

ON THE POSSIBILITY OF INDUCED SEX TRANSFORMATION IN UNDIFFERENTIATED SEX RACE OF *RANA CATESBEIANA* TADPOLES

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ABSTRACT

Tadpoles of undifferentiated sex race of *Rana catesbeiana* were treated with thiourea at stage II, and the development of gonads was observed. At the end of 5 months of treatment, when all control frogs and tadpoles were exclusively female, 30% of the experimental tadpoles showed hermaphroditic and male types of gonads. Therefore a sex transformation was induced in tadpoles of undifferentiated race by the treatment of thiourea. This was assumed to be the effect of hypothyroidism on the pituitary which in turn stimulated the rate of sex differentiation.

The differentiation of anuran sex gland as in other vertebrates is bipotential. Ovaries and testes arise respectively from the cortex and medulla of the indifferent gonad. The ultimate differentiation of sex depends on the dominance of one over the other of the 2 elements which is normally controlled by the sex genes during development. However, sex differentiation can deviate from the usual manner and be subjected to the influence of environmental as well as intrinsic physiological factors. One of such factors is the disturbance of the normally balanced thyroid-pituitary-gonad system.

Iwasawa made a series of studies on sex development as influenced by the treatment of goitrogen, thiourea or 2-mercapto-1-methylimidazole, in frog tadpoles of *Rhacophorus*

schlegelii, *Rana japonica*, *Rana temporaria* and *Rana nigromaculata* (1-6). Sex reversal from female to male was reported. However, only the first species was mentioned definitely as semidifferentiated sex race (7) whereas sex races of other species were unknown.

In Taiwan, *Rana catesbeiana*, the bullfrog, is not indigenous but was introduced from the United States via Japan in 1918 (8). For the past 8 years, not a single male has been found among all metamorphosing tadpoles, either raised in this laboratory by induced breeding or obtained from frog dealers, totaling more than 600. *Rana catesbeiana* in this island is thus classified as an undifferentiated sex race (9). The unique advantage of using these tadpoles as the materials for investigation on sex transformation, therefore, lies in the fact that even a single departure from the norm after experimental treatment will be readily observed in a normally 100% female population.

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The present paper reports such an experiment to seek the possibility of sex transformation in undifferentiated sex race of *Rana catesbeiana* tadpoles by the treatment of thiourea.

MATERIALS AND METHODS

Fertilized eggs of a single pair of *Rana catesbeiana* were obtained from a frog dealer in Taichung. Two months after fertilization, 600 tadpoles at stage II (10) with an average total length of 25 mm were randomly divided into 6 groups of 100 each. Two groups of experimentals were reared in an aquarium containing 3.3 gm of thiourea (Wako Pure Chemical Industries, Ltd, Osaka, Japan) in 10 liters of tap water. Four groups of controls were raised in plain tap water. The tap water was allowed to stand for 24 hours before use. Room temperature was averaged at 23.0 ± 1.5 C. The rearing medium either control or experimental was renewed daily except Sundays. The tadpoles were fed par-boiled leaves of water-convolvulus *ad libitum*.

A number of tadpoles in each group were examined for metamorphic progress and body weight increment monthly beginning from one month after the commencement of thiourea treatment. After examination, they were dissected and the sex was recorded according to the gross structure observed under a dissecting binocular microscope. Gonads were taken and weighed to the nearest 0.05 mg with a microtorsion balance. The chronic treatment of thiourea lasted for 5 months while the controls were observed for 12 months.

RESULTS

I. *Metamorphosis and growth*

Under the subtropical climate of Taiwan, tadpoles of *Rana catesbeiana* may metamorphose into froglets 3 or 4 months after fertilization if the eggs are laid during the spring

season. However, when induced breeding is practiced in late summer or autumn, the tadpoles will have to pass the winter and become froglets in the next spring. In the present experiment the controls began to metamorphose at the age of 3 months after fertilization (TABLE II) and other tadpoles (TABLE I) lived over winter unmetamorphosed.

Although the thiourea-treated tadpoles thrived well their metamorphosis was inhibited at stage III as long as they were immersed in the thiourea medium. This was evidently due to the effect of thiourea as a goitrogen to depress the synthesis of thyroxin. TABLE IV shows that the thiourea-treated tadpoles were heavier statistically than the control over-winter tadpoles when body weights were compared.

II. *Sex differentiation*

1. Normal tadpoles and froglets

As an undifferentiated sex race, bullfrogs in Taiwan pass their tadpole life exclusively as females with a pair of typical ovaries containing numerous oocytes. After metamorphosis, the genetically male ovaries begin to transform into testes with degeneration of oocytes and growth of medullary tissue. Macroscopically, young ovaries of the tadpoles stage are large, elongated and somewhat flattened bodies with corrugated margins. Sometimes the individual egg cells can be easily seen. The testes of the young frogs are small, cylindrical and oval in shape with smooth contour. Then there is a third type of gonads whose gross appearance lies in between ovaries and testes. They are the intersexes or transforming gonads. The onset of transformation depends on the temperature of the ambient water. Warm environment hastens the process.

