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ON THE EXTENT OF INFESTATION BY INTESTINAL HELMINTHS OF THE CHICKEN IN PEIPING*

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In the spring of 1936 when the writer began his study on the chicken intestinal round worm, *Ascaridia*, he became interested in the problem of the percentage of infestation by this parasite as well as by other helminths living in the intestines of chickens in Peiping. Ackert (1923) examined 1000 chickens from the vicinity of Manhattan, Kansas and found that 51% of them were infested with *Ascaridia perspicillum*. Wu (1936) also made a survey on the extent of infestation by intestinal nematodes in Nanking. He discovered that the percentages of infestation by *Heterakis*, *Ascaridia*, *Capillaria*, and *Acuaria* were 63.6%, 50.0%, 16.2%, and 1.3% respectively. In the present study the percentages of infestation by all the different intestinal helminths were determined. Besides, the helminths in other visceral organs were also subsidiarily discussed.

MATERIAL AND METHODS

Chicken intestines together with other visceral organs were collected from the kitchens of the students' dinning halls in the campus and from the restaurants around Yenching University. Sometimes, chickens were bought on the market and their viscera examined. As soon as the intestine was removed from the abdomen, it was immersed in normal saline solution. It was then cut open with scissors and searched carefully for parasites either with the naked eye or with the aid of a binocular microscope. During cold weather, after the large worms were removed, the whole intestine was immersed in warm normal saline so that all the small larvae of the round worms imbedded in the villi together with the cestodes would withdraw into the solution. They were then collected either with forceps or with a dropper. Cestodes with their scoleces firmly attached in the intestinal wall were dissected out carefully under the binocular microscope. Then each complete worm was put in tepid water for relaxation. After they had fully extended, they were transfered to Diedrich solution for fixation. The nematodes, after the adhering dirt had been washed away, were fixed in either Bouin, Diedrich, or hot alcohol. The trematodes, after relaxation in tepid water, were stretched out in between two slides and were fixed in Diedrich solution. As to staining, either alcoholic cochineal or Delafield haematoxylin and eosin were used. They all yielded good results.

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RESULTS AND DISCUSSION

Among the 145 intestines examined, the distribution of the various helminths is as follows :—

1. **Ascaridia lineata (Schneider).**

Cases, either male or female present	70	or 48.3%
Cases, both male and female present	48	or 33.1%
Total number of worms including males and females	633	
Average number of worms present in each individual	9.0	
The largest number of worms present in one case (42 males, and 73 females)	115	
Total number of male worms	252	
Number of chickens with male worm only	53	
Average number of male worms in each individual	4.8	
Total number of female worms	381	
Number of chickens with female worm only	65	
Average number of female worms in each individual	5.9	

Average seasonal percentages of infestation from February to October :

From February to May	26.3%
From June to August	44.4%
From September to October	62.9%

2. **Heterakis.**

Cases of *Heterakis* present	11	or 7.5%
Total number of *Heterakis*	40	
Average number of *Heterakis* present in each chicken	4	

3. **Cestodes.**

Cases of tapeworm present	95	or 56.5%
Cases, only one tapeworm present	10	or 10.5%
Cases, few tapeworms present	35	or 36.8%
Cases, many tapeworms present	35	or 36.8%
Cases, numerous tapeworms present	15	or 15.8%

(The last four percentages were calculated on the basis of 95 infected chickens).

Average seasonal percentages of infestation from February to October :

From February to May	50.0%
From June to August	77.7%
From September to October	66.1%

4. **Trematode (Harmostomum).**

Cases of trematode present	3	or 2.0%
Total number of trematodes	12	
Average number of trematodes present in each chicken	4	
The largest number of trematodes presnt in one case	10	

5. **Combined percentages of infestation :**

Presence of *Ascaridia*, cestode, and *Harmostomum*	1	or 0.7%
Presence of *Ascaridia*, cestode, and *Heterakis*	5	or 3.4%
Presence of *Ascaridia* and cestode	53	or 36.5%
Presence of *Ascaridia* and *Heterakis*	6	or 4.1%
Presence of *Ascaridia* and *Harmostomum*	1	or 0.7%
Presence of cestode and *Heterakis*	10	or 6.9%
Presence of cestode and *Harmostomum*	3	or 2.1%
Presence of *Harmostomum* and *Heterakis*	1	or 0.7%
Presence of *Ascaridia* only	15	or 10.4%
Presence of cestode only	36	or 24.8%
Presence of *Harmostomum* only	none	
Presence of *Heterakis* only	1	or 0.7%

From the above data it appears that the percentage of infestation by *Ascaridia* in Peiping which is about 48% or nearly the same as in Nanking and in the United States, while the percentage of infestation by *Heterakis* is much smaller than in Nanking. As for the cestodes, the percentage of infestation is greater than that of *Ascaridia*. In addition, each infected chicken often harboured quite a number of these parasites. This shows that infection by cestodes is easy. Perhaps this is due to the fact that the intermediate hosts are very common in poultry yards, or that the life histories of some of these species of cestodes are direct and involve no intermediate host. However, little is known about this. Besides, it is interesting to note that about 37% of the chickens harboured both *Ascaridia* and cestodes.

The other two parasites, *Heterakis* and *Harmostomum*, claim a much smaller percentage of infestation than either *Ascaridia* or cestodes. So far, other parasites than the four already mentioned, have not been found. In Nanking as reported by Wu, two additional nematodes were found, namely, *Capillaria* and *Acuaria*. Their percentage of infestation is small. Hsu (1935) found three kinds of trematodes in the intestine and bile passage in Soochow. They were *Echinostoma*, *Hypoderaeum* and *Opisthorchis* which have not been found in this locality. Guberlet (1928) studied the parasitic worms of Hawaiian chickens. He found four species of cestodes, three species of nematodes, including *Ascaridia* and *Heterakis*, and one new species of trematode, *Harmostomum* (*Postharmostomum*) *hawaiiensis*. The small amount of trematodes found here is probably due to the fact that the topographical conditions in this vicinity are not suitable for the completion for their life histories.

It is also interesting to note from the data given above that the average number of female *Ascaridia* in each chicken is greater than that of the male with a ratio of 4.8 to 5.9. This may be due to the male worm being less resistant or to an unknown genetical difference.

In regard to the seasonal percentage of infestation by *Ascaridia*, the greatest percentage for the period between February and October falls from September to October. From June to August the infection is also quite heavy, while from February to May the infection is reduced to its minimum. The explanation of these phenomena may be two fold: First, in the summer the development of the eggs of the parasite is faster than in other seasons owing to the warm weather. This is true for other ascarid eggs. For *Ascaridia* the same result was shown in another experiment made by the writer. Second, in the spring or summer most of the chickens are newly bred. They are young and have less resistance to infection by parasites, while the chickens in winter are older and have greater resistance. Herrick (1926) found that as chickens grew older, they became increasingly resistant to infection, and many lost all of their parasites before they were five months old. Ackert (1935) also discovered that the older chickens could develop more potent growth inhibiting factors in their bodies reacting against the development of the nematodes. These phenomena have also been observed by the writer.

The seasonal percentage of infestation by cestodes is a little different from that of *Ascaridia*. The greatest percentage is from June to August, while September and October have a smaller percentage; and from February to May it is least of all. However, these differences are not so significant as in *Ascaridia*. The greatest percentage of infestation in summer may also be explained by the warm weather which facilitates the completion of the life history of the parasite and the presence of the intermediate hosts; while the small differences in the percentages of infestation in these three seasons may be due to the more or less firm attachment of the parasites so that sometimes they can hardly be eliminated.

Ascaridia occurred most often in the upper part of the small intestine, the jejunum. It might lie in the lower part of the small intestine, but was rarely found in the other parts of the alimentary canal. Only two such cases, were found; one in the crop and the other one in the gizzard and in these cases the infection by the parasites was very heavy. *Heterakis* was usually found in the small intestine or the caeca, and only in one case, two were found in the oviduct. Cestodes were found either in the small intestine or in the caeca. They were firmly attached to the intestinal wall. Only in one case a single worm was found in the oviduct. As for the other visceral organs such as liver, lung, heart, spleen and kidney, neither adult worms nor larvae were found so far. This furnishes evidence that these nematodes generally have no migratory habits as already proved by Danheim (1923, '24) and Ackert (1923).

SUMMARY

The percentages of infestation by intestinal helminths of 145 chickens in Peiping were determined. They are 48.3%, 7.5%, 65.5%, and 2.0% for *Ascaridia*, *Heterakis*, cestodes, and *Harmostomum* respectively.

The average seasonal percentage of infestation for *Ascaridia* is 26.3%, from February to May; 44.4%, from June to August; 62.9%, from September to October.

The average seasonal percentage of infestation for cestodes is 50.0%, from February to May; 77.7%, from June to August; 66.1%, from September to October.

The detailed percentages of infestation and the location of the parasites are mentioned and discussed.

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TWO CASES OF ABNORMAL HERMAPHRODITISM IN RANA NIGROMACULATA

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Both normal and abnormal hermaphroditism are of interest, because they throw light on the problem of sex. Since the early work of Spengel ('76) on the urogenital system of amphibians, which included sex-organ anomalies in anurans, many cases have been reported concerning hermaphroditism in frogs and toads. An extensive review has been made by Crew ('21) of these cases among frogs and toads. Cheng ('32) has also made a summary of all the abnormal hermaphroditic cases in frogs belonging to the genus Rana up to 1932. Since then, occasional further records may be found in the literature.

During the course of an investigation on the seasonal changes of the gonads of Rana nigromaculata, the common North China frog, among the 250 specimens dissected, two hermaphrodites were found. A survey of the literature revealed that no record of hermaphroditism has ever been made on this species of frog in North China, although several cases have been reported on Rana esculenta, the European pond frog, which is closely related to this species. In one of the cases found, the condition seemed to be different from cases already described. Therefore, it seemed worthwhile to make a detailed study of these cases.

HERMAPHRODITE NUMBER I. (discovered and killed on October 15, 1937)

EXTERNAL CHARACTERS

The animal was an adult male frog measuring 6.3 cms. from the tip of the snout to the vent. There was no difference in general appearance and coloration as compared with the other normal males. The color of the back was yellowish gray, the typical color of the male frog at this season. Among the group, one could easily identify it as a male. Two vocal sacs, one on each side, were present and functional. Nuptial pads were also present but less pigmented than those of normal frogs. However, upon histological examination, projecting papillae of the epidermis and well-developed mucous glands were found to be present and showed no difference from those of the normal male.

PRIMARY SEX ORGANS

The right gonad was a testis which was oblong in shape but narrower at the anterior end. It measured 0.58 cm. in length which was about 0.1 cm. longer than the normal testis. The width of the anterior part was 0.23 cm., while that of the posterior part was 0.29 cm. The external appearance, like the normal, was quite smooth and yellowish in

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color without any trace of pigmentation. Upon sectioning, it appeared to be a perfect testis without even a single ovum (Plate I, fig. 1). Spermatozoa in bundles were found in the lumens of the tubules. The number seemed to be a little less than in the normal. Various stages of spermatogenesis could also be observed, but primary spermatocytes in synizesis stage were present in greatest number. The intertubular tissue was nearly the same in amount as in the normal frog.

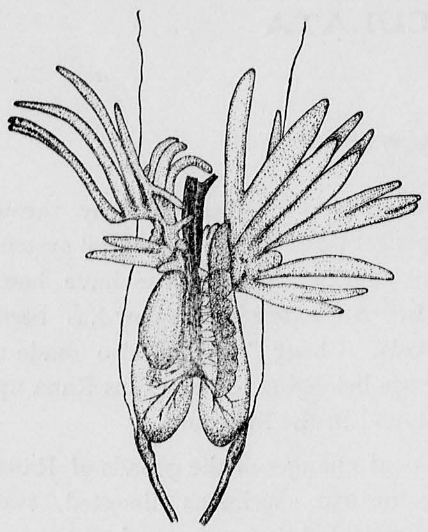

Fig. 1. Ventral view of urogenital organs of Hermaphrodite No. 1. x 2.5

The left gonad was an ovary composed of about nine lobes. Small ovocytes of yellowish color could be seen externally and they were lacking in pigment. The size of the whole ovary was 1.10 cm. in length, 0.16 cm. in average width and 0.09 cm. in mean thickness. Upon microscopic examination, the whole gonad was found to consist of a small number of ovogonia and many ovocytes without any yolk spherules in the cytoplasm (Plate I, fig. 2). The largest ovocyte measured 0.026 cm. in diameter, and the average size was 0.021 cm. The nuclei seemed to be somewhat degenerating although the chromatin granules were clear. More ovocytes showed shrinkage of the cytoplasm in comparison with the normal ovary of nearly the same age and of similar histological treatment, but follicle cells could be found around some of them. Nevertheless, the cells of the cyst wall were clear.

ACCESSORY SEX ORGANS

Thirteen vasa efferentia were present on the right side connected with the testis and four of them were bifurcated. Both the ureters were enlarged behind the kidneys to form seminal vesicles measuring about 0.7 mm. in diameter.

The rudimentary müllerian ducts on the two sides near the kidneys were just like those of normal frogs in size and structure, consisting of two layers only, a thin peritoneal covering and an epithelial lining formed by a single layer of cells.

The fatbodies at the anterior ends of the gonads were normal in size in comparison with those of the other males at this time of the year, except that the fatbody of the right side was considerably smaller than that of the left.

HERMAPHRODITE NUMBER 2. (discovered and killed on October 19, 1937)

EXTERNAL CHARACTERS

The animal was an adult frog measuring 5.9 cms. from the tip of the snout to the vent. The general appearance and coloration were just like the other normal males among the group. Vocal sacs and nuptial pads were present. The pads had nearly the same degree

of pigmentation as the others though they seemed to be lighter than the males which had specially deep pigmentation. But histologically, projecting papillae and well-developed mucous glands were found to be similar to those of the normal males.

PRIMARY SEX ORGANS

The gonads on both sides were testicular in appearance and had no pigment. The right one measured 0.40 cm. in length by 0.28 cm. in width, while the left one, which was a little larger than the right, measured 0.43 cm. in length by 0.30 cm. in width. Upon sectioning, no ovum was found in either of them (Plate I, fig. 3). Spermatozoa in bundles were present in the lumens of the tubules and were in nearly the same amount as in the normal testes. Different stages of spermatogenesis might be observed with a great number of primary and secondary spermatocytes crowded in the periphery of the tubules. The amount of the intertubular tissue was almost the same as that found in a normal testis at this season.

ACCESSORY SEX ORGANS

Eight vasa efferentia were present on the right side and ten on the left. There were no distinct enlargements of ureters to form seminal vesicles and the ducts emerged from the kidneys at a more anterior position than normal.

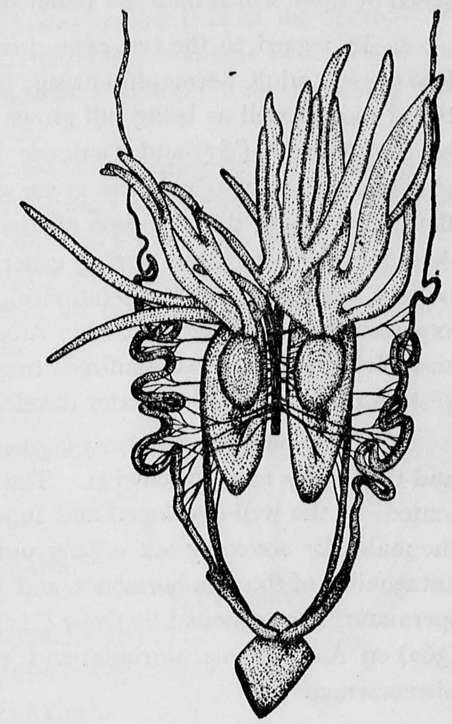

Fig. 2. Ventral view of urogenital organs of Hermaphrodite No. 2. x 2.5

The oviducts were moderately developed and were convoluted at their middle and lower portions. The anterior part of the oviduct was membranous and was somewhat flattened measuring 0.035 cm. in width and consisting of two thin layers of tissues, the outer peritoneal membrane and an inner epithelial lining (Plate 1, fig. 4). As the duct proceeded posteriorly, it gradually rounded up, although it was still composed of two layers (Plate 1, fig. 5). The diameter of this part was only 0.006 cm. Later, the duct became bigger and a third glandular layer was added in between the above two layers (Plate 1, fig. 6). The difference between this and the normal rudimentary oviduct is shown by comparing figs. 6 and 7. The inner epithelium became folded and distinctly ciliated. The average diameter of this part was about 0.049 cm. The posterior part of the oviduct measured about 0.081 cm. in diameter with the glandular layer crowding the whole lumen. There were distinct arteries and veins supplying the oviducts.

