and tissues. Besides, out of nine metamorphosed haploid *Rana nigromaculata* obtained gynogenetically by the present author (1960), two had a small number of diploid cells which were sporadically distributed in some organs such as the intestines and kidneys, and among red blood cells. However, no particular comment on the purity of their haploidy is made in the previous paper, because the percentages of diploid cells are so low (below about 0.5%) that their existence is considered negligible.

Most of the facts reported by the above investigators seem to suggest that the regulation from haploidy to diploidy frequently occurs in haploid amphibians. Such regulation will probably occur at various stages of development. When it occurs at an early stage of development, especially at the early cleavage stage, haploid-diploid bilateral mosaics or more irregular ones will be produced. Such mosaics have been found among tadpoles of several frog species (PARMENTER, 1933, '40; KAWAMURA, 1939; PORTER, 1939). In contrast with this, when the regulation occurs at a later developmental stage, mosaics consisting of a mixture of haploid and diploid cells are produced, as found in an androgenetic mosaic newt, *Triturus pyrrhogaster* (KAYLOR, 1940).

Concerning the 3-year-old haploid frog, it is supposed that the diploid cells in the intestines and pancreas have been produced from haploid cells by the regulation from haploidy to diploidy at a later developmental stage, probably at

the late tadpole stage or after metamorphosis. This supposition is based on the following facts: (1) in the intestines each of the diploid areas is very small in dimensions and has no connection with the others; (2) there are no great differences in size among the diploid areas of the intestines; (3) diploid areas are found in the intestines and pancreas alone. At the same time, it seems probable that the intestines and pancreas are the organs, in which the chromosomal regulation from haploidy to diploidy is apt to occur.

2. Viability of haploid frogs

In a previous paper, the present author (1960) suggested that the occurrence of viable haploids is related to the genetic constitution of mother frogs, in which lethal or deleterious genes are few. This suggestion is principally indebted to DARLINGTON's hypothesis (1937) that the poor viability of haploids was possibly due to the existence of certain recessive factors or genes.

In the year of 1961, nine metamorphosed haploid frogs were produced by the gynogenesis from eggs of two of ten *Rana nigromaculata* females. Seven of these haploid frogs developed from eggs of one and the same female, and the 3-year-old haploid frog was one of these seven haploids, as shown in Table 1. This state of affairs seems to give an additional support to the suggestion based on the genetic constitution of a special female.

On the other hand, it was noteworthy that the 3-year-old haploid frog together with four other haploid siblings developed from eggs reared at a comparatively high temperature 20~22°C until the feeding stage. A beneficial effect of a comparatively high temperature on the early embryonic development of haploids has been confirmed by serial experiments carried out by the author during the years 1961~1964 (unpublished). Concerning the effect of the environmental temperature on the development of haploid embryos, there seems to be no reports but HAMILTON's. Differing from the observation by the present author, she (1963) has stated that in *Xenopus laevis* no obvious morphological differences are found between haploid androgenetic tadpoles reared at 22°C and those at 16°C.

It may be worth-while discussing whether the excellent viability of the 3-year-old haploid frog was intimately related with the existence of diploid cells in the intestines and pancreas or not. KAYLOR (1940) has suggested that the excellent viability of a 120-day-old mosaic consisting of a mixture of haploid and diploid cells, as compared with a 47-day-old pure haploid in *Triturus pyrrhogaster*, partly depends on the existence of diploid cells. According to FANKHAUSER (1952), the normal development of haploid parts of bilateral mosaics is dependent on a beneficial "vitalizing" effect of normal diploid tissues on adjacent haploid ones. In connection with these matters, it seems very interesting that haploid androgenetic urodeles, *Pleurodeles waltlii*, joined in parabiosis with diploids of the same species lived for 2 or 3 years (GALLIEN and BEETSCHEN, 1960; GALLIEN, 1963, '67).

The 3-year-old haploid frog described in this paper had certainly the intestines with several small diploid areas and the pancreas consisting of a mixture of

haploid and diploid cells. However, it seems difficult to assume that the diploid cells in these organs improved the viability of this frog. This is based on the following reasons: (1) in the intestines, the diploid areas are too small to exert beneficial effects upon the viability of the haploid frog; (2) the irregular structure of the haploid-diploid areas in the pancreas is not considered to have a beneficial effect upon the viability of this animal.

In conclusion, the excellent viability of the 3-year-old haploid frog seems to be related to the genetic constitution of the mother, in which lethal or deleterious genes are few, and to the beneficial effects of a comparatively high temperature at the early developmental stage of this frog.

