Artificial Triploid Hybrids by Interspecific Mating of *Odontophrynus* (Amphibia, Anura)

Odontophrynus cultripes Reinhard and Lutken, 1862, and *O. americanus* (Duméril and Bibron), 1882, are 2 related species of the family *Ceratophrydidae*; while the former is a diploid species, the latter is a tetraploid one (Figure 1 a and c).

The karyotype of *O. cultripes* has 22 chromosomes consisting of 11 pairs of homologues (Figure 2a). These are represented by 11 bivalents in meiotic metaphase I and by 11 dyads in metaphase II. The karyotype of *O. americanus* has 44 chromosomes which may be sorted into 11 groups, each with 4 homologues (Figure 2b). 11 ring quadrivalents were counted in meiotic metaphases I and in metaphases II, 22 chromosomes were observed[1,2].

Comparative microphotometric DNA measurements as well as nuclear volume measurements of several tissues showed a 1:2 ratio between those 2 species[3].

Interspecific hybrids were obtained crossing diploid and tetraploid species; the present paper deals with these observations.

Specimens of *O. americanus* collected in São Paulo, Brazil, and specimens of *O. cultripes* collected in Belo Horizonte, Minas Gerais, Brazil, were maintained in water tanks containing also earth and sand. Spontaneous breeding of *O. americanus* was observed in October (spring), the tadpoles hatching 4 days after spawning, while in *O. cultripes* the spontaneous breeding and hatching has been observed during May and June (autumn).

In the interval between those spontaneous breedings, interspecific matings were induced by s.c. inoculation, in males as in females, of the macerated anterior pituitary lobe from *Bufo* specimens. Each *Odontophrynus* received about 30 mg of macerated material from 4 animals.

O. cultripes males and *O. americanus* females were induced to mate in October. 9 h after inoculation of hypophysis, spawning was observed. As many as 300 tadpoles hatched after 4 days. They developed normally, metamorphosis starting $4^1/_2$ months later. Their viability is apparently good, having only 5 animals perished up to the present time (Figure 1b).

Twenty-five experiments of *O. americanus* males × *O. cultripes* females cross were tried during the interval between January and June. The experiments between January and April, a total of 20, did not result in spawning. During April, May and June 5 experiments were done. All these crosses of *O. americanus* male × *O. cultripes* female resulted in ovulation, occurring 2–4 days after pituitary stimulation. The embryos from these matings did not survive gastrulation. At only 1 occasion, the hatching of 7 tadpoles was observed, which died, however, at stage 21 (after Shumway), less than 48 h after hatching.

Cytological studies were performed in 12 hybrids, *O. cultripes* males × *O. americanus* females, during different tadpole phases, and after metamorphosis. Four tadpole hybrids of *O. americanus* males × *O. cultripes* females were analysed soon after natural death.

The tadpoles, to be analysed, were immersed into a 1% solution of colchicine for $1^1/_2$–2 h prior to sacrifice. Some of them were analysed without the use of colchicine. In metamorphosed amphibians, an inoculation of a 0.5% solution of colchicine in a dosage of 0.2 ml/g was done 2 h prior to sacrifice. Small pieces of various tissues were fixed for 15 min in a 50% solution of glacial acid, after pretreatment in cold distilled water for 15 min. The fragments were

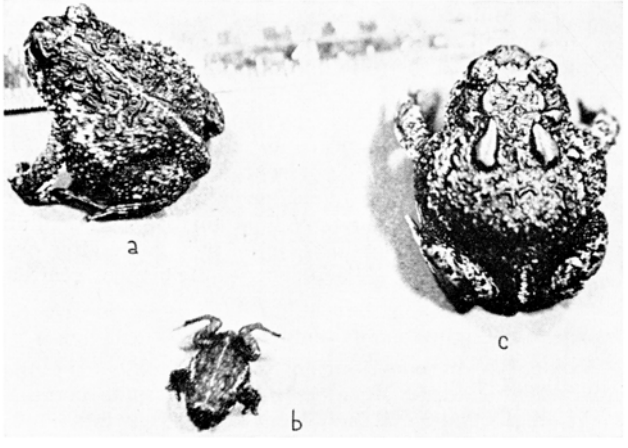

Fig. 1. (a) *O. americanus* adult tetraploid ($^3/_5$ natural size). (b) *O. cultripes* ♂ × *O. americanus* ♀ triploid hybrid, 9 months old, $4^1/_2$ months after metamorphosis ($^3/_5$ natural size). (c) *O. cultripes* adult diploid ($^3/_5$ natural size).

