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Lampbrush Chromosomes of an Allotriploid Frog Produced from (*Rana brevipoda*×*Rana nigromaculata*) ♀×*Rana brevipoda*♂

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ABSTRACT A female allotriploid frog was produced from a female diploid hybrid, *Rana brevipoda*♀×*Rana nigromaculata*♂, by mating with a male *Rana brevipoda*. The lampbrush chromosomes were examined in order to clarify their origin and behavior at the first meiotic division. Twenty-six chromosomes from *R. brevipoda* and 13 chromosomes from *R. nigromaculata* were observed. In most oocytes, only one to five triplets of homologous chromosomes formed trivalents, while the others formed bivalents and univalents of the same numbers. These bivalent and univalent chromosomes came from *R. brevipoda* and *R. nigromaculata*, respectively. The remaining oocytes contained 13 bivalents from *R. brevipoda* and 13 univalents from *R. nigromaculata*. (*Zool. Mag. 87: 64-68, 1978*)

In a previous paper, the writer (1975) reported on the lampbrush chromosomes of two Japanese pond-frog species, *Rana nigromaculata* and *Rana brevipoda*, and their hybrids. The oocytes of female hybrids had always 13 bivalents. The writer thereafter obtained a female allotriploid from a female diploid hybrid between these two species by backcrossing with a male *Rana brevipoda*.

The behavior of allotriploid chromosomes during meiotic divisions was observed by Beçak and Beçak (1970) in male triploid hybrids between *Odontophrynus cultripes* and *Odontophrynus americanus* and by Günther (1975) in male triploid „*Rana esculenta*". However, no report has been made on lampbrush chromosomes of allotriploid amphibians.

In this paper, the writer will report on the lampbrush chromosomes of the only female allotriploid obtained by him in order to clarify the origin and behavior of the three homologous chromosomes belonging to each of the 13 triplets.

Material and Methods

A female triploid hybrid between *Rana brevipoda* Ito and *Rana nigromaculata* Hallowell was used as material in this work. This female was accidentally produced from a female diploid hybrid between a female *Rana brevipoda* and a male *Rana nigromaculata* in 1975 by backcrossing with a male *brevipoda*. The female diploid hybrid laid a small number of large eggs besides numerous ordinary ones. The only female triploid hybrid arose from one of these large eggs. The triploidy of this female was noticed in the following year when the lampbrush chromosomes of her oocytes were observed after being preserved by Gall's method (1966).

The most suitable oocytes for observing lampbrush chromosomes were 1.6 to 1.8mm in diameter. In order to disperse lampbrush chromosomes, a mixture of 5 parts of 0.075M KCl solution, one part of 0.075M NaCl solution and 0.12 parts of 10% Formalin was used. Analyzable preparations were obtained from 31 oocytes.

Observation

The lampbrush chromosomes of this female triploid consisted of 13 triplets of homologous chromosomes, Nos. I to XIII. On the basis of landmarks along chromosome axes (cf. Fig. 3, Ohtani, 1975), it was found that each of the triplets was composed of two *Rana brevipoda* and one *Rana nigromaculata* chromosomes (Fig. 1). However, the three chromosomes of