3. Characters of haploid frogs

The morphological characters of the 3-year-old haploid frog were different from those of the control diploid frogs in many respects. Some of them are considered to be the characteristics which are directly connected with the haploidy in *Rana nigromaculata*, because they have also been observed in young haploid frogs reported in a previous paper (MIYADA, 1960). Such characters are as follows:

- (1) The body is dwarf, about two-thirds of that of the control diploids.
- (2) The bodily form is pudgy, owing to that the proportions of the snout and limb lengths to the body length are smaller in haploid frogs than those in the control diploids.
- (3) The dorso-lateral folds on the back of the body are less prominent than those of diploid frogs.
- (4) The retinae of the eyes are reduced in thickness, being about two-thirds of those of diploid frogs.
- (5) The ovaries are extremely delayed in development and differentiation.

Up to the present, twelve androgenetic *Pleurodeles waltlii* joined in parabiosis with diploids of the same species are the only haploids which were raised near to their sexual maturity (GALLIEN and BEETSCHEN, 1960; GALLIEN, 1963, '67). Of these haploid parabiotic newts, four were males with submature testes, five were males with immature or vestigial testes, two had indifferent gonads and the remaining one was a hermaphrodite with an ovotestis. In the submature testes, the spermatogenesis was abortive and failed at the stage of primary spermatocytes. Accordingly, the male haploid urodeles had no reproductive ability.

In anurans, the 3-year-old haploid frog obtained by the present author is probably the first individual raised near to the stage of sexual maturity. This haploid was a female. Although female diploid *Rana nigromaculata* reared in the laboratory usually attain their sexual maturity at the age of two years, the female haploid did not produce mature ova in spite of the age of three years. She remained at the submature stage, as it were. The ovaries of this frog were principally normal in histological structure. However, they contained a large number of degenerating auxocytes besides growing auxocytes. There were no auxocytes which had begun to deposit yolk granules in the cytoplasm. This seems

to suggest that in the haploid ovaries oogenesis was blocked at a certain stage before yolk accumulation. Accordingly, this female haploid frog did not seem to have a reproductive ability, even if she lived longer.

SUMMARY

1. A number of viable gynogenetic haploid tadpoles were produced from *Rana nigromaculata* eggs by gynogenesis. The haploidy of these tadpoles was identified by the tail-tip method. Seven of them completed their metamorphosis. One of these metamorphosed haploids lived for 3 years.

2. The 3-year-old haploid frog together with six other haploids was produced from eggs of one and the same mother. This haploid was fed on flies and spiders which were made inactive by removing the wings and some legs, respectively, because it was too slow to take the actively moving food.

3. The haploidy of the 3-year-old haploid frog was cytologically confirmed on various kinds of organs and tissues after its death. This frog was actually a pure haploid, except that several small areas in the intestines consisted of diploid cells and that the pancreas was mostly constructed of a mixture of haploid and diploid cells.

4. The haploid frog was a female. It differed from the control diploid female of the same age in the following points: (1) it was a dwarf, being about two-thirds of the control diploid in body length, and looked pudgy; (2) it did not reveal the sexual color and pattern; (3) dermal ridges on the back were very few and small; (4) the dorso-lateral folds were less prominent.

5. The visceral organs of the haploid frog were of approximately normal size in proportion to its body size, except the liver, spleen and intestines which were abnormally small. Besides, the retinae were about two-thirds in thickness of those of the control diploids.

6. The ovaries of the haploid female were filled with a large number of growing auxocytes which contained no yolk granules and a considerable number of degenerating auxocytes. The oviducts were normal in histological structure.

7. The haploid female did not seem to have a reproductive ability, even if she lived longer.

ACKNOWLEDGMENTS

The author wishes to express his gratitude to Dr. Toshijiro KAWAMURA, Professor Emeritus of Hiroshima University, for his kind guidance during the course of this work and kindness in reading the original manuscript.

LITERATURE

- BALTZER, F. 1922. Ueber die Herstellung und Aufzucht eines haploiden *Triton taeniatus*. Verh. Schweiz. Naturf. Ges. **103**: 248-249.