The uteri which were quite well-developed lay side by side with the ureters and measured 0.117 cm. in diameter (Plate 1, fig. 8). A distinct dilated portion was seen at the junction of the oviduct and the uterus. The wall of the uterus was thin and made of two layers, an outer peritoneal covering and an inner folded ciliated epithelium.

DISCUSSION

The hermaphrodites of frogs have been classified into two groups, namely, the Pflüger's or juvenile hermaphrodites and the Crew's adult hermaphrodites. The former is confined mostly to the undifferentiated and semi-differentiated races, while the latter is composed of those which undergo female-to-male sex reversal.

In regard to the two cases described in this present paper, they are most likely to fall into Crew's adult hermaphroditism, for according to Tsai ('30) they belong to the differentiated race as well as being full grown. Hermaphrodite number 2 is similar to that described by Tichomirow ('87) and Gerhartz ('05) in Rana esculenta, and Witschi ('21) in Rana temporaria. Crew ('21) has given some evidence to show that "the müllerian ducts are developed under the direction of the ovaries, but that the degree of their development is determined by the time during general development at which the spermatic tissues first become expressed". The relationship of the oviducts and the ovarian tissues has also been experimentally demonstrated by Adams ('28) on some castrated male Triturus viridescens and Wolf ('28) on ovariotomized frogs of Rana pipiens. The absence of distinct seminal vesicles may be due to the later development.

Hermaphrodite number 1 is more peculiar, due to its unilateral arrangement of gonads, and thus needs more discussion. The dominance of the testis over the ovary which is represented by the well-developed and functional testis as compared with the rudimentary ovary, the male-like accessory sex organs and secondary sex characters, indicate that "there is an antagonism of the sex-hormones and that the ovarian is powerless in the presence of the spermatic", as suggested by Crew ('21). Experimental results obtained by Humphrey ('31, '36a) on Amblystoma tigrinum and parabiotic twins of the same animal showed the same phenomenon.

Furthermore, the similarity of the accessory organs (except the fatbody) and the secondary sex characters on both sides of the body of this hermaphrodite with a typical bilateral arrangement of gonads, furnishes strong evidence to support the idea of symmetric hormonic influence. There are contradictary cases described by Cole ('96), Lavroff ('08), Bhattacharya and Das ('20), Woronzowa ('26), Christensen ('29) and Cheng ('29) in which the oviducts developed more on the side which had a larger amount of ovarian tissue. Crew ('21) explained the asymmetric condition by the relative amount of ovarian tissue and Witschi ('21, '25) by the difference of rate in the sex transformation of the two gonads. But how the hormonic influence may be exerted on one side and not the other has not been satisfactorily explained. The comparatively smaller size of the fatbody on the right side might be due to its constant consumption by the neighboring testis to carry out the testicular activity as has been demonstrated experimentally by Adams and Rae ('29).

Nevertheless, the completely unilateral arrangement of the gonads may lead to another possible explanation. Champy ('21) described a case of total sex inversion from male to

female in Triton alpestris; and Burn ('28) also found that ovarian cortex had appeared in one male host of Amblystoma through the presence of a large female graft. Witschi ('29) in the artificial self-fertilization experiment of two frog hermaphrodites, got a result which was somewhat diverse from that of regarding the hermaphrodites as genetic females. Humphrey ('36b) found that in fourteen out of 187 heterosexual pairs of parabiotic Amblystoma, the testes were undergoing reversal.

Again, Champy ('13) suggested that the testis-ova might be derived from an oviform transformation of indifferent germ cells; and King ('10) also insisted that as the spermatozoa are a much more specialized type of cell than the ova, therefore, it might be expected that male germ cells would assume the characteristics of ova more frequently than the female germ cells would be able to develop into spermatozoa.

It is therefore possible that due to some genetic defect or abnormality of the germ cells of one side during the young stage of development, the gonad which should be a testis like that of the other side underwent transformation into an ovary and that Hermaphrodate 1 may be genetically a male. Unfortunately, no breeding experiment can be done to show whether this assumption is correct. The final solution of problems like this depends on future experimental, genetic and cytological researches.

SUMMARY

Two adult hermaphrodites of Rana nigromaculata have been found. The sex characters are as follows :—

HERMAPHRODITE NUMBER 1

PRIMARY SEX ORGANS

RIGHT SIDE	LEFT SIDE
Well-developed testis	Rudimentary ovary
Mature spermatozoa present	Absence of spermatic tissues

ACCESSORY SEX ORGANS

RIGHT SIDE	LEFT SIDE
Thirteen vasa efferentia present	No vas efferens
Seminal vesicle present	Seminal vesicle present
Oviduct rudimentary—same as normal	Oviduct rudimentary—same as normal
Fatbody comparatively smaller	Fatbody comparatively larger

SECONDARY SEX CHARACTERS

Male-like general appearance on both sides of the body
Two vocal sacs present
Nuptial pads present and histologically normal

HERMAPHRODITE NUMBER 2

PRIMARY SEX ORGANS

RIGHT SIDE	LEFT SIDE
Well-developed testis	Well-developed testis
Mature spermatozoa present	Mature spermatozoa present

ACCESSORY SEX ORGANS

Eight vasa efferentia present	Ten vasa efferentia present
No distinct seminal vesicle	No distinct seminal vesicle
Oviduct and uterus moderately developed	Oviduct and uterus moderately developed
Fatbody comparatively smaller	Fatbody comparatively larger

SECONDARY SEX CHARACTERS

Male-like general appearance with two vocal sacs and well-developed nuptial pads

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EXPLANATION OF FIGURES IN THE PLATE

1 Photomicrograph of transverse section of a portion of the right testis of Hermaphrodite 1, showing the normal condition of the spermatic tubules and the intertubular tissue. X 100.

2 Photomicrograph of transverse section of a portion of left rudimentary ovary of Hermaphrodite 1, showing the degenerating ovocytes, the ovogonia (ovg), and the ovarial sac (ovs). X 100.

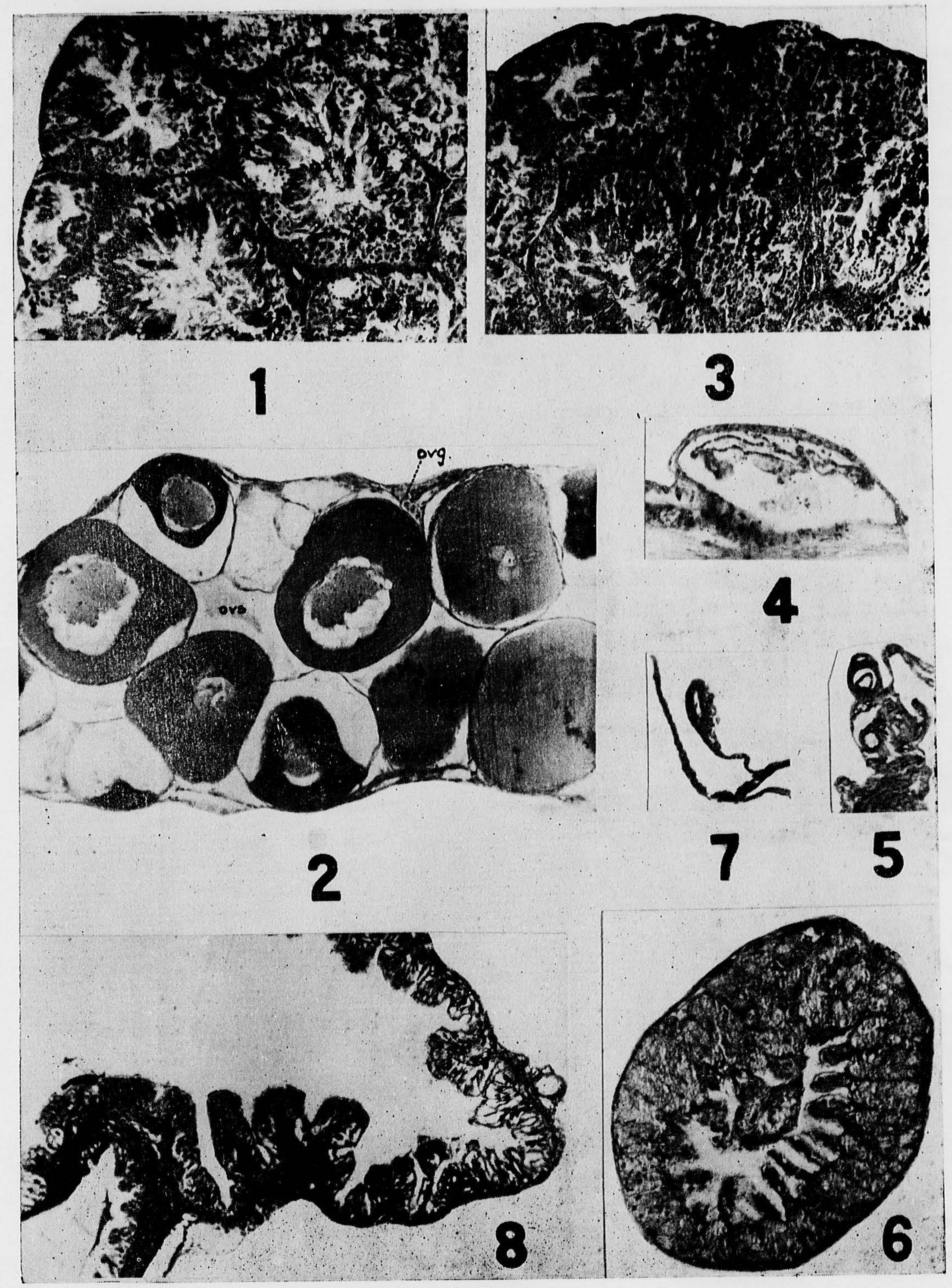
3 Photomicrograph of transverse section of a portion of right testis of Hermaphrodite 2, showing the normal condition of the spermatic tubules and the intertubular tissue. The left testis is in the same condition as that of the right one. X 100.

4, 5, 6 Photomicrographs of transverse sections of oviduct of Hermaphrodite 2 at three successive regions. X 100.

7 Photomicrograph of transverse section of the lower part of the rudimentary oviduct of normal male frog. X 100. Compare with fig. 6.

8 Photomicrograph of transverse section of a portion of uterus of Hermaphrodite 2, showing the well-developed ciliated epithelium. A portion of the ureter is also shown at the left corner. X 100.

Peking Nat. Hist. Bull. 1937-38, Vol. 12. PLATE



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A STUDY OF BIDDER'S ORGAN IN CHINESE TOADS

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INTRODUCTION

Bidder's organ is the ovary-like structure situated at the anterior end of each gonad in the toad. It was discovered in 1758 by Rösel von Rosenhof who considered it as a part of the fatbody. Since then, different theories have been developed to explain its nature.

Ratke (1825) and Bidder (1846) believed that this organ is related to the formation of testicular tissue; while Jacobson (1828), Von Wittich (1853), La Valette St. George (1874), Stephan ('01), Ceruti ('05), Ognew ('08), King ('08), Ponse ('27) and Witschi ('33) considered it a rudimentary ovary. However, Spengel (1876) denied the strictly ovarian character of this organ, but maintained that it takes part in some way in gametogenesis in both sexes. Hoffman (1886) assumed this organ to be a "rudimentary hybrid gland" containing both ova and spermatozoa. Although Knappe (1886) maintained that this organ is a rudimentary ovary, he believed that spermatozoa may develop from the follicle cells of the degenerating ova.

Interpreting from the point of view of embryology, Swingle ('22) considered it as a persisting embryonic gonad (progonad), in which the cells have undergone oviform degeneration. King ('08) found that the germ-cells of Bidder's organ are similar in character to those forming the functional gonads and their development is the same up to synizesis stage, that the gonia stages are located similarly at the periphery of the organ, and the oocytes toward the center. Beccari ('25, '29) found that it is the time difference in the development of the anterior and the posterior gonads that determines the formation of this organ, and Ponse ('27) concluded that it is the mesogonad which is destined to form the greater part of the definitive organ of Bidder.

During recent years, experimental methods have been adopted in attacking this problem. Harm ('21-'26) and Guyénot and Ponse ('23-'27) found that after the removal of the testes or ovaries, this organ will produce mature eggs. Harm ('14, '26) also showed that this organ has an endocrine function in the male.

From the above brief history, it can be seen that the nature of this organ is still not certain. The writers took this problem with the hope that from the study of Chinese material some facts might be observed which would help in the understanding of this peculiar organ.

The writers wish to express their thanks to Dr. J. C. Li for his kindness in reading the manuscript and for his criticisms. Thanks are also due to Dr. Chenfu F. Wu and Mr. Y. L. Ts'ui for making the photomicrographs.

MATERIAL

The material used was from the three most common species of Chinese toads, namely Bufo bufo gargarizans (B. bufo asiaticus), Bufo raddei, and Bufo melanostictus. The former two species are present in North China, while the latter one is the South Chinese form.

Bufo bufo and Bufo raddei were collected in this neighborhood at different ages beginning with metamorphosis. Adult individuals were killed at different times to observe whether any abnormalities occur in their reproductive organs. Specimens of Bufo melanostictus had been collected in Fukien and are part of the Yenching Amphibian Collection.

OBSERVATIONS

TYPICAL REPRODUCTIVE ORGANS OF THE THREE COMMON SPECIES OF CHINESE TOADS

1. **BUFO BUFO.** This is the largest among the three species. The average size is about 9 cm. The male gonad (fig. 3) is cylindrical in shape and yellowish in color. The Bidder's organ, which is present in all individuals, may be either closely connected with the anterior end of the testis or completely separated from it. The shape of the Bidder's organ is not round but somewhat flattened (fig. 3) and the color is yellowish red. The reddish color is due to the rich supply of blood vessels. This organ is larger and thicker in younger individuals and becomes thinner in adult ones. The rudimentary oviducts of this species are much developed and more conspicuous than in the other two.

The female gonad of the adult individual is large during the breeding season. It requires three or four years for the gonad to grow to maturity, when the toad has a body size to over 9 cm. (snout to vent). The Bidder's organ in younger individuals is large (figs. 4, 5) and like that of the males, but it becomes much reduced in size as the individual reaches maturity (fig, 6). The organ is reddish in color and closely associated with the fatbody. The reduction of the organ may be so great that it becomes merely a strip of leaf-like tissue or disappears completely. However, the majority keep this organ in a more or less reduced condition.

2. **BUFO RADDEI.** This species is smaller than Bufo bufo. The average size is about 6.5cm. The testes (fig. 10) are bean-shaped instead of cylindrical as in Bufo bufo. The maturity is reached about the third year with a size of over 6 cm. in body length (snout to vent). The Bidder's organ of the male, unlike that in Bufo bufo, is long, light yellowish red in color and closely associated with the testis (fig. 10), and sometimes Bidder oocytes may be seen scattered on the surface of the anterior end or even the lower portion of the testis. It is somewhat flattened either dorso-ventrally or laterally.

Bidder's organ is not present in the adult female because it degenerates at the end of the first year.

3. **BUFO MELANOSTICTUS.** The size of the adult is about the same as in Bufo bufo. The maturity is reached when the individual attains a size of about 7 cm. in length. The male has a Bidder's organ like that of Bufo bufo in shape (fig. 15) but with light yellow color in material preserved in formalin. The testis is also like that of Bufo bufo except that it is slightly constricted into several lobes (fig. 15).