[1] M. L. BEÇAK, W. BEÇAK and M. N. RABELLO, Chromosoma *19*, 188 (1966).
[2] M. L. BEÇAK, W. BEÇAK and M. N. RABELLO, Chromosoma *22*, 192 (1967).
[3] W. BEÇAK, M. L. BEÇAK, D. LAVALLE and G. SCHREIBER, Chromosoma *23*, 14 (1967).

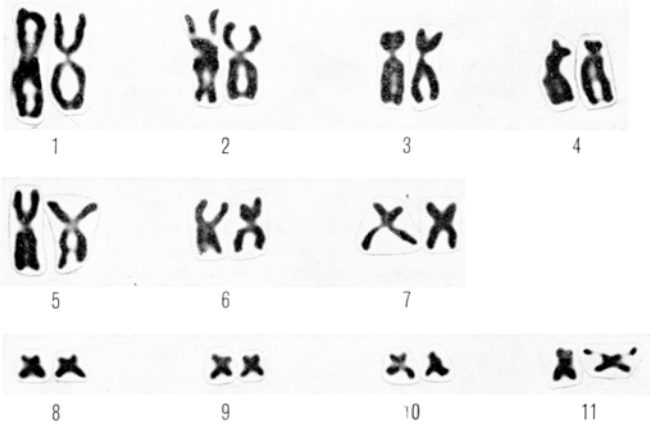

Fig. 2a. Karyotype of a diploid *O. cultripes* male showing 11 groups of 2 homologues. × 1750.

squashed and the coverslips removed in a mixture of methanol and dry ice. The cytological preparations were stained with Giemsa after hydrolysis for 10 min in *N* HCl at 60 °C.

In the hybrids, obtained by both types of inter-specific cross-breeding, the karyotypes and chromosome counts show 33 chromosomes (Figure 3b). The triploid nature of both types of hybrids was demonstrated. The karyotypes consist of 11 groups of 3 chromosomes (Figure 3a). The correspondent chromosomes of the 2 species in each group are apparently similar, but they can be distinguished by their peculiarities (Table).

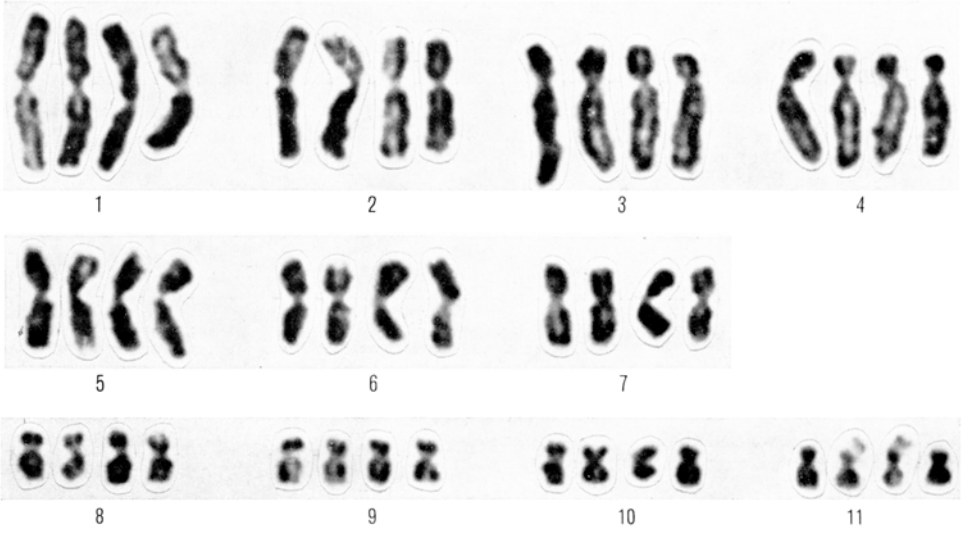

Fig. 2b. Karyotype of a tetraploid *O. americanus* male showing 11 groups of 4 homologues. × 2250.