- BRIGGS, R. 1946. Effects of the growth inhibitor, hexenolactone, on frog embryos. I. Effects on diploid embryos. II. Differential effects on haploid and diploid embryos. *Growth* **10**: 45-73.
- 1949. The influence of egg volume on the development of haploid and diploid embryos of the frog, *Rana pipiens*. *J. Exp. Zool.* **111**: 255-294.
- DARLINGTON, C. D. 1937. Recent advances in cytology. 2nd ed. Philadelphia.
- FANKHAUSER, G. 1938. The microscopical anatomy of metamorphosis in a haploid salamander, *Triton taeniatus* LAUR. *J. Morph.* **62**: 393-413.
- 1945. The effects of changes in chromosome number on amphibian development. *Quart. Rev. Biol.* **20**: 20-78.
- 1952. Nucleo-cytoplasmic relations in amphibian development. *Internat. Rev. Cytol.* **1**: 165-193.
- FISCHBERG, M. 1948. Experimentelle Auslösung von Heteroploidie durch Kältebehandlung der Eier von *Triton alpestris* aus verschiedenen Populationen. *Genetica* **24**: 213-329.
- GALLIEN, L. and J. C. BEETSCHEN. 1960. Différenciation sexuelle et gamétogenèse abortive chez un mâle haploïde d'Urodèle (*Pleurodeles waltlii*), élevé en parabiose. *C. r. hebd. Séanc. Acad. Sci., Paris* **251**: 1655-1657.
- GALLIEN, L. 1963. Différenciation sexuelle d'individus haploïdes du triton *Pleurodeles waltlii* MICHAH. mis en parabiose. Effets de compétition dans les associations ♀ 2n : ♂ n. *C. r. hebd. Séanc. Acad. Sci., Paris* **257**: 2890-2893.
- 1967. Développement d'individus haploïdes adultes élevés en parabiose chez le triton *Pleurodeles waltlii* MICHAH.: Syndrome de l'haploïdie et différenciation sexuelle. *J. Embryol. exp. Morph.* **18**: 401-426.
- HAMILTON, L. 1963. An experimental analysis of the development of the haploid syndrome in embryos of *Xenopus laevis*. *J. Embryol. exp. Morph.* **11**: 267-278.
- 1965. The development of haploid and diploid embryos of *Xenopus laevis* after treatment with sodium thiocyanate and lithium chloride. *Expl. Cell Res.* **38**: 684-685.
- 1966. The role of the genome in the development of the haploid syndrome in Anura. *J. Embryol. exp. Morph.* **16**: 559-568.
- KAWAMURA, T. 1939. Artificial parthenogenesis in the frog. I. Chromosome numbers and their relation to cleavage histories. *Jour. Sci. Hiroshima Univ., Ser. B, Div. 1*, **6**: 115-218.
- KAYLOR, C. T. 1940. Studies on experimental haploidy in salamander larvae. I. Experiments with eggs of the newt, *Triturus pyrrhogaster*. *Biol. Bull.* **79**: 397-408.
- MIYADA, S. 1953. The oxygen consumption of haploid larvae in the frog *Rana nigromaculata*. (In Japanese) *Sci. Rep. Hyogo Univ. Agric. (Series Natur. Sci.)* **1**: 9-12.
- 1960. Studies on haploid frogs. *Jour. Sci. Hiroshima Univ., Ser. B, Div. 1*, **19**: 1-56.
- PARMENTER, C. L. 1933. Haploid, diploid, triploid and tetraploid chromosome numbers and their origin in parthenogenetically developed larvae and frogs of *Rana pipiens* and *Rana palustris*. *J. Exp. Zool.* **66**: 409-453.
- 1940. Chromosome numbers in *Rana fusca* parthenogenetically developed from eggs with known polar body and cleavage history. *J. Morph.* **66**: 241-260.
- PORTER, K. R. 1939. Androgenetic development of the egg of *Rana pipiens*. *Biol. Bull.* **77**: 233-257.
- SUBTELNY, S. 1958. The development of haploid and homozygous diploid frog embryos obtained from transplantations of haploid nuclei. *J. Exp. Zool.* **139**: 263-306.

EXPLANATION OF PLATE

PLATE I

1. Three-year-old haploid frog No. CH-n5. $\times 1$.
2. Three-year-old control diploid frog No. CH-2n3. $\times 1$.
- 3 and 4. Visceral organs of the same haploid and diploid frogs as (1) and (2). 3, Haploid frog. $\times 2$. 4, Control diploid frog. $\times 1.5$
- 5 and 6. The ovaries of the same haploid and diploid frogs as (1) and (2). 5, Haploid frog. $\times 2$. 6, Control diploid frog. $\times 1.5$
7. The oviducts (Müllerian ducts) of the haploid frog. $\times 2$.