The female also possesses a Bidder's organ similar to that of Bufo bufo in shape and size (fig. 16). This organ is present in all younger specimens, but is much reduced in the adult (fig. 17); and in one of the several adult specimens examined with mature eggs, it is missing. Whether most of the adults keep this organ or not is hard to decide due to the limitation of material available. But according to Alexander ('31, '33) this organ in the female has a tendency to disappear after the individual attains maturity.

CHANGES IN BIDDER'S ORGAN AFTER METAMORPHOSIS

1. **BUFO BUFO.** Just after metamorphosis, both males and females measure 18mm. in body length. The female can be distinguished from the male by the longer and more lobed gonad (figs. 1, 4) and also histologically by the small oocytes and small ovarian cavities, while in the male spermatogonia are arranged to form tubules. The outer active growing layer in both sexes is continuous with the outer layer of the Bidder's organ, whose gonia cells are also in active mitosis.

During this stage the male Bidder's organ is oval in shape measuring 0.8mm. in length by 0.4 mm. in width with a yellowish red color (fig. 1). It is connected with the testis which is about three times the length and one third the width of Bidder's organ (fig. 1). When the individual grows to about 32 mm. in body length, the Bidder's organ measures 1.5 mm. in length by 0.8 mm. in width, and is separated from the testis by a little constriction (fig. 2). Eventually, when the individual reaches the adult stage, the constriction between the Bidder's organ and the testis becomes deeper and they may be completely separated from each other. The Bidder's organ is no more oval in shape but appears more or less triangular in cross section, while the testis grows in size and becomes wider and longer (fig. 3).

The female Bidder's organ in the younger stage is like that of the male (fig. 4). When the individual reaches about 36 mm. in body length, the true ovary is much more developed and the constriction between it and the Bidder's organ becomes more conspicuous (fig. 5). The shape of the Bidder's organ begins to become irregular or flattened. And then it is gradually reduced in size when the gonad grows into maturity as already described.

2. **BUFO RADDEI.** Both males and females measure 16 mm. in length after metamorphosis. The male has yellowish pear-shaped Bidder's organs measuring 1.8 mm. in length by 0.5 mm. in width and short light yellowish testes measuring only 0.8 mm. in length by 0.2 mm. in width (fig. 7). The Bidder's organ is closely connected with the testis and upon histological examination, the outer active dividing layer of the former is found to be continuous with the latter and some of the Bidder oocytes of the lower portion are mixed with the germ-cells of the testis, which is solid with cells which are still indistinguishable from those of the ovary (fig. 42). This species, therefore, differentiates a little later than Bufo bufo.

As the male reaches about 31 mm. in body length, the constriction between the Bidder's organ and the testis becomes less, and the total size is 3.5 mm. in length by 0.7 mm. in width (fig. 8). Histological study shows that the Bidder's oocytes at the junction begin to degenerate and some of them are changed into spermatic tissue in various stages of maturation division (fig. 43). Remnants of the Bidder's oocytes may sometimes be found in a degenerate Bidder's oocyte "area".

In males about 38 mm. in body length, the testes grow longer, reaching about one half of the total length of the mature gonad (fig. 9). The Bidder's organ which becomes somewhat flattened is still closely associated with the testis and may overlap its anterior part. From now on the testes grow larger and larger while the Bidder's organ remains stationary or degenerates a little.

The female after metamorphosis has a Bidder's organ 1.5 mm in length by 0.4 mm. in width (fig. 11). The ovary which is connected with the Bidder's organ measures 1.7 mm. in length by 0.2 mm. in width. It is slightly lobed with interrupted cavities and is continuous with the outer actively dividing layer of the Bidder's organ (fig. 44). The lower portion of the Bidder's organ is completely surrounded by the ovarian tissue.

As the individual reaches 24 mm. in length, the ovarian portion becomes more lobed (fig. 12). Two distinct parts can be made out in the Bidder's organ : the outer layer which is continuous with the lower ovarian tissue consisting of newly grown oocytes, light yellowish in natural color and more bluish with basic dye, and an inner solid portion, which is deeper yellowish in natural color and more reddish with acid dye, consisting of degenerating Bidder oocytes (fig. 45).

When the individual attains a size of 32 mm., the ovarian portion and the outer layer of the Bidder's organ become more developed, while the inner portion of the Bidder's organ appears more degenerate (fig. 13). And as it reaches 36 mm. in body length (near the end of the first year), nearly all the Bidder "areas" are filled in by the follicle cells which originally surrounded these oocytes (figs. 14, 46). During the second year the inner portion completely disappears.

3. BUFO MELANOSTICTUS. No young specimens are available. The development is probably like that of Bufo bufo except that the rudimentary oviduct in the male is not so much developed.

ABNORMAL CASES

1. BUFO BUFO. Abnormality of the Bidder's organ or of other reproductive organs was rarely found by external examination, although hundreds of toads have been killed. Nevertheless, in the male, after sectioning the Bidder's organ and testes, the occurrence of testicular tissue in the former is not unfrequently found. Altogether seven cases have been found (figs. 23-29). The position of the testicular tissue in the Bidder's organ is not necessarily near the testis, as it may be in the center or anterior part near the fatbody. Mature spermatozoa and other maturation stages are found in this testicular tissue. And it is worthwhile noticing that the size of the spermatic "tubules" is always the same as that of the nearby Bidder oocytes.

So far only two peculiar cases in the female have been discovered: Case A is the occurrence of a single mature egg at the anterior periphery of the degenerating Bidder's organ (fig. 30). This egg is of the same size and contains the same amount of pigment and yolk as the eggs in the true ovaries. Case B shows the presence of growing oocytes in the

basal portion of the fatbody connecting with the anterior end of the Bidder's organ and also in the right periphery of the Bidder's organ (fig. 31). These oocytes are also in the same stage of development as the others in the ovaries of the same individual (which being collected in July has smaller ovaries than in Case A).

2. BUFO RADDEI. Abnormality in the reproductive organs of this species is quite frequently found, more so than in Bufo bufo. Four abnormal cases will be described. The first three of these were noticed with naked eye, while the fourth was discovered after sections were made; therefore, no drawing was made to show the exact external appearance of the latter. All four of these cases were found during July and August.

CASE A. This measures 49 mm. in body length (snout to vent) and looks like a normal male externally with dark thumb pads. Internally, however, there is no testis on the left side of the animal, but the left Bidder's organ is curved at the anterior end and situated in the middle of the gonad region (fig. 18). The color and surface appearance of the left Bidder's organ, like that of the right one, is yellowish gray and rough. As to the size, the left Bidder's organ measures 4.9 mm. in length by 0.8 mm. in width, while the right one measures only 3.5 mm. in length by 1.2 mm. in width. The one testis which is on the right side is bean-shaped and yellowish in color, measuring 48 mm. in length by 33 mm. in width with Bidder oocytes scattered on the surface of the upper portion. The rudimentary oviducts of the two sides are equal in size and like the ordinary ones. Moreover, the Wolffian ducts of the two sides are also equal, measuring 0.3 mm. in diameter.

However, upon sectioning, it is found that spermatic tissue is present in the lower portion of the left Bidder's organ and some of the Bidder oocytes in other regions have begun to degenerate (fig. 32). After a careful examination of the degenerating Bidder oocytes and those which contain spermatic tissue, it is found that the former are filled either by follicle cells (figs. 33, 34) or follicle cells together with remnants of the oocytes (fig. 37), while the latter consist of germ-cells in all stages of development from spermatogonia to spermatozoa in addition to red blood corpuscles and follicle cells (figs. 35, 36).

CASE B. This is a male externally measuring 55 mm. in length. But internally there is no testis on the right side, only a somewhat lobed Bidder's organ measuring 8.0 mm. in length by 1.5 mm. in width (fig. 19). The left Bidder's organ which measures 4.1 mm. in length by 1.0 mm. in width is normal in appearance. In comparing the color and size of the Bidder oocytes of the two sides, they are found to be the same. The color is light yellowish red. The one testis which is on the left side is yellowish in color and bean-shaped, somewhat broader at the anterior end. The rudimentary oviducts, like the normal, are not specially enlarged on the right side and the Wolffian ducts are also equal in size, measuring 0.5 mm. in diameter.

Serial sectioning of both Bidder's organs shows no spermatic tissue present and no special enlargement of the central cavity. (figs. 47, 48). The testis is normal in development in comparison with that of the ordinary toad at this season. Scattered Bidder oocytes are also found at the periphery of the testis (fig. 39).

CASE C. This individual measures 64 mm. in length and is a normal male. However, comparatively larger oocytes with gray or black pigment occur in both Bidder's organs (fig. 20). The right and left Bidder's organs measure 5. omm. in length by 1.2 mm. in width and 5.5 mm. in length by 1.4 mm. in width respectively. They are situated on the ventral side of the fatbodies which extend more posteriorly than usual, reaching the testes. The two testes, which join the Bidder's organs, are well developed. The right one measures 6.5 mm. in length, by 2.6 mm. in width, while the left one measures 5.5 mm. in length by 2.4mm. in width.

Upon sectioning, yolk granules arranged in a zone near the periphery of the cytoplasm are found in the majority of the Bidder oocytes (fig. 49). But a few of the Bidder oocytes degenerate either by the growing in of the follicle cells or by the production of black pigment. No central cavity is found in between the oocytes. However, both testes appear normal histologically.

CASE D. This individual measures 36 mm. in length. Externally, it can not be identified as male or female since it is only one year old and the seconday sex characters are not yet differentiated. However, the general appearance of the gonads was somewhat similar to that in a female of the same size with yellowish Bidder's tissue enclosed by cream-colored oocytes, although no accurate examination was made.

After serial sectioning, the right gonad was found to be normal containing large degenerating Bidder oocytes in the anterior part and normal oocytes surrounding the degenerating Bidder's tissue and occupying the posterior portion of the gonad. But the left gonad is hermaphroditic. It has large degenerating Bidder oocytes surrounded by the normal transformed oocytes in the anterior portion of the gonad (fig. 50), and only Bidder oocytes and spermatic tissue in the posterior region (fig. 51). The spermatic tissue lies on the hilus side and starts from the middle of the gonad, gradually increasing in area toward the posterior, until it occupies nearly the whole cross section area at the extreme posterior end. The spermatic "tubules" in this tissue are of the same size as the degenerating Bidder oocytes alongside of them. "Tubules" containing remnants of Bidder oocytes, follicle cells and spermatic tissue are often found in the region between the Bidder oocytes and spermatic tissue (fig. 51).

3. BUFO MELANOSTICTUS. Among the preserved specimens available, two abnormal cases in the reproductive organs were found.

CASE A. The individual measures 54 mm. in body length and is a male. The right gonad consists of an anterior Bidder's organ which is half-darkened by the development of pigment and a three-lobed testis measuring 9.0 mm. in total length (fig. 21). The left gonad consists of a distinctly three-lobed Bidder's organ, which measures 5.3 mm. in length, and a small posterior testis measuring only 2.6 mm. in length and lying just opposite the last right testicular lobe (fig. 21). The three lobes of the left Bidder's organ, instead of lying opposite to the right Bidder's organ, lie opposite to the two right anterior testicular lobes. The color of the testes of both sides and the first lobe of the left Bidder's organ is light yellow, while that of the second and third lobes of the left Bidder's organ and that of the right Bidder's

organ is yellowish red. Histological study shows that the three lobes of the left Bidder's organ contain Bidder oocytes only, and no spermatic tissue is found among them.

CASE B. This is a male measuring 54 mm. in body length. Both Bidder's organs are closely associated with the short bean-shaped testes; and since there is no constriction between the testes and the Bidder's organs, they are hard to distinguish from one another externally (fig. 22). The color of the testes is light yellow, while the color of the Bidder's organs is only slightly deeper. Since the right testis together with the Bidder's organ measures only 4.3 mm. in length and the left ones together measure also only 6.0 mm. in length, they are very much shorter than the usual gonads of this species. In addition, sagittal sections show that the anterior portions of both testes are wrapped around by the Bidder's tissue, similar to what occurs in Bufo raddei (figs. 40, 41).

DISCUSSION

From the above study of the normal development and the abnormal cases of Bidder's organ, it is shown that this organ generally differentiates earlier than the definitive functional gonad in either sex, and is arrested in development later when the definitive gonad becomes differentiated. The Bidderian tissue, then, may remain as such or degenerate or transform into either functional ovarian or testicular tissue of the corresponding sex.

There are three ways observed by the writers by which Bidder oocytes degenerate. The first is the invasion by the follicle cells which gradually absorb the cytoplasm of the Bidder oocytes; the second is the invasion by follicle cells and blood capillaries; and the last is the development of black pigment which gradually destroys the oocytes. These three ways are similar to what was observed by Knappe (1886) and King (1908).

Knappe (1886) suggested that the inability of the Bidder oocytes to become functional eggs is due to the fact that they are not able to form yolk, while King (1908) found that yolk granules may be present in the Bidder oocytes but the arrangement is irregular because they never collect in a zone midway between the nucleus and periphery of the egg as in normal ova. However, the writers have observed that yolk granules may be present in some individuals with an arrangement like that of the normal ova, and also that Bidder's cells are able to grow to maturity like normal eggs. This agrees with the experiments of Harm ('21-26) and Guyénot and Ponse ('23-'27) in which after ablation of the testes of the male or of the ovaries of the female, the Bidder's cells produce yolk and become mature eggs.

The development of yolk granules in the Bidder oocytes in one male (Bufo raddei, Case C) may be due to the presence of an especially large quantity of food material, since the large fatbodies here are closely associated with the Bidder's organs. This agrees with Harm's experiment ('23) in which he found in a castrated male which had been fed on diet containing an excess of fat, lipoids and lecithin that the caudal portion of the Bidder's organ could become an ovary.

The possibility of the transformation of Bidder's tissue into normal ovarian oocytes and ova has been proved beyond doubt by experiments done by Harm ('21-'26) and Guyénot and Ponse ('23-'27) and by the observation of the writers in the normal development of the female gonad of Bufo raddei and in the two peculiar female cases of Bufo bufo. However, in the development of the female gonad of Bufo raddei only the thin outer active growing layer of Bidder's organ becomes the anterior part of the adult ovary, and in the two cases of female Bufo bufo the transformed ova lie at or near the periphery of Bidder's organ. From the development of the female gonad of Bufo raddei, it is shown definitely that only the Bidder gonia are able to be transformed into normal oocytes, while the inner Bidder oocytes degenerate. Therefore, this would lead to the suggestion that the transformed ova in the two cases of female Bufo bufo observed and in the experiments of Harm, Guyénot and Ponse may have developed from Bidder gonia instead of from Bidder oocytes. But these workers give no definite statement about this point.

Moreover, the possiblility of the transformation of Bidder oocytes into spermatic tissue is shown to be a fact by the study of the normal development of the male gonad of Bufo raddei and by many of the abnormal cases presented in this paper. The few most important facts may be briefly summarized into three points: (1) The occurrence of both spermatic tissue and remnants of the cytoplasm of the Bidder oocyte in the space of one oocyte ("tubule"). (2) The presence of spermatic "tubules" inside of the Bidder's organ quite far away from the testis. (3) The size and shape of spermatic "tubules" in the Bidder's organ are always the same as that of the Bidder oocytes. This last fact was long ago pointed out by Bidder (1846) but unfortunately his interpretation was opposed by later workers.