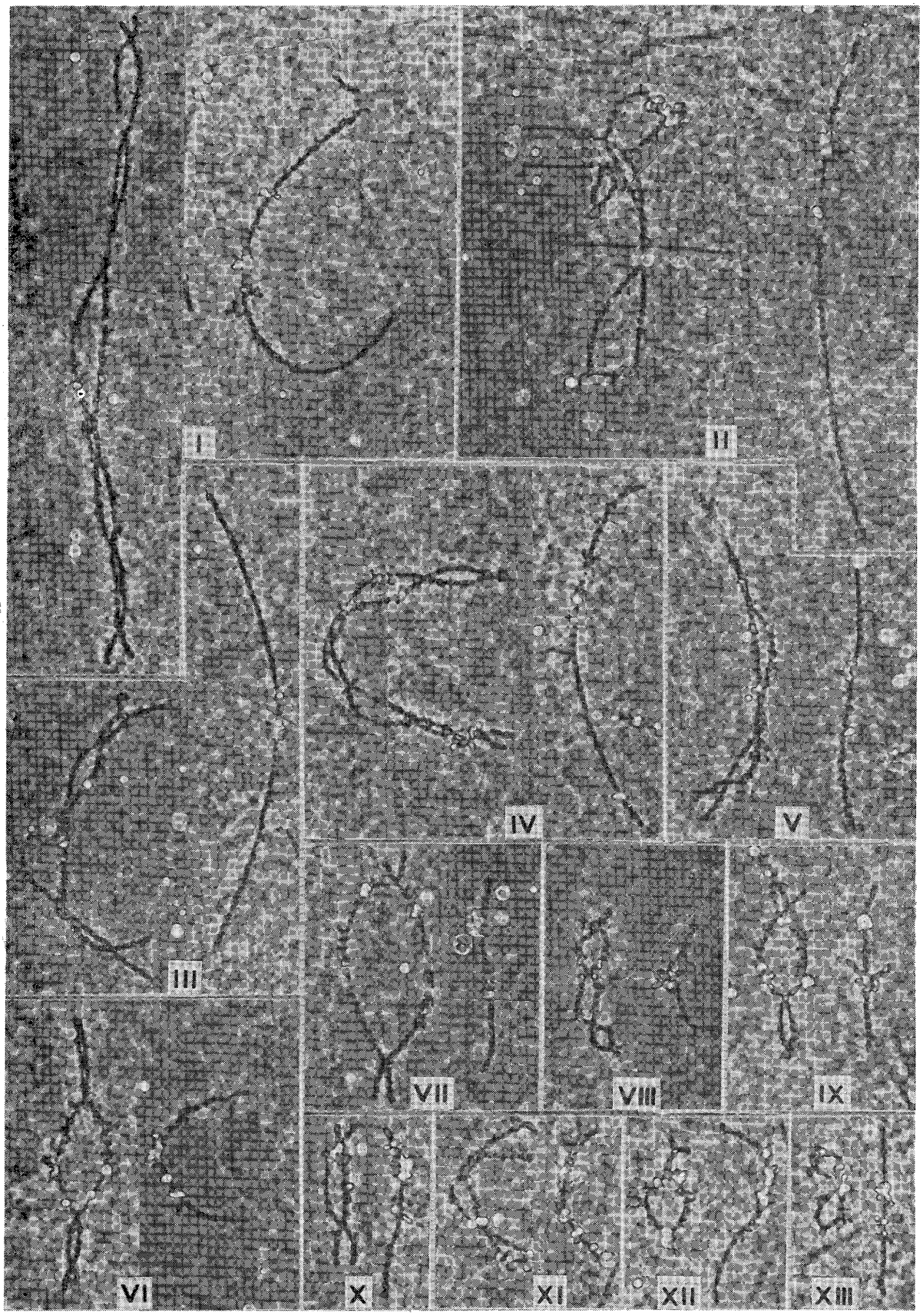

Table 1. Numbers of uni-, bi- and trivalent chromosomes in 31 oocytes of a female allotriploid between *Rana brevipoda* and *Rana nigromaculata*

Univalents	Bivalents	Trivalents	Oocytes
13	13	0	8
12	12	1	7
11	11	2	10
10	10	3	5
8	8	5	1

each triplet usually formed a trivalent in a few cases. The frequencies of uni-, bi- and trivalent chromosomes in 31 oocytes are presented in Table 1. There were one to five trivalents in 23 oocytes, while the remaining eight oocytes contained no trivalents. All the chromosomes other than those of trivalents formed bivalents and univalents of the same numbers. Each of the bivalent consisted of two *Rana brevipoda* chromosomes which joined with each other by one to nine chiasmata and occasionally by a telomere fusion. Each univalent came from *Rana nigromaculata*.

In each trivalent, the *nigromaculata* chromosome joined to one of the two *brevipoda* chromosomes by a certain number of chiasmata and frequently by a telomere fusion in addition. The five triplets of large-chromosomes Nos. I~V formed 19 trivalents in the 31 oocytes (Table 2). In eleven of these trivalents, the *nigromaculata* chromosome joined to one of the two *brevipoda* chromosomes by one to three chiasmata which were situated in the subterminal or terminal region, while in three other trivalents the joining occurred by one chiasma situated in the median region. In the remaining five trivalents, the *nigromaculata* chromosome joined to one of the two *brevipoda* chromosomes by a telomere fusion alone. The eight triplets of small-chromosomes Nos. VI~XIII fromed 28 trivalents in the 31 oocytes (Table 2). In 19 of them, the *nigromaculata* chromosome joined to one of the *brevipoda* chromosomes by one chiasma which was situated in the terminal region, while in the other nine trivalents the joining occurred by a telomere fusion alone.