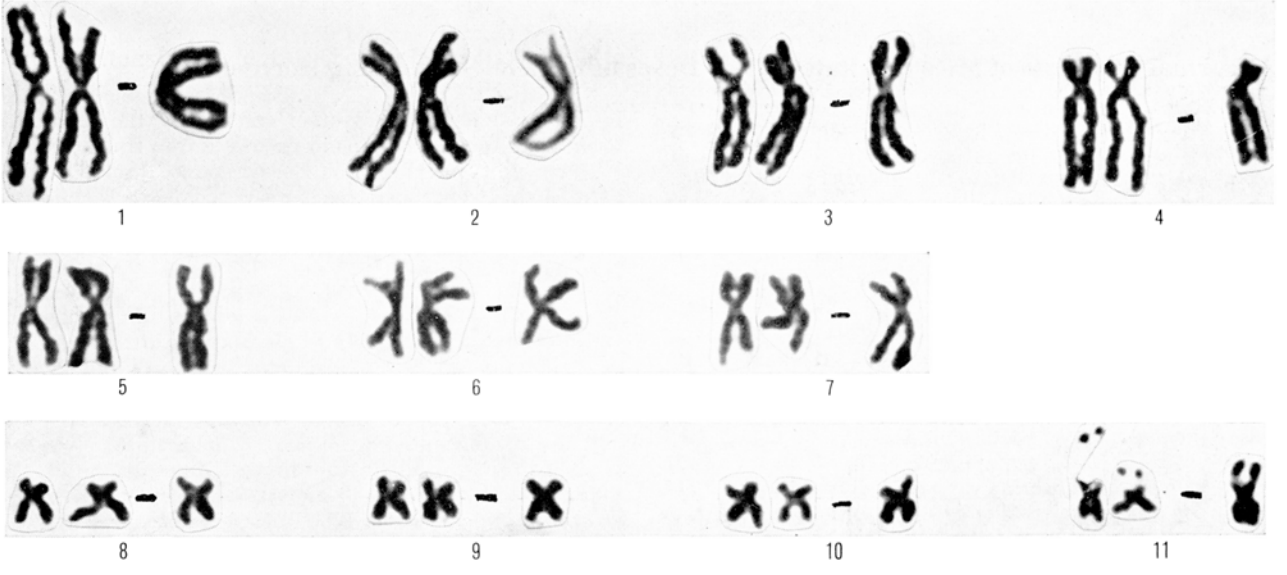

Fig. 3a. Karyotype of the triploid hybrid *O. cultripes* ♂ × *O. americanus* ♀, consisting of 11 groups of 3 chromosomes. × 3250.

Chromosome measurements of *O. cultripes* and *O. americanus*, based on 10 karyotypes of each species (Beçak[4])

	Species	1	2	3	4	5	6	7	8	9	10	11
Relative	*O. cultripes*	20	13	12	12	10	9	8	5	4	4	3
length	*O. americanus*	17	15	13	12	10	10	8	4	4	4	3
Arm	*O. cultripes*	1.2	1.6	2.0	4.0	1.3	1.0	1.4	1.1	1.3	1.2	2.0
ratio	*O. americanus*	1.3	1.7	2.3	4.0	1.2	1.3	1.1	1.5	1.5	1.5	2.8
Centromere	*O. cultripes*	0.46	0.38	0.34	0.20	0.43	0.50	0.41	0.48	0.43	0.45	0.33
index	*O. americanus*	0.44	0.37	0.31	0.20	0.45	0.43	0.48	0.40	0.40	0.40	0.26

Histological sections of the *O. cultripes* males × *O. americanus* females hybrids, up to 7 months after hatching, were analysed. The specimens apparently do not show abnormalities in their somatic development.

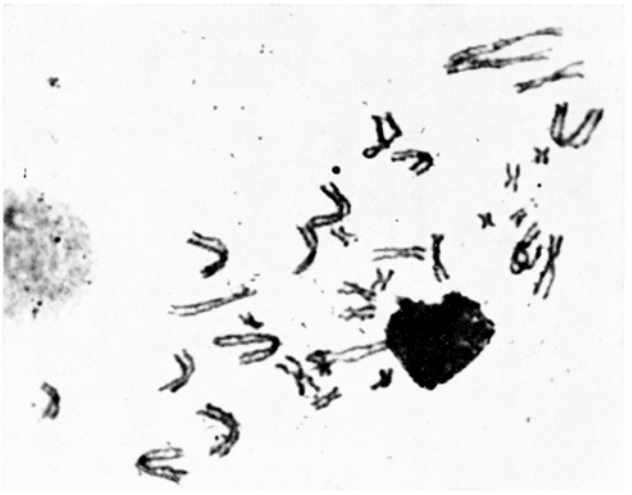

Fig. 3b. Somatic metaphase of the triploid hybrid *O. americanus* ♂ × *O. cultripes* ♀ showing 33 chromosomes. × 1350.