But the transformation of Bidder oocytes or where the spermatic tissue comes from needs some discussion. Knappe (1886) also found spermatozoa in the Bidder's organ and he believed that the spermatozoa are developed from the follicle cells. Unfortunately, he was opposed by King ('07, '08) who considered that what Knappe called spermatozoa were sporozoan parasites, simply because she found some such parasites in Bidder's organ in one Bufo lentiginosus. Beccari ('25, '29) has observed that the germ-cells of the presumptive Bidder's organ reach their site in the gonadal folds earlier than those of the definitive ovaries and testes. He suggests that the occasional finding of testicular islands in Bidder's organ or of oocytes in the testis is due to the fact that sometimes the primordial germ-cells have failed to reach their proper site in the gonadal fold. But the facts observed by the writers clearly indicate that the spermatic tissue occurs inside of the degenerating Bidder oocytes, so the germ-cells would have to migrate into the "tubules" after the degeneration of the Bidder oocyte has started. Nevertheless, there are two possible ways by which the spermatic tissue may be derived. The first is from germ-cells, which are hidden in between the Bidder oocytes, penetrating into the degenerating oocytes as the follicle cells grow in. The germ-cells then divide and become spermatic tissue. The second is the transformation of follicle cells into spermatogonia as suggested by Knappe (1886). However, if these two ways are compared, the first one seems to be more reasonable, because so far there is no direct evidence or experiment to show that the follicle cells can be changed into germ-cells. On the other hand, we do find active germ-cells in the outer layer of the Bidder's organ and it is very possible that there are some young germ-cells scattered in between the oocytes. The reason why they cannot be seen normally may be that they are pressed in between the growing Bidder oocytes. But as the Bidder oocytes degenerate, they may be set free and migrate into the space of the Bidder oocytes together with the follicle cells or along with the blood capillaries.

Going a step further, we need to know why these new germ-cells in the Bidder's organ differentiate into spermatic tissue in the adult male individual instead of differentiating again into Bidder oocytes. In order to explain these facts, we must assume that the male functional gonads after their differentiation may influence the Bidder's organ anterior to them through some "field influence". Therefore, in all the cases met, only excepting case D of Bufo raddei, spermatic tissue never occurs in the Bidder's organ of the female nor ova in the male. In addition, there is another significant fact, in Bufo raddei the outer layer of the Bidder's organ of the female becomes the anterior part of the definitive ovary and the lower portion of the Bidder's organ of the male is changed into spermatic tissue and is added to form the anterior part of the testis; while in other species, the whole Bidder's organ remains as such or degenerates completely. This phenomenon can also be explained if we assume that there is a "field influence" exerted by the differentiated testis or ovary which can effect the transformation of the Bidder's organ into the corresponding tissues, and this "field influence" varies in extent with each species. If this assumption is right then the condition in the female Bufo raddei can easily be explained by the fact that in this species the ovarian "field influence" can extend to the anterior end of the Bidder's organ. Consequently, the outer layer of the Bidder's organ becomes the ovary while the inner portion which can no more grow into eggs degenerates. A similar explanation can be applied to the male Bufo raddei:— that the "field influence" can extend to the lower portion of the Bidder's organ. The occasional occurrence of Bidder oocytes at the surface of the testis is because some of them may remain unchanged. Witschi's ('33) attempt to explain similar facts by assuming that there is a sex-specific controlling factor of gonad development present in the hypophysis, simply shifts the difficulty to another body.

Case D of Bufo raddei shows a hermaphroditic condition of the gonads, which needs some further explanation as all hermaphrodite cases do.

SUMMARY

1. Both the normal development and some anomalies in the Bidder's organ of Bufo bufo gargarizans (asiaticus), Bufo raddei, and Bufo melanostictus are described and discussed.

2. The male Bidder's organ of these three species is present throughout their lives; while the female Bidder's organ of Bufo bufo and Bufo melanostictus may disappear or become very much reduced in the adult, while that of Bufo raddei always disappears at the end of the first year.

3. The outer active growing layer of the Bidder's organ of Bufo bufo and Bufo raddei is continuous with the lower outer ovarian or testicular tissue. In the female Bufo raddei this outer active growing layer of the Bidder's organ becomes the anterior part of the definitive ovary, while the inner portion degenerates; and in the male Bufo raddei the lower inner portion of the Bidder's organ becomes the anterior part of the definitive testis.

4. The transformation of Bidder's organ into ovarian tissue in the normal female Bufo raddei is by means of the growing of the Bidder gonia, while the transformation of Bidder's organ into testicular tissue in the normal male Bufo raddei is accomplished by the degeneration of the Bidder oocytes and then by the multiplication of spermatogonia, which have been lying in reserve.

5. Seven cases of the presence of testicular tissue in the Bidder's organ of the male Bufo bufo and two cases in that of Bufo raddei were met, and two cases of the presence of normal oocytes or ova in the Bidder's organ of the female Bufo bufo were also observed.

6. This testicular tissue in the Bidder's organ of the abnormal individuals is present in the space of the original degenerated Bidder oocytes. It is suggested that it may come from the Bidder gonia hidden in between the Bidder oocytes and migrating in after degeneration has started.

7. The normal oocytes and ova in the Bidder's organ of the abnormal individuals have most probably come from the transformation of Bidder gonia.

8. "Field influence" is suggested to explain the presence of testicular tissue in the Bidder's organ of the abnormal male individuals, the presence of normal ova in the Bidder's organ of the abnormal female individuals, and the transformation of the outer layer of the Bidder's organ of the normal female Bufo raddei into the anterior part of the definitive ovary and the transformation of the inner part into part of the definitive testis.

POSTSCRIPT: After this paper had gone to press, a paper by Horié appeared on the anatomy of Japanese toads in which his observations on Bufo bufo (vulgaris) and Bufo melanostictus agree with those of the writers except that in his summary he states that there is no Bidder's organ in the adult females of Bufo melanostictus.

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EXPLANATION OF PLATES

Plate I

Fig. 1. Urogenital organs of male Bufo bufo 18 mm. in body length. 4x.
2. Urogenital organs of male Bufo bufo 32 mm. in body length. 4x.
3. Urogenital organs of male Bufo bufo 87 mm. in body length. 2x.
4. Urogenital organs of female Bufo bufo 16 mm. in body length. 4x.

5. Urogenital organs of female Bufo bufo, 36 mm. in body length. 4x.
6. Bidder's organ and part of ovary and fatbody of female Bufo bufo 93 mm. in body length. 2x.
7. Urogenital organs of male Bufo raddei 16 mm. in body length. 4x.
8. Urogenital organs of male Bufo raddei 31 mm. in body length. 4x.
9. Urogenital organs of male Bufo raddei 38 mm. in body length. 4x.
10. Urogenital organs of male Bufo raddei 64 mm. in body length. 2x.
11. Urogenital organs of female Bufo raddei 16 mm. in body length. 4x.
12. Urogenital organs of female Bufo raddei 24 mm. in body length. 4x.
13. Urogenital organs of female Bufo raddei 32 mm. in body length. 4x.
14. Urogenital organs of female Bufo raddei 36 mm. in body length. 4x.
15. Urogenital organs of male Bufo melanostictus 70 mm. in body length. 2x.
16. Urogenital organs of female Bufo melanostictus 55 mm. in body length. 4x.
17. Bidder's organ and part of ovary and fatbody of female Bufo melanostictus 72 mm. in body length. 2x.
18. Abnormal urogenital organs of male Bufo raddei 49 mm. in body length. 4x.
19. Abnormal urogenital organs of male Bufo raddei 55 mm. in body length. 4x.
20. Abnormal urogenital organs of male Bufo raddei 64 mm. in body length. 4x.
21. Abnormal urogenital organs of male Bufo melanostictus 54 mm. in body length. 4x.
22. Abnormal urogenital organs of male Bufo melanostictus 50 mm. in body length. 4x.

Plate II

Fig. 23-29. Sections through the Bidder's organs of adult male Bufo bufo showing the occurrence of testicular tissue in the Bidder's organs.
30-31. Sections through the Bidder's organs of adult female Bufo bufo showing the occurrence of normal ovum and oocytes in the Bidder's organs.
32. Longitudinal section through the left Bidder's organ of an abnormal male Bufo raddei (Case A, fig. 18).
33. "Tubule" (A) in the upper portion of figure 32—under high power.
34. A few follicle cells of figure 33 enlarged.
35. "Tubule" (B) in the lower portion of figure 32—under high power.
36. "Tubule" (C) in the lower portion of figure 32—under high power.
37. "Tubule" (D) in the lower portion of figure 32—under high power.
38. A "tubule" in the lower portion of the left gonad of Case D of Bufo raddei—under high power.
39. Longitudinal section through the left testis of the abnormal male Bufo raddei (Case B, fig. 19) showing the position of the Bidder oocytes in the testis.
40. Median sagittal section of the right gonad of abnormal male Bufo melanostictus (Case B, fig. 22).

41. Median sagittal section of the left gonad of abnormal male Bufo melanostictus (Case B, fig. 22).

Plate III (Photomicrographs)

Fig. 42. Longitudinal section through the junctional region between Bidder's organ and testis of male Bufo raddei 16 mm. in body length (fig. 7).

43. Longitudinal section through the junctional region between Bidder's organ and testis of male Bufo raddei 31 mm. in body length (fig. 8).

44. Longitudinal section through the junctional region between Bidder's organ and ovary of female Bufo raddei 16 mm. in body length (fig. 11).

45. Longitudinal section through the upper portion of the Bidder's organ of female Bufo raddei 24 mm. in body length (fig. 24).

46. Cross section through the Bidderian region of female Bufo raddei 36 mm. in body length (fig. 14).

47. A portion of the longitudinal section of the right Bidder's organ of the abnormal male Bufo raddei (Case B, fig. 19).

48. Longitudinal section through the upper portion of the left Bidder's organ from the same individual as in fig. 47.

49. A portion of the longitudinal section through the Bidder's organ as well as the closely associated fatbody of the abnormal male Bufo raddei (Case C, fig. 20).

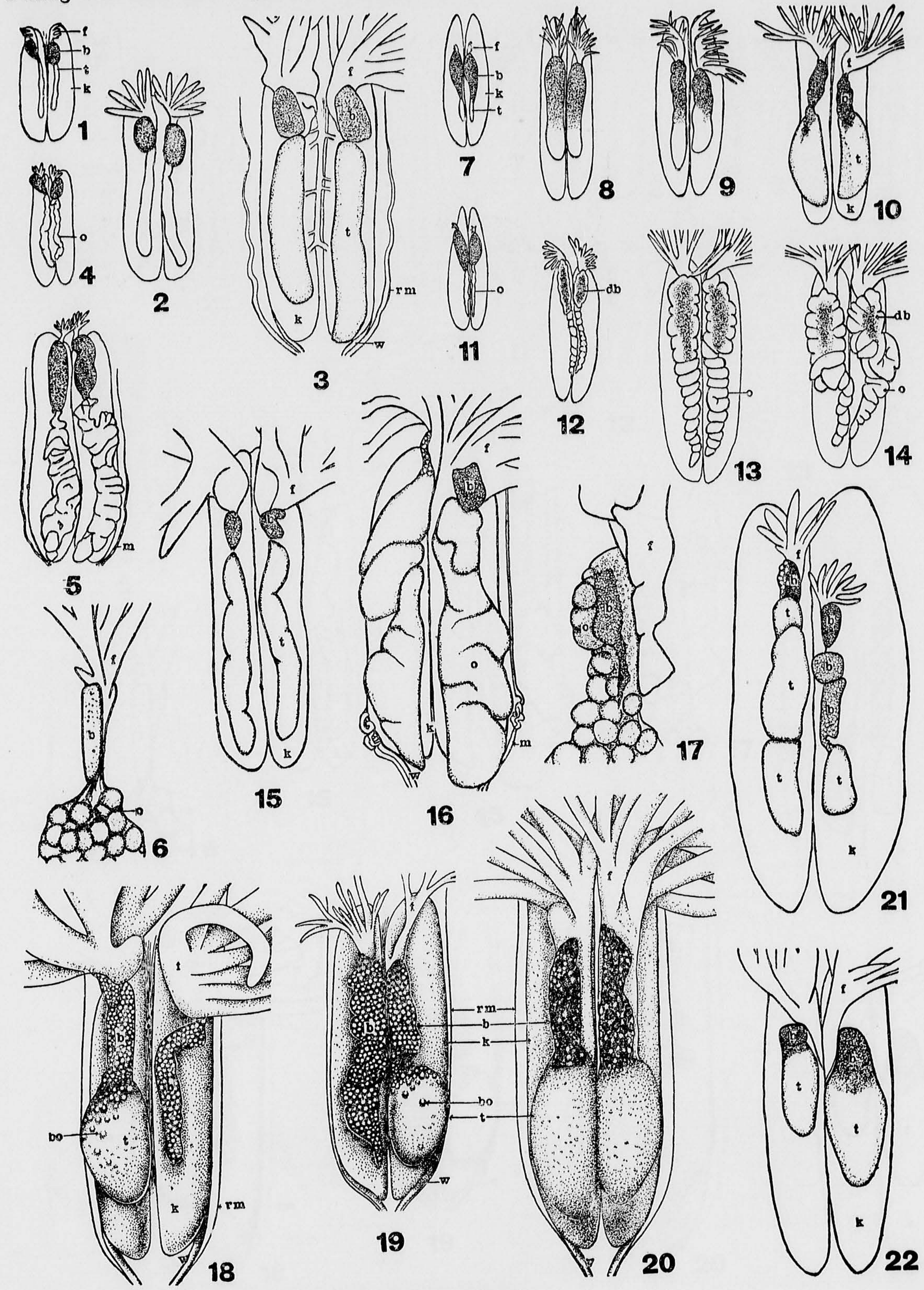
50. Cross section through the upper portion of the left gonad of the abnormal Bufo raddei (Case D).

51. Cross section through the lower portion of the left gonad from the same individual as in fig. 50.

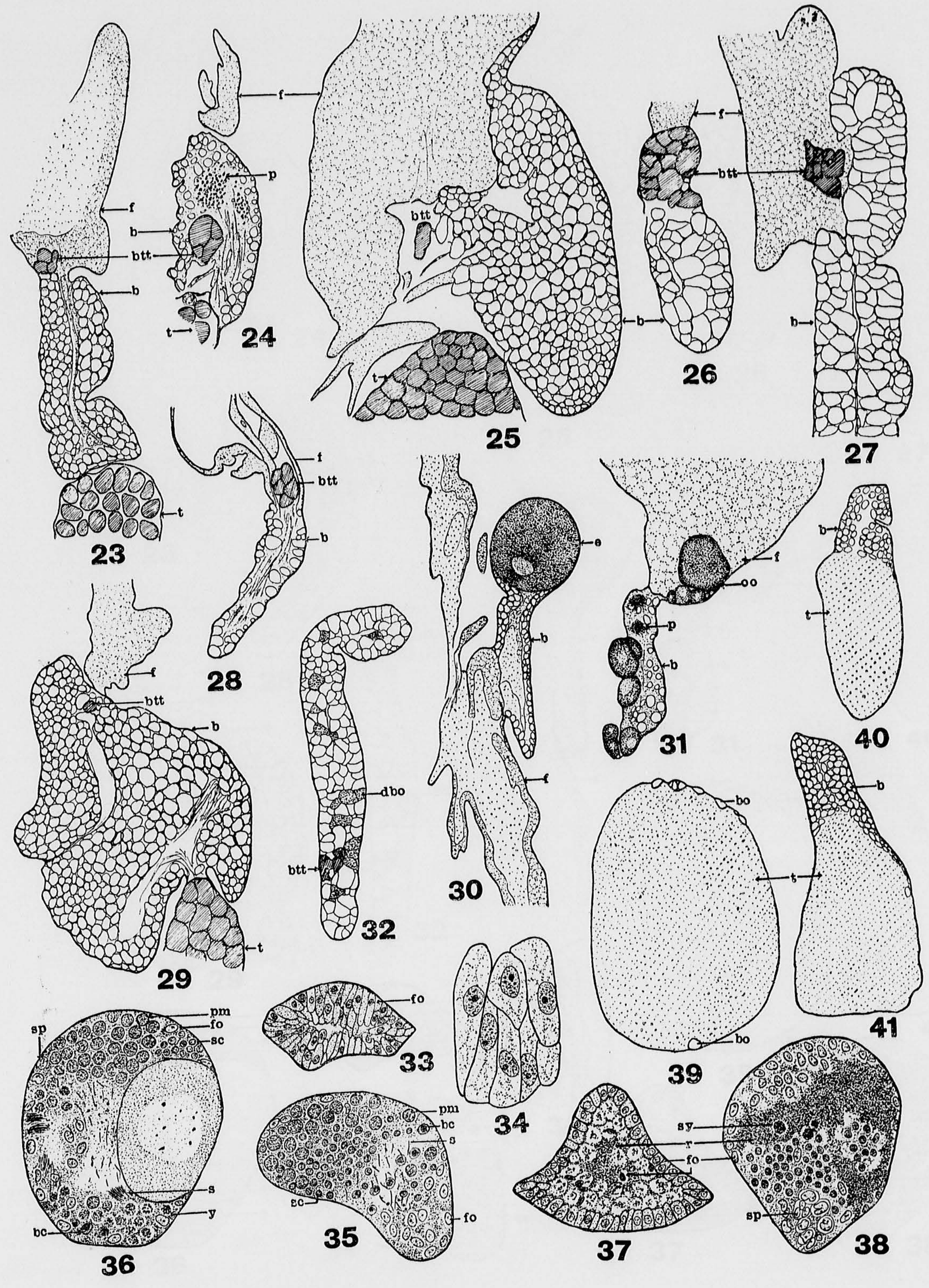
INDEX TO FIGURES

b......Bidder's organ
bc .....blood cell
bo ....Bidder oocyte
bt.....Transforming Bidder oocyte
btt.....Bidder's testicular tissue
db.....degenerating Bidder's organ
dbo....degenerating Bidder oocyte
e......ovum
f......fatbody
fo......follicle cell
i.......inner portion of Bidder's organ
k......kidney
l.......outer layer of Bidder's organ
m.....Müllerian duct
o.......ovary
oc.....ovarian cavity
oo.....oocyte
ot......ovarian tissue
p......pigment
pm....primary spermatocyte
r.......remnants of Bidder oocyte
rm....rudimentary Müllerian duct
s......spermatozoan
sp.....spermatogonium
sy.....synapsis stage of primary spermatocyte
t.......testis
tt......testicular tissue
ttt.....transformed testicular tissue
w......Wolffian duct
y......yolk