Table 2. Number of trivalents in 31 oocytes of a female allotriploid between *Rana brevipoda* and *Rana nigromaculata*

Chromosome No.	Trivalent	Bivalent + Univalent	Total
I	6	25	31
II	6	25	31
III	1	30	31
IV	4	27	31
V	2	29	31
VI	11	20	31
VII	1	30	31
VIII	0	31	31
IX	4	27	31
X	1	30	31
XI	4	27	31
XII	2	29	31
XIII	5	26	31

The frequency of trivalents of chromosomes Nos. I~XIII in 31 oocytes is presented in Table 2. The trivalents of chromosomes Nos. I, II, VI and XIII were found in more than five oocytes; especially, those of No. VI were in eleven oocytes. No trivalents of chromosome No. VIII were found in 31 oocytes. The remaining eight kinds of chromosomes formed trivalents in less than four oocytes.

Discussion

Although triploidy is established by fertilization of a diploid ovum with a haploid spermatozoon or by fertilization of a haploid ovum with a diploid spermatozoon, the following three pathways are conceivable in produc-

Fig. 1. Photographs of chromosomes Nos. I~XIII in an oocyte of a female allotriploid between *Rana brevipoda* and *Rana nigromaculata*. ×400.

ing diploid ova. Firstly, a diploid ovum is produced by retention of the second polar body immediately after insemination, secondly by retention of the first polar body and release of a diploid second polar body, and thirdly by regular meiotic divisions of a tetraploid primary oocyte produced accidentally in the ovary of a diploid female. Triploid frogs produced from fertilized eggs by refrigeration or a heat-shock were presumed to arise through the first pathway (Kawamura, 1941a, b; Sato, 1952; Muto, 1952). Through a similar pathway, allotriploid frogs were also produced by Kawamura (1952a, b). Nishioka (1971) produced two kinds of autotriploids and four kinds of allotriploids from *Rana nigromaculata* and *Rana brevipoda* by various methods. Female allotriploids obtained from a female or male tetraploid by mating with a male or female diploid produced mature ova.

On the other hand, spontaneous occurrence of triploid frogs has been reported by Hertwig and Hertwig (1920) and Dalcq (1930). Hertwig and Hertwig found that all the control tadpoles of a crossing experiment between a female *Rana esculenta* and a male *Bufo viridis* were triploid. These triploids surely arose from diploid eggs by fertilization with haploid spermatozoa. Hertwig and Hertwig presumed that the diploid eggs were produced by the lack of the second maturation division or retention of the second polar body, or from a tetraploid or triploid female. Wickbom (1945) described three adult triploid *Rana esculenta*. Günther (1970) found that seven of 24 *Rana esculenta* examined by him were triploid. Günther (1975) reported again that spontaneous triploids sometimes amounted to more than 80% of *Rana esculenta* distributed in a district of the German Democratic Republic. According to Berger (1966, 1967, 1968, 1973), *Rana esculenta* is a hybrid between *Rana ridibunda* and *Rana lessonae*. Uzzell *et al.* (1975) clarified that triploid *Rana esculenta* are largely produced from unreduced ova of female diploid *Rana esculenta* by fertilization with sperm of *Rana ridibunda* or *Rana lessonae*.

In the present study, it was clarified that the female triploid produced from a large ovum of a female diploid hybrid, *Rana brevipoda* ♀ × *Rana nigromaculata* ♂, by fertilization with sperm of a male *Rana brevipoda* consisted of two genomes from *Rana brevipoda* and one genome from *Rana nigromaculata*. Thus, the large ovum was surely an unreduced one consisting of one genome from *Rana brevipoda* and one genome from *Rana nigromaculata*. Such a diploid ovum can be produced by regular meiotic divisions of a tetraploid primary oocyte which appears accidentally in the ovary of a diploid female, as insisted by Uzzell *et al.* (1975). The writer really found primary oocytes with 26 pairs of bivalent chromosomes in the ovary of the female hybrid which produced the present allotriploid (unpublished).