Development of gonads has been followed; the results will be published elsewhere, together with the results of sex-ratio analysis in hybrids[5].

Résumé. Des hybrides triploïdes ont été obtenus par croisement de *Odontophrynus cultripes*, amphibien diploïde, avec *O. americanus*, tétraploide. Les hybrides interspécifiques présentent normalement 33 chromosomes dans les cellules somatiques. Les hybrides *O. cultripes* ♂ × *O. americanus* ♀ sont plus viables que les *O. americanus* ♂ × *O. cultripes* ♀.

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Secção de Genética and Secção de Fisiopatologia, Instituto Butantan, São Paulo (Brazil), 8 June 1968.

[4] M. L. BEÇAK, *Cariótipos e evolução cromossômica em Amphibia Anura* Thesis, São Paulo (1967).

[5] This work was supported by Public Health Service Research Grant No. GM-14577-02 from the National Institute of General Medical Sciences and by Fundo de Pesquisas do Instituto Butantan.

Abnormal Response of Mice and Rats to Low Doses of Follicle-Stimulating Hormone

An examination of the response of mice to low doses of follicle-stimulating hormone (FSH) by the method we described previously[1], led to the surprising result that, when the dose was reduced below a certain level, a significant lowering of intravaginal triphenyltetrazolium chloride (TTC) reduction response was observed as compared with controls treated with 20 IU HCG only.

Below 1 μg of FSH (lyophilized pig FSH, Mann Research Laboratories, New York) this fall reaches a maximum at 0.25 μg (approximately 25%, $P < 0.01$) (Figure 1).

The same phenomenon occurs with another sample of FSH (Ormonoterapia Richter, Milan; sample 223 MMP), of high content (2–2.5 times higher than the first). In this case, the response lowering range is below 0.5 μg of FSH, with a maximum fall value at 0.125 μg, as expected, in view of the greater specific activity of this sample, equivalent to 23% ($P < 0.01$) (Figure 1).

This anomaly is encountered also in the classic STEELMAN and POHLEY test[2], where a decrease of the ovarian weight can be noticed, compared with controls treated with HCG alone (40 IU).

When using sheep NIH-FSH-S2, such a decrease is significantly below 22.5 μg of FSH. The same result can be obtained with other samples of FSH (Ormonoterapia Richter, sample 223 MMP; lyophilized pig FSH, Mann Research Laboratories); in the latter case the decrease is reported below 5 μg of FSH (Figure 2).

The result may be considered as being evidence of an interaction between exogenous hormone administered and the endogenous follicle-stimulating system. This interaction indicates the presence of a direct (or indirect, acting at hypothalamic level instead of directly on the hypophysis) feed-back system controlling the secretion of pituitary gonadotropins in the rat and in the mouse.

If we now interpret the dose-response curves in the light of this hypothesis, we can see that each is the sum of 2 curves which represent the endogenous and the exogenous contribution to the circulating level of follicle-stimulating activity.

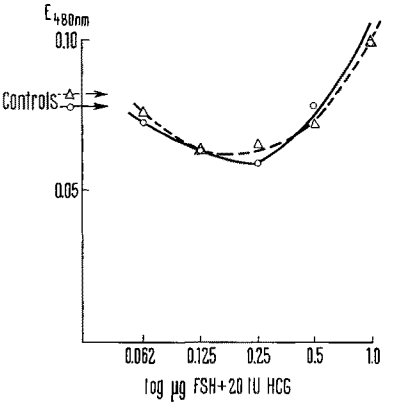

Fig. 1. Evaluation of FSH activity by intravaginal TTC reduction[1]. ○——○ Pig FSH (Mann Res. Lab.); △——△ FSH (Ormonoterapia Richter).

[1] M. M. CASELLATO, G. LUGARO and K. WEYDANZ, Experientia *23*, *1078* (1967).

[2] S. L. STEELMAN and F. M. POHLEY, Endocrinology *53*, 604 (1953).