The external structure of the different types of gonads of *Rana catesbeiana* in Taiwan agrees well with the description of Swingle for the same species in the United States(11).

In the present experiment, tadpoles which did not metamorphose in the first year and those over-winters still breathing with

gills in the second year all showed female type of gonad except one out of 158 tadpoles exhibiting testes (TABLE I). This

TABLE I.
Sexes of control over-winter tadpoles examined

Month after fertilization	Stage	Sex			Total
		♀	♂	♂	
7	IX-XVII	40	0	0	
8	VIII-XV	30	0	0	
11	VIII-XIV	22	0	0	
12	XVII-XX	14	0	0	
13	VI-XIII	6	0	0	
14	VI-XX	45	0	1	
	Total	157	0	1	158

exceptional tadpole was considered normal biological variation. The young frogs were also 100% female by gross inspection. However, they began to show the intersex type of gonad at the age of 12 months after fertiliza-

tion or 8 months after metamorphosis (TABLE II). Whether or when 1:1 sex ratio would be achieved remains to be solved. The data indicate that Taiwan bullfrog belongs to the undifferentiated sex race.

TABLE II.
Sexes of control frogs examined

Month after fertilization	Sex			Total
	♀	♂	♂	
3	54	0	0	
4	61	0	0	
5	36	0	0	
7	11	0	0	
12	7	3	0	
13	18	0	1	
14	25	1	0	
Total	212	4	1	217

2. Thiourea-treated tadpoles

TABLE III shows that all treated tadpoles remained in female phase until 5 months after thiourea treatment when they indicated a sex differentiation of 69% females, 24% males and 7% intersexes by gross examination. This was clearly a deviation from the norm when compared with control tadpoles and frogs of the same age in TABLES I and II.

Thus sex transformation from female to male gonad was obtained in undifferentiated sex race of *Rana catesbeiana* tadpoles after thiourea treatment under the present experimental condition. However, due to the limited number of tadpoles used, it was dubious whether an ultimate result of all male population would be obtained if the treatment was prolonged.

TABLE III.
Sexes of thiourea-treated tadpoles examined

Month after fertilization	Month after treatment	Stage	Sex			Total
			♀	♂	♂	
3	1	III	9	0	0	
4	2	III	15	0	0	
5	3	III	64	0	0	
7	5	III	50	5	17	
		Total	138	5	17	160

3. Comparison of gonadal weight between control and experimental tadpoles.

The effect of thiourea on sex differentia-

tion was not confined to the process of sex reversal only but the rate of gonadal development was also influenced as shown in TABLE IV.

TABLE IV.
Comparison of gonadal weight between control and thiourea-treated tadpoles at the same age

	Month after fertilization	No. of tadpoles	Body weight Mean±SE, gm	Stage	Gonadal weight Mean±SE, mg/gm BW		
					♀	♂	♂
Control	7	37	3.91±0.10	IX-XVII	0.76±0.06	—	—
Experimental	7	39	5.18±0.18	III	2.01±0.13	0.77±0.36	0.27±0.17
Difference			1.27±0.21		1.25±0.14	—	—
P			<0.0001		<0.0001		

Thus it was evident that the weight of the ovaries was significantly increased after thiourea treatment and that between the experimentals themselves the relative weights of ovaries, intersexes and testes were decreased in that order.

DISCUSSION

The present result not only confirmed Iwasawa's finding of phenotypic sex transformation from female to male in frog tadpoles induced by thiourea treatment but also showed that sex transformation could occur in undifferentiated sex race as well as in other races. However, histological studies on the gonads of *Rana catesbeiana* should be followed.

The significant increase of relative ovar-

al weight after thiourea treatment indicated an accelerated growth of the treated ovaries. This might indicate an overall increase of developmental rate of gonads, either ovaries or testes. Thus transformation of genetically male ovaries into testes, which normally began to occur at the age of 12 months after fertilization under the present experimental condition, was hastened to the age of 7 months after fertilization by the treatment of thiourea for 5 months. In this sense the action of thiourea in sex transformation was probably an acceleration of sex differentiation.

According to Iwasawa (2, 6), sex transformation induced by thiourea is not a direct effect of the goitrogen on gonad but results from the influence of hypothyroidism on the pituitary which in turn rendered gonadal

transformation possible. The present authors agree to the point that balanced status of the normal thyroid-pituitary-gonad system is triggered off by the goitrogen. However, a correlation of the histological pictures of the distal lobe of frog pituitary to the gonadal transformation under controlled temperature must be ascertained.

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