All the mature allotriploids between *Rana japonica* and *Rana ornativentris* obtained by Kawamura (1952b) were sterile males; the first reduction divisions of spermatocytes were always very abnormal. Unlike these allotriploids of brown frogs, those obtained by Beçak and Beçak (1970) from crossing between *Odontophrynus cultripes* (2n=22) and *Odontophrynus americanus* (2n=44) were not sterile. In male triploids, uni-, bi- and trivalent chromosomes appeared in various frequencies in the first metaphase plates of spermatogenesis. These frequencies were very similar to those found in the oocytes of the female allotriploid between *Rana brevipoda* and *Rana nigromaculata*. This situation in these two kinds of allotriploids seems to differ from that in male triploid *Rana esculenta* described by Günther (1975). In the latter triploids, first metaphase plates with diploid chromosomes were always found in addition to those with 13 bivalents and 13 univalents or with various numbers of univalents, bivalents or multivalents.

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References

BEÇAK, M.L. AND W. BEÇAK (1970) Further studies on polyploid amphibians (*Ceratophrydidae*) III. Meiotic aspects of the interspecific triploid hybrid: *Odontophrynus cultripes* (2n=22)×*O. americanus* (4n=44). *Chromosoma* (Berl.) 31: 377-385.

BERGER, L. (1966) Biometrical studies on the population of green frogs from the environs of Poznan. *Annal. Zool.* (*Warszawa*) 23: 303-324.

——— (1967) Embryonal and larval development of F_1 generations of green frogs different combinations. *Acta. Zool. Crac.* 12: 123-160.

——— (1968) Morphology of the F_1-generation of various crosses within *Rana esculenta* complex. *ibid.* 13: 301-324.

——— (1973) Systematics and hybridization in European green frogs of *Rana esculenta* complex. *J. Herpetol.* 7: 1-10.

DALCQ, A. (1930) La formule chromosomiale chez la grenouille. *Ann. Bull. Soc. Roy. Sci. Méd. Nat. Bruxelles* 1930: 15-22.

GALL, J.G. (1966) Techniques for the study of lampbrush chromosomes. *Methods in Cell Physiology* (edited by D.M. Prescott), Academic Press, New York. pp. 37-60.

GÜNTHER, R. (1970) Der Karyotyp von *Rana ridibunda* Pall. und das Vorkommen von Triploidie bei *Rana esculenta* L. (Anura, Amphibia). *Biol. Zbl.* 89: 327-342.

——— (1975) Untersuchungen der Meiose bei Männchen von *Rana ridibunda* Pall., *Rana lessonae* Cam. und der Bastardform „*Rana esculenta*" L. (Anura). *ibid.* 94: 277-294.

HERTWIG, G. UND P. HERTWIG (1920) Triploide Froschlarven. *Arch. mikr. Anat.* 94: 34-54.

KAWAMURA, T. (1941a) Triploid frogs developed from fertilized eggs. *Proc. Imp. Acad. Tokyo* 17: 523-526.

——— (1941b) On the sex of triploid frogs in *Rana nigromaculata*. (In Japanese) *Zool. Mag.* (Tokyo) 53: 334-347.

——— (1952a) The sex of triploid frogs, *Rana japonica* Günther. *J. Sci. Hiroshima Univ., Ser. B, Div.* 1, 13: 121-128.

——— (1952b) Triploid hybrids of *Rana japonica* Günther×*Rana temporaria ornativentris* Werner. *ibid.* 13: 129-138.

MUTO, Y. (1952) Production of triploid toads, *Bufo vulgaris formosus* (Boulenger), by a temperature-shock on fertilized eggs. *ibid.* 13: 163-171.

NISHIOKA, M. (1971) Abnormal combinations of the nucleus and cytoplasm and their effects in amphibians. (In Japanese) *Symposia Cell. Biol.* 22: 189-203.

OHTANI, H. (1975) The lampbrush chromosomes of the sibling species, *Rana nigromaculata* and *Rana brevipoda*, and their hybrids. (In Japanese) *La Kromosomo* 100: 3162-3172.

SATO, M. (1952) The sex of triploid frogs, *Rana limnocharis*. *J. Sci. Hiroshima Univ., Ser. B, Div.* 1, 13: 155-161.

UZZELL, T., L. BERGER AND R. GÜNTHER (1975) Diploid and triploid progeny from a diploid female of *Rana esculenta* (Amphibia Salientia). *Proc. Acad. Nat. Sci. Phila.* 127: 81-91.

WICKBOM, T. (1945) Cytological studies on Dipnoi, Urodela, Anura, and *Emys*. *Hereditas* 31: 241-346.