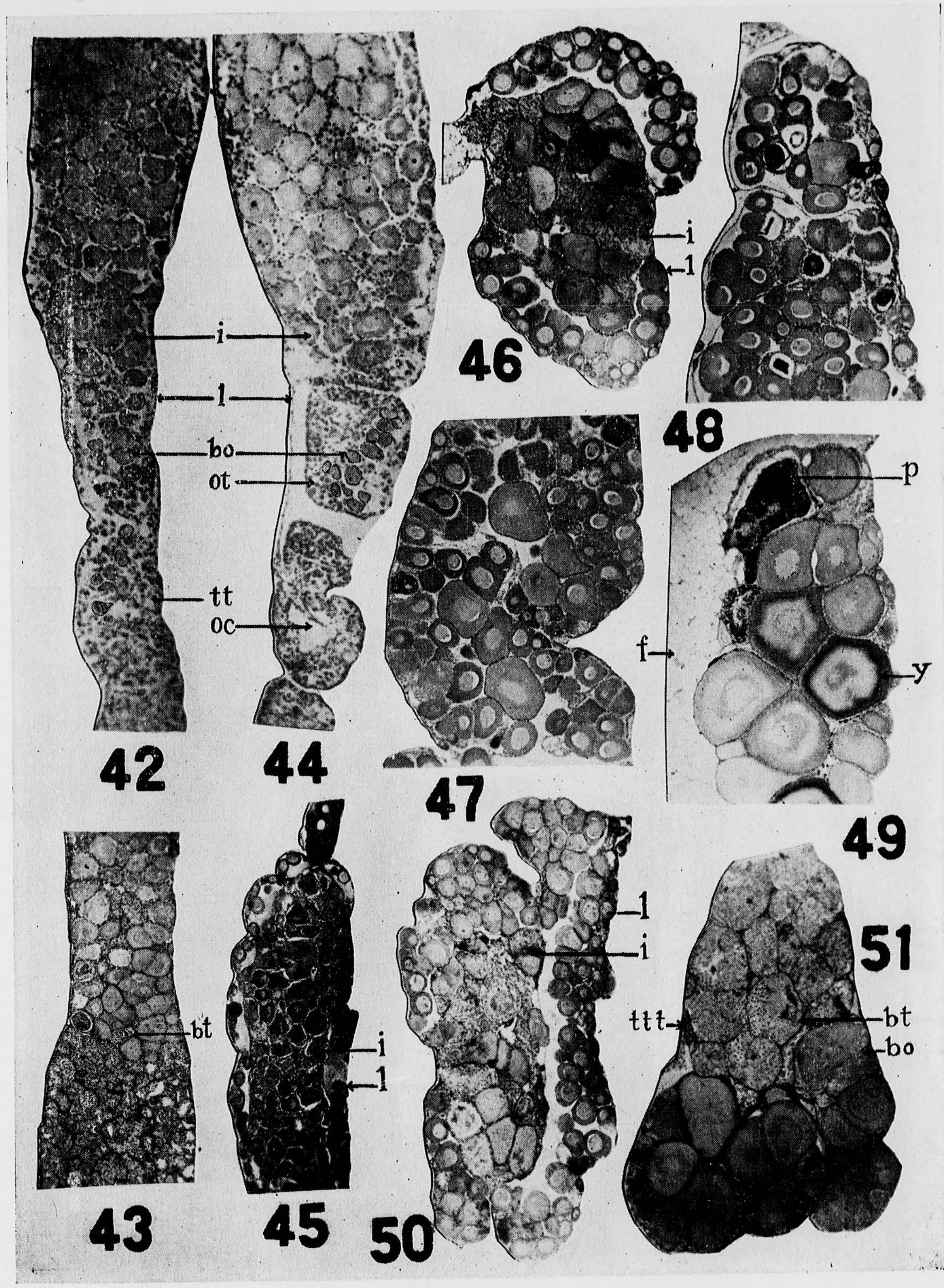
Peking Nat. Hist. Bull. 1938-39, Vol. 13, Part 3. PLATE I



Ting and Boring, Bidder's Organ in Chinese Toads.



Ting and Boring, Bidder's Organ in Chinese Toads.



Ting and Boring, Bidder's Organ in Chinese Toads.

Peking Natural History Bulletin, 1938-39, Vol. 13, Part 3, p. 181.

A STUDY OF THE RECIPROCAL HYBRIDS OF TWO SPECIES OF FROGS, RANA NIGROMACULATA AND RANA PLANCYI

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INTRODUCTION

Experiments on hybridization are valuable for genetics, cytology, and a study of species relationships. During the past hundred years many such experiments have been made on echinoderms, fishes, and amphibians. Among the amphibians, although hybrids between species, genera, and even families have been reported, yet the production of true hybrids has been rare and most of the crosses have resulted in the formation of false hybrids or abnormal young which were unable to develop to maturity. Among the anurans, in the past only two combinations have succeeded in which healthy true hybrids were produced, and in those cases the reciprocal cross was not successful. Therefore the work reported in this paper is the first in which healthy true hybrids have been produced reciprocally.

Experiments on the hybridization of anurans were started as early as the end of the eighteenth century. Spallanzani (1787), after his successful experiments on artificial fertilization, attempted to produce hybrids of anurans but without results. About half a century later, Rusconi (1840) tried to fertilize the eggs of the green frog (Rana esculenta) with the sperm from a toad (Bufo vulgaris). The eggs cleaved but went no further.

During the years 1867-1872, De l'Isle made many attempts to hybridize different French species of frogs and toads, namely, Rana esculenta, Rana agilis, Rana temporaria*, Bufo vulgaris*, and Bufo calamita. However, among all the different crosses, only in the case of Bufo vulgaris and Bufo calamita were the eggs able to develop into tadpoles and these soon died; while in the other cases, even cleavage was not obtained. Lataste (1878) also succeeded in fertilizing eggs of Pelobates fuscus with the sperm of Pelobates cultripes. But the tadpoles produced were monstrous or dropsical and could not develop far.

Pflüger (1882), Pflüger and Smith (1883), and Born (1883,'86) performed repeated experiments attempting to cross anurans. Eventually, they simultaneously succeeded in obtaining some perfect hybrids from the crosses of Rana arvalis (female) and Rana temporaria (male). In addition, Born was able to produce a few perfect hybrids from the cross of Bufo vulgaris (female) and Bufo viridis* (male), but they died soon after passing through metamorphosis.

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*In the original papers, Rana fusca, Bufo cinereus, and Bufo variabilis were used instead of Rana temporaria, Bufo vulgaris, and Bufo viridis respectively. Since the latter are the present day systematic names, they are adopted for use here.

Héron Royer (1883) described a case of the interfamily crossing of Pelobates fuscus (female) and Rana temporaria (male) in nature. He brought the eggs into his laboratory and watched them. Two of them metamorphosed. It is curious to read that they were indistinguishable from pure Rana temporaria, so perhaps some confusion might have taken place. He again in 1891 made crosses with different species of Discoglossus and Bufo, and this time the hybrid larvae produced showed some male characters. Gebhardt (1894) also obtained hybrid embryos of Rana esculenta and Rana arvalis.

Since the beginning of the twentieth century, interest has turned to the behavior of chromosomes in the hybrid. G. Hertwig ('13, '18), after his crossing of Rana esculenta (female) and Bufo viridis (male) which produced tadpoles like those of Rana esculenta, was able to distinguish the true hybrid from the false hybrid because in the latter the sperm takes no part in the development but only activates the egg. By means of radium emanations on sperm, he could prove his explanation. Bataillon and Tchou-su ('29) and Tchou-su ('31) made crosses of French species of frogs and toads and the embryos were studied cytologically.

For the Chinese species of frogs and toads, Tchou-su ('34) has performed different crosses among eleven species in Canton. However, the hybrids produced developed only to various early stages in the different crosses, and in only two crosses resulting in false hybrids were they able to go through metamorphosis.

The writer wishes to express his thanks to Prof. Alice M. Boring for her helpful suggestions and kindly criticisms throughout the preparation of this paper. He is also indebted to Prof. J. C. Li for his kindness in reading the manuscript and for his valuable criticisms.

MATERIAL

The material used was the two most common species of frogs occurring in North China, Rana nigromaculata Hallowell and Rana plancyi Lataste. They are both pondfrogs inhabiting quiet water and ricefields. However, the former is the commonest among the pondfrogs and is closely related to Rana esculenta in Europe, while the latter is found only in China proper and no further north than Hopei and Korea (Boring '38).

The external appearance, size, and habitat of these two species is quite different. The color of Rana nigromaculata varies from yellowish green to greenish gray with dark colored markings on the dorsal side, while the ventral side is white (figs 21, 22). There are two narrow longitudinal dorso-lateral folds and scattered short longitudinal ridges on the back between the dorso-lateral folds. The male has two external vocal sacs and is a little smaller than the female in body size (fig. 22). The color of Rana plancyi is green on the back and yellow on the belly. There are inconspicuous dark spots and small roundish tubercles scattered on the back between the two dorso-lateral folds which are wide and golden. (figs. 23, 24). The male of this species is much smaller than the female and has no external vocal sacs (fig. 24).

As to their habitats, Rana nigromaculata prefers ricefields and low bushes, while Rana plancyi prefers ponds with waterplants. The spring appearance of Rana nigromaculata is in the beginning of April, while that of Rana plancyi is about a half month later. The same is true for the time of the starting of egg laying (Liu '31).

The eggs of both species have three layers of jelly envelopes and are laid in masses. The total number of eggs laid by both species is about the same, around 5000. But the egg of Rana nigromaculata measures about 1.7 mm. in diameter with the animal pole black or dark gray in color and the vegetative pole creamy, while the egg of Rana plancyi measures only 1.4 mm. in diameter with brown color at the animal pole and creamy at the vegetative pole.

The tadpoles of Rana nigromaculata and Rana plancyi according to Pope ('31) and Boring, Liu, and Chou ('32) are also different in appearance and coloration. The tadpole of Rana nigromaculata has flaring lips, two rows of upper teeth (the inner row is interrupted), and three rows of lower teeth, while the tadpole of Rana plancyi has rounded lips, one row of upper teeth, and two rows of lower teeth. The tail in Rana plancyi is broader and tapers more abruptly. As to the coloration, the tadpole of Rana nigromaculata varies from greenish gray to light gray, while the tadpole of Rana plancyi is greenish brown.

Both species of frogs used in the experiments were obtained from the vicinity of West Peking.

HYBRIDIZATION EXPERIMENTS AND OBSERVATIONS

A. METHODS FOR ARTIFICIAL FERTILIZATION

There were two ways by which unfertilized eggs were obtained. The one was by induced ovulation similar to that used by Rugh ('35), the other was directly from the uteri of a frog which was ready to lay. The detailed methods are described in the following paragraphs.

1. INDUCED OVULATION. Frogs whose ovaries contained mature eggs were used. The writer found that frogs caught at the end of September already contained mature eggs which could be fertilized and could develop into normal young. The hormone used for induction was the anterior pituitary gland from the frog. The gland after being taken out was teased in one cc. of distilled water and then was quickly injected into the abdominal cavity by a large hypodermic needle. Care was taken not to injure the viscera, especially the bilobed urinary bladder attached to the ventral body wall. The minimal dose was one female gland or two male glands injected daily or on alternate days until the eggs were laid. The jar in which the frog was put was allowed to have only a very small amount of water, because too much water would cause the jelly of the laid eggs to swell and thus would hinder the artificial fertilization. If the induction was performed in winter, the induced frog was put in a warm chamber at a temperature of about 25°C. For early spring induction frogs were caught in reserve before the hibernating period, since in the early spring it is very hard to obtain frogs and one species may come out while the other species is still in hibernation.

2. EGGS FROM UTERI. Eggs from the uteri of the frog can be obtained only in the breeding season. Female frogs were collected and were washed thoroughly with water to get rid of any possible spermatozoa adhering to the body. Then the abdominal wall was cut open to see whether there were eggs in the uteri or not. If there were, they were taken out carefully by the help of a pair of clean forceps into a clean dry Petri dish. Artificial fertilization was performed as follows :

The desired number of eggs was put in one or two layers in two dishes about 4 inches in diameter and 2 inches in height, one dish used for hybridization, the other for control. At the same time two male frogs, one for hybridization the other belonging to the same species as the eggs, were washed thoroughly, killed, the testes removed and placed in the dishes with the eggs to be fertilized. In removing the testes from the two different species, separate instruments should be used so that the spermatozoa of one could not be mixed with the other. Now a small amount of distilled water was poured into the dishes, just enough to cover the eggs, and the testes were teased quickly with separate pairs of forceps in the water in the corresponding dishes. The dishes were let stand for about a quarter of an hour. During this period, the gelatinous envelopes of the eggs would swell up gradually at the same time that the sperms were penetrating, fertilizing the eggs. Finally more water was added and the fertilization process was completed. If the eggs were properly fertilized, cleavage would begin after about 3-4 hours at laboratory temperature (about 20°C). On the second day the uncleaved eggs, which usually became whitish at the surface, should be removed to prevent decay and to minimize the growth of bacteria which would hinder the development of the normal eggs. Pond water should be used and should be changed daily. In the winter time, the developing eggs were put in a warm chamber at about 28°C and the pond water was also warmed up to that temperature before it was used.

B. METHODS FOR REARING TADPOLES AND YOUNG FROGS

As the eggs hatched out into tadpoles they were transferred to bigger containers such as glass jars or an aquarium so equipped that the water could be changed automatically. If glass jars were used the water should be changed daily. The writer has found that tap water containing even a small amount of lime is not harmful to the tadpoles. The major food was green algae, yolk, and liver. The latter two were put into a small glass container which was then sunk into the aquarium so that the decayed food might be taken out and new food provided. As the tadpoles were ready to metamorphose, floating wood or Spirodela leaves were put on the surface so that tadpoles with forelimbs could have a place to rest without being drowned. Then they were removed immediately to another vessel filled with sand and shallow water. The tail now became shorter and shorter and the metamorphosing tadpole began to take insects as food. Therefore fruit flies (Drosophila) were fed to them. When the metamorphosis was completed, they were again removed to a larger vessel filled with soil to a level two inches below the rim. At the center of the vessel, a dish of water was placed, the rim of which was on the level of the soil, so that the young frogs might not be dried up due to lack of water. The vessel was covered with cloth and fruit flies or house flies (for the older ones) were fed to them. When the winter approached, the larger frogs burrowed down to hibernate while the smaller ones remained at the surface. The ones which did not hibernate were then removed to a warm lighted chamber adjusted at 25°C. They ate well and were very active.

C. EXPERIMENTS AND OBSERVATIONS

.1 RANA NIGROMACULATA ♀ x RANA PLANCYI ♂—INDUCED OVULATION. On April 1 1938, a female R. nigromaculata was injected daily with 2 male anterior pituitaries. On April 3 about 1000 eggs were laid. 200 eggs were used as control (i.e. to be fertilized by the sperm of the same species) while the rest were fertilized with the sperm of R. plancyi. Cleavage began after four hours in both groups of eggs. On the next day, about one third of the eggs were found undeveloped in both groups. On the 5th day they all reached the yolk plug and neural fold stages, and on the 6th day some of them were out from the jelly envelopes. The color of the tadpoles in both groups was dark gray. On the 12th day they measured 8 mm. in body length (from the tip of the snout to the tip of the tail), and on the 21st day the hybrid group measured 9 mm. in average body length, while the control group measured 10 mm. On the 30th day the hybrid group measured 13 mm., while the control group measured 17 mm. From this rate of growth, it looks as though the hybrids grew more slowly, but this is not necessarily true since the number of tadpoles in the hybrid group was much larger and thus each might not have had as much food as in the control group. This question of hybrid vigor will be tested later. As to the body color, the hybrid tadpoles were lighter than the control and the ventral side was more golden due to the presence of more lipophores. The two caudal whitish lines of muciferous crypts (Boulenger) or lateral-line organs were very conspicuous in the hybrids (figs. 2, 26) but were covered by the melanophores in the controls (figs 1, 25). However, the mouthparts were almost the same at the early stage of development, consisting of two rows of lower teeth and one row of upper teeth (figs. 4, 5). But later one inner broken row of teeth was added to the upper teeth and one outer shorter row was added to the lower teeth (fig. 2). When this added row of the lower teeth of the hybrid was compared with that of the control, it was found to be a little shorter (fig. 3). The tail of the hybrid was a little wider than that of the control and tapered more abruptly, like R. plancyi (figs. 1,6,9,25.26,27). The first completely metamorphosed young in the hybrid group appeared on the 110th day, while that in the control group appeared on the 195th day. The newly metamorphosed young of the hybrid measured on an average 16 mm. for body length (snout to vent) and 20 mm. for hind limbs, while that of the control measured 24 mm. for body length and 30 mm. for hind limbs. About 130 hybrid tadpoles and 20 normal tadpoles completed their metamorphoses in their respective hybrid and control groups.

Concerning the coloration and appearance of the young frogs, the ground color on the back of the hybrid frogs varied from sap green with a little metallic luster in the just metamorphosed young to grayish brown with less luster in the older ones (figs. 8, 33). There were deep gray irregular spots scattered on the back and sides and many tubercles on the back. The two dorso-lateral folds were narrow and slightly lighter in color. In some of the older individuals, a narrow lighter median dorsal line was present. The ventral body wall varied from smooth, and more golden in color in younger ones to more rough, less yellowish in the older ones. Dark spots were found on the ventral body wall especially in the pharyngeal region of the younger individuals, but these spots on the pharyngeal region disappeared as the individuals grew older. At the posterior sides of the thighs there was a poorly defined dark line edged by an upper whitish line (fig. 20); and on the dorsal side of the hind legs, there were sharply defined cross bars.

The normal control frogs were a dark greenish gray color on the back with dark green cross bands and inconspicuous longitudinal ridges (figs. 3, 35). The two dorso-lateral folds were very narrow and there sometimes was a narrow light gray or a broad light green middorsal line present. The ventral body wall was smooth, and slightly golden in the just metamorphosed young but became whitish as they grew older. There were no lines along the posterior surface of the thighs (fig. 18), but there were also sharply defined cross bars on the dorsal surface of the hind legs.

2. RANA PLANCYI ♀ x RANA NIGROMACULATA ♂—INDUCED OVULATION—APRIL. On April 2, a female R. plancyi which was caught in reserve in the last winter was injected with 2 male pituitaries, and on April 4 it was again injected with 2 male pituitaries. On the morning of April 7, it laid about 200 eggs which were all fertilized with R. nigromaculata sperm, since no male R. plancyi was available. Cleavage began in 3½ hours after the fertilization. Eight cells resulted in 4½ hours, and 32 cells in 6 hours. On the 2nd day, only 54 eggs were found developing while the rest died uncleaved. On the 5th day the neural groove stage was found, and on the 8th day they were out from the jelly envelopes measuring 4 mm. in length. 10 individuals died at this stage. On the 11th day, they measured 8 mm. in length; and on the 20th day they measured 10 mm. in length. The body color was cream yellow and was very much lighter then the hybrids in (1). On the 30th and 39th days, they measured 15 mm. and 19 mm. respectively. The body color now became darker and darker until it reached nearly the same degree of darkness as the hybrids in (1). For the sake of comparison, R. plancyi tadpoles from outside were collected and reared These showed that the color of the hybrids was more grayish instead of brownish as in the normal tadpoles from outdoors. The ventral body wall of the hybrid was also a little less yellowish than that of the normal. The mouthparts of the hybrids were different from those of the normal tadpoles and were the same as those of the (1) hybrids, although in the early stages they had only 1 row of upper teeth and 2 rows lower teeth (figs. 12, 13). The larger tadpoles had laterally flaring lips, 2 rows of teeth on the upper jaw and 3 on the lower, instead of round lips, 1 row of upper teeth, and 2 rows of lower teeth as in the normal Rana plancyi tadpoles (figs. 10, 16). Nevertheless, both groups had two conspicuous caudal lines of muciferous crypts and had the same form of tail (figs. 9, 14, 15, 27, 28). Unfortunately, on the 42nd day only 3 individuals were left since the others died because of the pollution of the water. These three completed metamorphosis on the 100th day, measuring 20 mm. for body length and 24 mm. for the length of the hind limbs. The normal metamorphosed young of Rana plancyi measured 18 mm, and 22 mm. for the body length and hind limb respectively.

The metamorphosed young hybrid was grayish brown with very dark irregular spots and rough tubercles on the back (fig. 17). There might be a narrow light colored median dorsal line present. The two dorso-lateral folds were narrow. The ventral body wall was lightly spotted and slightly golden in color in younger individuals, but became light yellowish in older ones. The dark and whitish lines at the posterior surface of the thighs were not as clearly defined as in R. plancyi, while the cross bars on the hind legs were sharp and conspicuous.

The metamorphosed young R. plancyi was dark green in color with small spots of raw umber color and a few tubercles on the back (fig. 11). There was no middorsal line present

in either the younger or older individuals and the two dorso-lateral folds were slightly broader in their anterior part behind the eyes (fig. 36). The spotted pattern on the lateral abdominal wall became continuous as if there was an irregular line present on each side. The dark line on the posterior surface of the thighs was very sharp and well defined, but the bars on the dorsal surface of the legs were faint and dispersed and were not complete across the legs. The ventral body wall was golden and spotted in newly metamorphosed young, and became yellowish and lightly spotted in the older ones.

3. RANA NIGROMACULATA ♀ x RANA PLANCYI ♂—EGGS FROM UTERUS. On May 8, about 2000 eggs were obtained from the uteri of a R. nigromaculata collected from outside. Half of the eggs were fertilized with the sperm of R. plancyi, and half with sperm of R. nigromaculata. But the number of eggs which cleaved were very few, and only 5 in the hybrid group and 2 in the control group developed and completed their metamorphosis. The tadpoles of the hybrids showed more brownish coloration on the dorsal side and more golden on the ventral side in comparison with those of the control. The two caudal lines of muciferous crypts were also conspicuous in the hybrid and could hardly be found in the control. Nevertheless, the mouthparts of the tadpoles of these two groups had the same tooth formula (2-3) and the same form of lips. The metamorphosed young of the hybrid measured 18 mm, in body length and 23 mm. for length of the hind limbs, while those of the control measured 23 mm. in body length and 28 mm. for the length of the hind limbs. The color and appearance of the hybrids were nearly the same as those of (I), while the control was grayish in body color, lacking the dark line behind the thighs and had sharply defined cross bars on the legs.

4. RANA PLANCYI ♀ x RANA NIGROMACULATA ♂—SEPTEMBER. Two female R. plancyi (M, N) were injected daily from Sept. 25th to 29th with 2 female pituitaries. Individual M laid eggs on Sept. 30th. Three male R. nigromaculata (X,Y,Z) were used to fertilize the eggs, and their sperm were examined before they were used in fertilization. The sperm from these males were active in distilled water. 320 eggs were fertilized by male X, 600 by male Y, and 48 by male Z. 500 eggs were kept for control. No cleavage was observed in the eggs fertilized by males Y and Z; while with sperm from male X, cleaved eggs were found after 2 hours in the warm chamber. On the 2nd day 160 eggs were found decayed, while the rest were in the neurula stage with partly closed neural tube. The control group showed that 21 eggs died, while the rest reached the yolk plug or neurula stage with partly closed neural tube. On the 3rd day only 14 individuals were left in the hybrid group: 4—6 mm., 6—3 mm., and 4—closed neural tube stage; while in the control, 40 individuals were left: 6—6 mm., 19—3 mm, and 15—closed neural tube stage. On the 5th day only 8 were left in the hybrid group: 7—7 mm., 1—6 mm. with external gills developed; and the others died at the 3 mm. embryo stage. The body color of these small tadpoles was cream yellow. In the control, 22 were left: 2—7 mm., and 20—6 mm. with external gills also and with the same body color as the hybrids. On the 9th day, only 7 were left in the hybrid group. The largest measured 15 mm., the smallest measured 10 mm., and the average was 13 mm. The color of the dorsal side of the tadpole became darker than that of the control which is light yellowish. In the control group, only 19 were left: the largest being 11 mm., in length, the smallest being

9 mm., while the average was 10 mm. On the 12th day, the average size of the hybrid tadpoles was 15 mm. The color became darker and gray. The average size of the control was only 12 mm. The smaller size in the control did not necessarily mean that they were weaker, because the difference in size might be due to the difference in number of the two groups. Unfortunately, on the 15th day, the control group died due to the pollution of water, while in the hybrid group, only 5 were left measuring 22 mm. On the 18th day, the hybrids were examined, they had 2 rows of teeth on the upper jaw and 3 rows (2 long and 1 short) of teeth on the lower jaw, flaring lips and two conspicuous caudal lines of muciferous crypts. The tail was also wide and tapered abruptly. On the 23rd, 27th, and the 32nd day, they measured on an average 34 mm, 40 mm, and 43 mm. respectively. On the 38th day, one of the tadpoles had its forelimbs out, a grayish green color and round spots on the back, and a light golden and faintly spotted belly. The average length was 45 mm. The first one completed its metamorphosis on the 46th day with its body length measuring 17 mm, and hind legs 20 mm. The rest metamorphosed within one week.

The color of the metamorphosed young frogs was light brown with many conspicuous tubercles on the back. They became light greenish gray as they grew older (fig. 34). The two dorso-lateral folds were narrow. The belly was light yellowish in color and rough in appearance. There was also a poorly defined dark line behind the thighs and sharp cross bars on the legs.

Female N was injected daily with 2 female pituitaries beginning from Oct. 6. On Oct. 8, she laid about 1500 eggs. They were then divided into three equal parts which were fertilized with the sperm of two R. nigromaculata (1, 2) and one R. plancyi respectively. 15 minutes later the pigment of some of the eggs showed movement toward the animal pole and small black dots showing the entrance of the sperm were also found on the surface. However, none of the eggs cleaved.

5. YOUNG HYBRID FROGS. Up to the time of the writing of this paper, the hybrids are still very active and feed well (fig. 33, 34). They appear in no point weaker than the normal young frogs. The oldest is about six months old counting from the time since metamorphosis. The total number of the living hybrids is still about 30 in spite of the fact that many have been killed for the study of sex and chromosomes, and some of them died because of the unnatural environment. It appears that a few of the hybrids which did not hibernate and which have been reared in the incubator during the winter have been attacked by some disease so that their heads are bent to one side. After examining a few of the dead ones, nothing abnormal was found in the viscera. The disease is probably connected with the nervous system.

DETAILED DESCRIPTION OF YOUNG INDIVIDUALS OF THE PARENT SPECIES AND OF THE HYBRIDS

A. MEASUREMENTS

Measurements of body size were made with a steel vernier caliper. The length of the head was measured perpendicularly from the tip of the snout to a line between the posterior borders of the two tympanums, and the width of the head was measured between the angles of the jaws.

All the measurements have been calculated into index values from each individual and the average values are tabulated in Table I. It can be seen that the fore and hind limbs in the young of the two parent species are nearly the same in length. But from the average ratio of the head, it shows that the head of young R. nigromaculata is longer than wide, while in that of young R. plancyi the width is approximately the same as the length. In comparing the hybrids it can be seen that all different measurements in both crosses are nearly the same. But if they are compared with the parent species, the size of the head of the hybrids (although they vary greatly) is more like that of R. plancyi. The hind limbs are shorter than in either parent species.

TABLE I. AVERAGE INDEX VALUES CALCULATED FROM EACH INDIVIDUAL OF PARENT SPECIES AND HYBRIDS

	R. nigro.	R. plancyi	Hybrids: R. nigro. ♀ x R. plancyi ♂	Hybrids: R. plancyi ♀ x R. nigro. ♂
No. of individuals	12	11	15	5
Range of body length in mm. (snout to vent)	20.5—33.5	17.3—31.0	14.5—24.0	17.0—23.4
Width of head / Length of head	1.017	1.100	1.119	1.100
Fore limb / Body length	0.588	0.580	0.533	0.530
Hind limb / Body length	1.51	1.53	1.36	1.28

B. CHROMATOPHORES

The coloration of the frog depends mainly on the distribution, arrangement, and contraction or dispersion of the chromatophores which are of three kinds, namely, the melanophores (black), the guanophores (white), and the lipophores (yellow or golden). For studying the yellow and white chromatophores, the skin was studied in the fresh condition under the binocular microscope, for fixing the skin even in formalin dissolves some of these pigments. But for the melanophores, since some of them may lie beneath the guanophores and lipophores, they were studied in absolute alcohol which had already dissolved the other two kinds.

In observing groups of these young frogs of both parent species and of the hybrids, it was apparent that the general coloration of each group was for the most part consistent. Therefore an analysis of the chromatophores in the skin of a few individuals in each group was made and the results recorded in Table II.

TABLE II. DISTRIBUTION OF CHROMATOPHORES IN YOUNG PARENT SPECIES AND HYBRIDS.

	Ventral body wall	Lower lateral body wall	Dorso-lateral folds	Upper lateral and dorsal body wall
R. nigro.	lipophores + (dispersed) guanophores +++ (dispersed)	lipophores + (dispersed) guanophores +++ (contracted) melanophores + (less contracted)	lipophores +++ (less dispersed) melanophores +++ (less contracted)	lipophores +++ (contracted) melanophores +++ (less contracted)
R. plancyi	lipophores +++ (dispersed) guanophores + (dispersed)	lipophores +++ (contracted) guanophores + (dispersed) melanophores +++ (contracted)	lipophores +++ (dispersed) melanophores +++ (contracted)	lipophores +++ (less contracted) melanophores +++ (contracted)
Hybrids	lipophores ++ (dispersed) guanophores ++ (dispersed)	lipophores +++ (contracted) guanophores +++ (dispersed) melanophores ++ (less contracted)	lipophores +++ (less dispersed) melanophores +++ (less contracted)	lipophores +++ (contracted) melanophores +++ (less contracted)

C. INTEGUMENTAL GLANDS

For studying the glands in the skin, both paraffin sections and total mounts were employed, but the writer has found that soaking the skin that had been fixed in formalin in absolute alcohol was the best way for studying the general distribution of the glands.

As to the integumental glands, they are of two kinds: one is the simple saccular mucous gland and the other is the simple saccular granular gland probably poisonous in nature. The mucous gland is generally smaller producing a transparent secretion, while the granular gland is usually larger and secretes a yellow substance which is not transparent. The distribution of these two kinds of glands is summarized in Table III.

TABLE III. DISTRIBUTION OF THE INTEGUMENTAL GLANDS IN THE BODY WALL.
(M. represents mucous glands, G represents granular glands.)

	Mid-ventral body wall		Latero-ventral		Lower lateral		Upper lateral		Dorso-lateral folds		Dorsal	
	M	G	M	G	M	G	M	G	M	G	M	G
R. nigro.	many	—	few	—	—	—	few	—	very few	many, occupy narrow space	many	few
R. plancyi	many	few	many	quite a number, larger	few	few	many	few	very few	many, larger, occupy wide space	many	quite a number, mostly in groups
Hybrids	many	few	few	quite a number	—	—	many	few	very few	many, occupy narrow space	many	few, mostly in groups

A SUMMARY OF THE DISTINGUISHING CHARACTERS OF THE HYBRIDS

From the above observations on the hybrids produced reciprocally, it is shown that the appearance of the reciprocal hybrids is almost the same in spite of minor differences which may be due to variation in the particular parents used. A careful comparison of the characters of the hybrid tadpoles and young with those of the controls shows that there are certain characters in the hybrids like each parent and certain characters which are intermediate between the two parents or slightly different from both, no matter in which way they are crossed. The characters which resemble those of either parent should be called dominant characters, while those which are intermediate or somewhat modified should be called hybrid characters. They are summarized in Table IV.

TABLE IV. DISTINGUISHING CHARACTERS OF THE TADPOLES AND YOUNG OF THE HYBRIDS.

	Tadpole	Young
R. nigro. characters	1. Flaring lips 2. Tooth formula : ⅔	1. Narrow dorso-lateral folds 2. Middorsal line usually present 3. Distinct cross bars on legs 4. Color of back—dark greenish gray*
R. plancyi characters	1. Shape of tail : more abruptly tapering 2. Conspicuous caudal lines of muciferous crypts	1. Shape of head : wide as long 2. Black line on back of thighs * 3. More granular poison glands in skin 5. Dorsal tubercles = groups of glands 6. Color of side = more lipophores
Modified characters	1. Color of back intermediate 2. Color of belly intermediate	1. Color of belly intermediate 2. Length of hind limbs : shorter

THE CHROMOSOMES OF THE PARENT SPECIES AND THE HYBRIDS

A. METHOD

For studying the chromosomes, especially for counting the number, the aceto-carmine technique used by Bridges ('36) for studying the salivary chromosomes of Drosophila and by Li ('38) for insect chromosomes was adopted with some modifications. Testes were taken out from freshly killed individuals. After they were cut into small pieces, they were put immediately into aceto-carmine fluid (this special carmine is produced by Coleman and Bell

* Slightly less distinct

Co., U. S. A.) which served for both fixing and staining. Two hours later, a few pieces were put on the center of a glass slide together with a drop of the fluid. Then a cover-slip was put on and it was pressed firmly between several layers of absorbing paper with the palm of the hand. The cells were thus crushed and the chromosomes in some of the cells, especially those lying at the peripheral zone of the crushed piece, might be spread out. Since the germ-cells of the frog are small and incapable of adhering to the slide, in making permanent slides they will often be lost. Therefore, the edges of the cover-slip were sealed with paraffin, which can make the slide last for several weeks. After the desired figures had been drawn, permanent mounts were tried out, but only a few cells remained.

This method is much better than fixing the testes in Bouin's, Flemming's or other fixing fluids and making paraffin sections, because complete sets of chromosomes are very hard to find in s ctions and all the chromosomes are crowded together and it is impossible to count so many small chromosomes unless spread apart. However, there is one defect in that the crushing will cause the chromosomes to become bigger and that may change their normal shape.

B. OBSERVATIONS

Due to the limited material of the hybrids a thorough study of their chromosomes has been impossible at the present moment. Furthermore, the gonads of the hybrid are still not yet matured, so that synaptic stages can hardly be found, although a few synaptic rings were present in one of the older individuals killed.

The chromosome number of the two parent species appears to be the same, 26, which is the same as that in two American species, Rana pipiens (Parmenter '25, '33) and Rana palustris (Parmenter '33). In the the metaphase plate, both species have 5 pairs of long rod-shaped or V-shaped chromosomes, and 8 pairs of short rod-shaped or V-shaped chromosomes (figs. 37-39, 43). Among the larger 5 pairs, there is one largest pair which forms the biggest ring during synapsis. The sex chromosomes can not be distinguished. In the synaptic stage, there is always one largest ring, 4 medium rings, 5 smaller rings, and 3 which do not form rings, since the chromosomes are too short (figs. 40-42, 44-47).

As to the hybrid chromosomes, at present, there has been only one figure found belonging to the prophase of the spermatogonia (?) which is separate enough to make a count possible (fig. 48). It shows that there are also 26 chromosomes. And among them, 5 longer pairs can also be found. But concerning the synaptic figures of the hybrid, so far there was only one individual which was old enough, from which a few synaptic figures could be found (figs. 49, 50). They showed that there is also ring formation, while the exact number and shape (normal or abnormal) of the rings are hard to decide at the present time because of the limited material and damage by the crushing method. These points, can not be solved until the frogs are old enough for the gonads to be matured.

THE SEX OF THE HYBRIDS

The sex of the hybrids was determined by examining the gonads of the older tadpoles and of the metamorphosed young. The distribution is as follows : Among the 26 older tadpoles examined, there were 12 males to 14 females; while among the 61 metamorphosed young, there were 24 males to 37 females. When they are added together, there are 36 males to 51 females. These numbers are too small to prove anything except that the two sexes are present in fairly even numbers.

DISCUSSION

The striking facts to be noted in the present experiment are :—first the development of paternal characters in the hybrids no matter which way the crosses were made; and second, the consistent expression of dominant characters in both reciprocal crosses. These facts must be taken to mean that the hybrids are really the result of the combination of the chromatin material of the two parents of different species. The results also show that these two parent species, although they are different in appearance and in habitat, are closely related to each other, since hybrids can be produced in reciprocal crosses and develop normally without showing any sign of weakness. However, in order to make sure of this point, a thorough study of the behavior of the chromosomes from the two parent species is necessary, paying special attention to the stages of union of the pronuclei and of the maturation divisions. If these stages are normal, then either one of the species might have arisen from the other species by means of mutation. Since Rana nigromaculata is more widely distributed than Rana plancyi, which is present in only a very limited area of China, it is more likely that Rana plancyi sprung from Rana nigromaculata.

Pflüger (1883) in his experiments on hybridization, where the crosses were not reciprocal, explained this fact by the difference in the shape of the heads of the sperm of the two parent species. The sperm of a species having a pointed head was claimed to penetrate into the egg of the other species more easily than one with a blunter head. However, an examination of the sperm heads of these two species used for crossing, shows that they have nearly the same size and shape, both being rather blunt (figs. 51, 52). All modern studies of fertilization emphasize the fact that it is the chemical nature rather than the shape of the sperm which is important in controlling the possibility of fertilization.

The time of the development of the paternal characters is of interest. Conklin ('08) stated "that the early development of animals is of purely maternal type, and that it is only in stages later than the gastrula that the influence of the spermatozoön begins to make itself felt". But Fischel ('06) after his study of echinoderm hybridization, claimed that the role of the spermatozoön is from the beginning, while the egg cytoplasm furnishes material only for the formative operation of the combined nuclear material. Newman ('08, '10) found that the influence of the hybrid spermatozoön in the teleost starts probably long before gastrulation and can be detected as early as 14 hours after fertilization. In regard to the coloration of our tadpoles, since the egg color of R. nigromaculata is dark gray while that of R. plancyi is light brown, the early development of the tadpole is either dark gray or cream yellow in color according to the maternal species. And in the hybrid the paternal color characters are not noticed until the 9th day when the tadpole has reached a size of about 13 mm. (see p. 187). The development of the paternal coloration is probably associated with the development of the chromatophores.

The possibility of the occurrence of hybrid anurans in nature has long been considered. Remarks have been made by v. Mehely that "in the few localities where the two species co-exist, intermediate specimens occur which are no doubt to be regarded as hybrids."* For instance, Rana arvalis in Europe has been thought of as being the hybrid of Rana temporaria and Rana esculenta by Koch (1871) and others, since it has many aspects intermediate between the supposed parents. Nevertheless when Pflüger and Smith (1883) tried to hybridize the above mentioned two species, the eggs developed only to the blastula stage in the combination of Rana esculenta (female) with Rana temporaria (male), and reciprocally not even cleavage was obtained. To decide whether Rana arvalis can be considered as this supposed hybrid more data are needed.

Concerning Rana nigromaculata and Rana plancyi, at the locality in which the writer is working, Boring ('38) has just published a paper on the great variation in the pattern of Rana nigromaculata and the writer's observations corroborate this. But among the writer's whole collection, not a single specimen has been found in which the pattern looks like that of an artificially produced hybrid. For this reason, it is quite safe to say that hybridization in nature is very rare (if it ever occurs). The amplexus between male Rana nigromaculata and female Rana plancyi has been produced experimentally in the laboratory by Liu ('31) but the occurrence of amplexus between these two species in nature has not been noted until the spring of 1938, when our collector brought into the laboratory a pair in amplexus of which the male was Rana plancyi and the female was Rana nigromaculata. The writer put them in a jar, but on the next day they separated.

Pope ('29) has described a species in Fukien which has the characters of both Rana nigromaculata and Rana plancyi to which he gave a new name Rana fukienensis. It has the general coloration of Rana nigromaculata with a light middorsal longitudinal line. It resembles Rana plancyi by the faint cross bars across the tibia, the white stripe along the back of the thighs and the lack of external vocal sacs. He ('31) also suggested that it may have originated from Rana plancyi and probably is only a variety of that species. A series of studies on this Fukien frog in relation to Rana plancyi by Boring and Chang ('33, '36) lead to this same conclusion that Rana fukienensis is very close to Rana plancyi, and probably a variety of it. Hybridization experiments and a study of the nature of the chromosomes of this Fukien frog may throw more light on this problem.

SUMMARY

True hybrids from the reciprocal crosses of Rana nigromaculata and Rana plancyi have been produced and they are similar in appearance.

The development of the hybrid is normal throughout as compared with its parent species.

The hybrid shows some characters which resemble each parent and some characters which are intermediate between or modified from the parent species.

The diploid chromosome number both of the parent species and of the hybrid is found to be 26. It consists of 2 longest chromosomes, 8 medium chromosomes and 16 shorter chromosomes.

* Quoted indirectly from Boulenger (1896)

13 synaptic pairs have been found in meiosis of both the parent species consisting of 1 largest ring, 4 medium rings, 5 smaller rings and 3 which do not form rings. Synaptic rings regardless of their nature are also found in the hybrid.

The proportion of the sexes of the hybrids is 36 males to 51 females.

Both paternal and maternal chromatin materials play their rôle in the development of the characters of the hybrid.

It is suggested that Rana plancyi is a species which has arisen by mutation from Rana nigromaculata.

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EXPLANATION OF PLATES

PLATE I

Fig. 1. Tadpole, R. nigromaculata, 48 mm. body length. 2x.
2. Mouthparts, tadpole, R. nigromaculata, 46 mm. body length.
3. Young R. nigromaculata, 20 mm. body length. 4x.
4. Tadpole, R. nigromaculata hybrid (R nig. ♀ x R. plancyi ♂). 13 mm. body length. 4x.
5. Mouthparts, R. nigromaculata hybrid, 18 mm. body length.
6. Tadpole, R. nigromaculata hybrid, 45 mm. body length. 4x.
7. Mouthparts, R. nigromaculata hybrid, 45 mm. body length.
8. Young R. nigromaculata hybrid, 18 mm. body length 4x.
9. Tadpole, R. plancyi, 50 mm. body length. 2x.
10. Mouthparts, tadpole, R. plancyi, 49 mm. body length.
11. Young R. plancyi, 18 mm. body length. 4x.
12. Tadpole, R. plancyi hybrid (R plancyi ♀ x R. nig. ♂) 13 mm. body length. 4x.
13. Mouthparts, R. plancyi hybrid, 13 mm. body length.
14. Tadpole, R. plancyi hybrid, 36 mm. body length. 2x.
15. Metamorphosing tadpole, R. plancyi hybrid, 42 mm. body length. 2x.
16. Mouthparts, R. plancyi hybrid, 48 mm. body length.
17. Young R. plancyi hybrid, 20 mm. body length. 4x.
18. Back of thighs of young R. nigromaculata.
19. Back of thighs of young R. plancyi.
20. Back of thighs of young hybrid.

PLATE II (Photographs)

Fig. 21. Adult female R. nigromaculata, $\frac{1}{2}$ natural size.
22. Adult male R. nigromaculata, $\frac{1}{2}$ natural size.
23. Adult female R. plancyi, $\frac{1}{2}$ natural size.
24. Adult male R. plancyi, $\frac{1}{2}$ natural size.
25. Tadpole, R. nigromaculata, natural size.
26. Tadpole R. nigromaculata hybrid, natural size.
27. Tadpole, R. plancyi, natural size.
28. Tadpole, R. plancyi hybrid, natural size.
29. Metamorphosing tadpole, R nigromaculata, natural size.
30. Metamorphosing tadpole, R. nigromaculata hybrid, natural size.
31. Metamorphosing tadpole, R. plancyi, natural size.
32. Metamorphosing tadpole, R. plancyi hybrid, natural size.

33. Young R. nigromaculata hybrid, natural size.
34. Young R. plancyi hybrid, natural size.
35. Young R. nigromaculata, natural size.
36. Young R. plancyi, natural size.

PLATE III

All figures were drawn with the aid of a camera lucida under a Leitz oil-immersion objective, 1/12, aperture 1.30, (100x); oc. 10x; tube length, 170 mm.; paper 106 mm. below the stage. Magnification : 1870 x.

Fig. 37-39. Metaphase of spermatogonia of R. nigromaculata.

40-42. Ring-shaped chromosomes in the primary spermatocytes of R. nigromaculata (the broken rings are due to mechanical injury).

43. Metaphase of spermatogonium of R. plancyi.

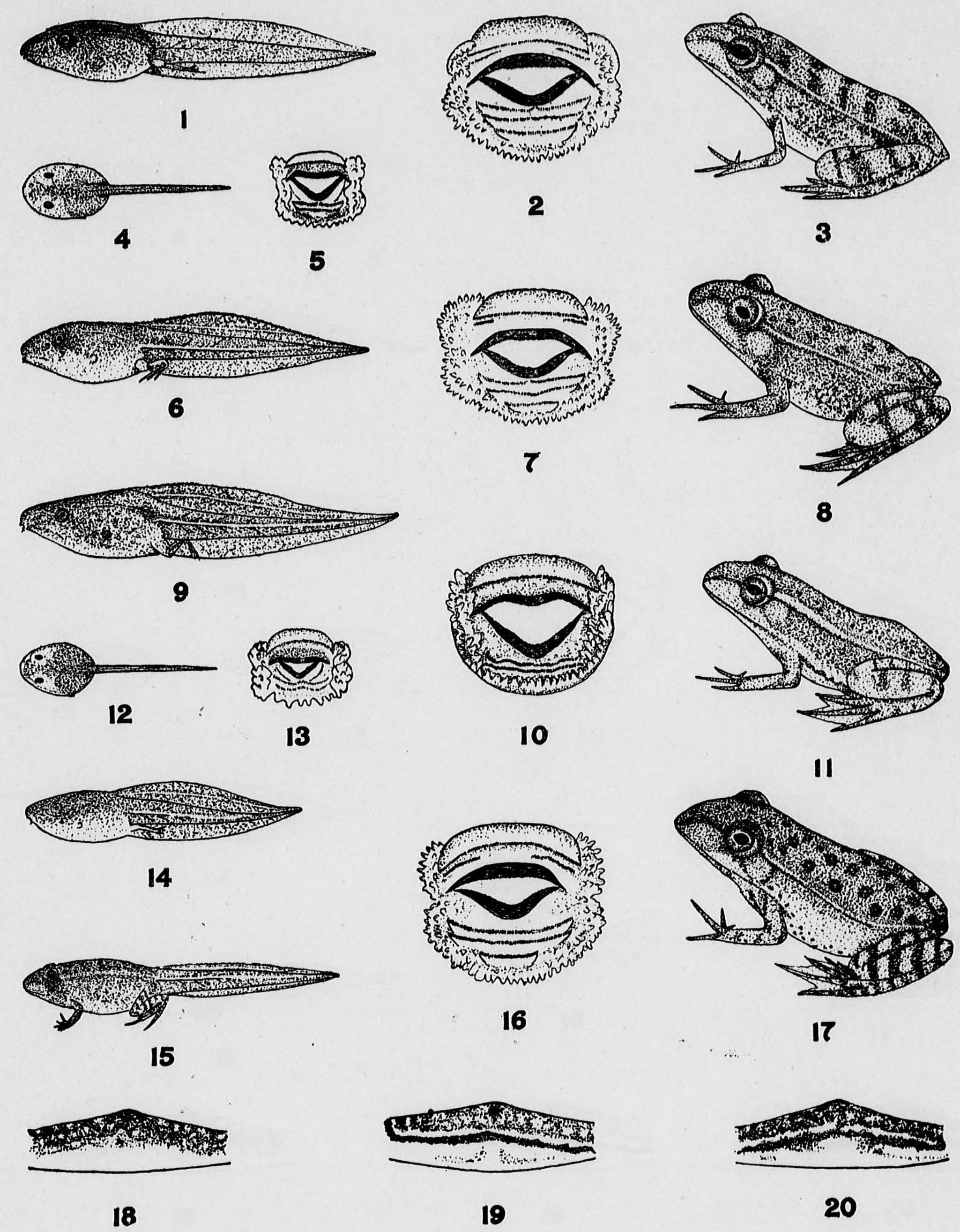
44-47. Ring-shaped chromosomes in the primary spermatocytes of R. plancyi (the broken rings are due to mechanical injury).

48. Prophase of spermatogonium (?) of the hybrid.

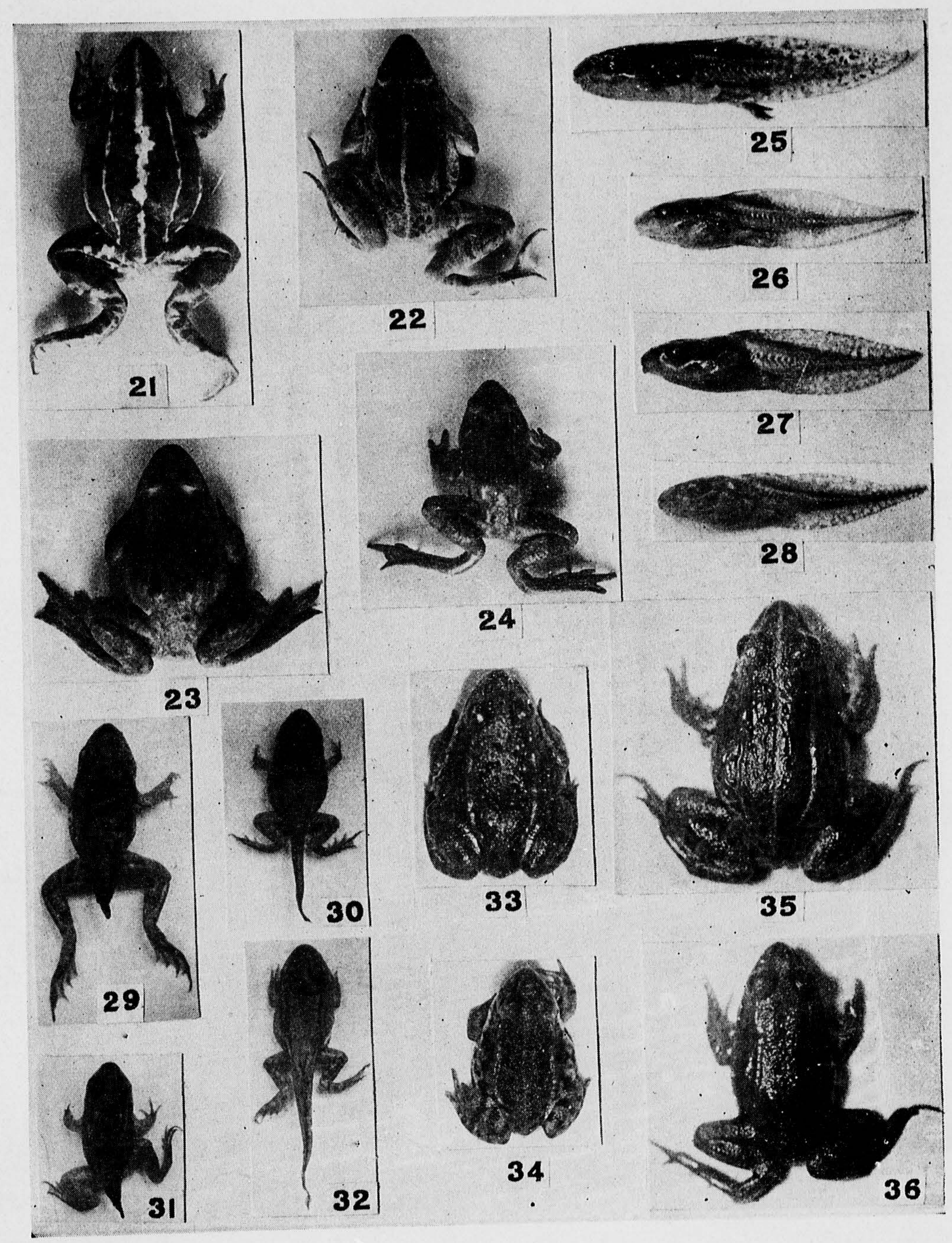
49-50. Synaptic stage in the primary spermatocytes of the hybrid.

51. Spermatozoön of R. nigromaculata.

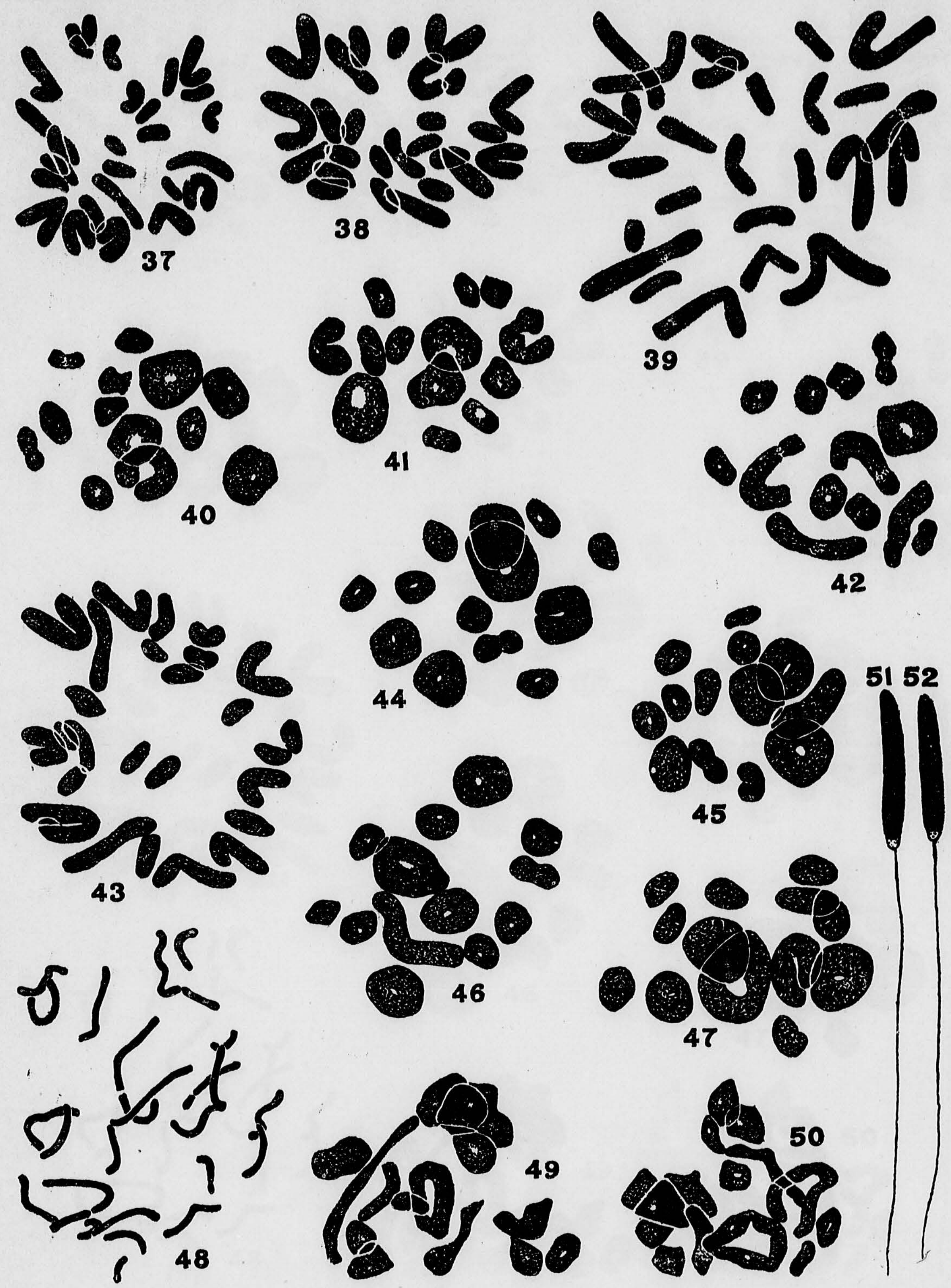
52. Spermatozoön of R. plancyi.



Ting, Reciprocal Frog Hybrids.



Ting. Reciprocal Frog Hybrids.



Ting, Reciprocal Frog Hybrids.

Peking Natural History Bulletin, 1939-40, Vol. 14, Part 1, p. 49.

THE SEASONAL CYCLE IN THE REPRODUCTIVE ORGANS OF THE CHINESE TOAD BUFO BUFO AND THE POND FROG RANA NIGROMACULATA

BY HAN-PO TING AND ALICE M. BORING

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I INTRODUCTION

Seasonal sexual rhythm in various classes of animals has been a subject of interest during recent years and has been studied by Turner ('19), Craig-Bennett ('31), Matthews ('38), Aron ('24), Alexander ('33), Blount ('29), Risley ('38), Rowan ('28), Bissonette ('30), Rasmussen ('17) and Well ('35). This is not only because the sexual rhythm itself in different animals needs to be understood, but also because it is desirable to find the controlling factors. Among the vertebrates, except in some birds and mammals, the sexual cycle generally occurs but once each year. This sexual rhythm is different with different animals, with different climates and with different environmental conditions. Even when animals are closely related, there may be certain differences in the sexual cycle.

For the controlling factors, temperature, food and light have been found to be the three most important external ones, although light may be more important in one animal than another. As to the internal factors, the hormones probably play the most important rôle in governing sexual activity. The anterior pituitary hormone controls the gonads which in turn govern all the secondary sex-organs. The source of the gonadic hormone especially in the male, has been a subject of dispute during recent years. The interstitial cells are suggested by one group of workers, while the Sertoli cells or germinal epithelium by others.

The purpose of this study therefore can be summed up under three points:

1. To study the sexual rhythm in the primary and secondary sex-organs of Bufo bufo and Rana nigromaculata as common representatives of Amphibia in this temperate Asiatic region.
2. To compare the sexual rhythm of the toad with that of the frog.
3. To discover the relationship between the testicular constituents and the period of sexual activity.

II MATERIAL AND METHODS

A. MATERIAL

Bufo bufo and Rana nigromaculata were the two species used. They were collected in the fields, ponds or on water banks in the vicinity of Yenching University, West Peking. In the winter season hibernating toads could be collected easily at Wen Chuan under the shallow water, since the water there does not freeze because of the presence of hot springs. About 50 toads and 70 frogs were collected each month. Among these a number of mature individuals of approximately the same size were selected, and the average size of the group of each month was kept as nearly as possible the same as that of the previous month.

B. METHODS

1. **For keeping frogs in winter.** In order to keep frogs during the winter under a natural environment the following method was devised and was found to be quite successful. During the early part of October a few days before the animals went down for hibernation, they were captured. Several ditches were dug 4 ft. deep and 2 ft. in diameter. A wire cage of small mesh about 2 ft. high filled with soil was put in each ditch. Then about 30 frogs, half males and half females, were put on the surface. Because of the cold outdoor temperature they would bury themselves into the soil within a few minutes. The cage was closed and the ditch was covered with a board. As the ice began to form the space in the ditch was filled with straw so that the temperature would not be too low, since ordinarily, frogs bury themselves much deeper down in the ground. When frogs were needed, one ditch was opened. They were found lying at the bottom of the cage. The death rate was about 15 % of which the females were always more in number.

2. **For general dissection and fixation of material.** The selected individuals, after being etherized, were measured with a steel vernier caliper for body length (from the tip of the snout to the vent). Then the abdominal cavities were cut open. For the male the whole urogenital system was removed and put in normal saline to wash away the blood. Two specimens were picked out and were fixed in modified Bouin's fluid for histological study as representatives of the group. The testes and fatbodies were removed for quantitative measurements. For the female, the ovaries, the fatbodies, Bidder's organs and the oviducts were removed. A portion of the ovary and Bidder's organ were fixed in Bouin's fluid, while the fatbodies were fixed either in Bouin's fluid or 10% formalin. As to the oviduct, it was fixed in Gilson's fluid, since Bouin's fluid would make it more brittle and formalin would cause it to swell up because of the hydrophilous condition of the jelly. The ovaries and oviducts for quantitative measurement were not put in normal saline before the determination was made because the weight would be changed.

The fore-arms of the male were also cut off from some individuals and were fixed in 10% formalin, while a piece of the dermal and epidermal layer of the pad from the second finger was removed from the skeleton and was fixed in Bouin's fluid for the histological study of the seasonal changes of this secondary sex-character.

3. **For quantitative measurements.** The length and width of the testes were measured under the binocular microscope. The measurement of the width of the testes was at right angles to the hilum. The weight of the testes, fatbodies, oviducts and ovaries with smaller eggs was taken in an analytical balance to the nearest milligram, while the ovaries with larger eggs were weighed on an ordinary balance. But before weighing they were rolled on a towel until the extra body fluid was absorbed without drying the specimens. The volume of the testes, ovaries and oviducts was determined by displacement in a finely graduated cylinder.

4. **For histological preparations.** The paraffin method was chiefly employed for the histological study. Sections were made 10 μ in thickness and were stained with haematoxylin and eosin. For the testes, sections 7 μ in thickness and stained with iron haematoxylin and orange G were also made.

5. **For calculating the quantity of the intertubular tissue and the spermatic tissue.** For the quantitative measurement of the intertubular tissue and the spermatic tissue of the testis, the principle was adopted from Bascom ('25) but with somewhat different procedure. This method can be described as follows:

A representative field was drawn from the section of the testis on graph paper with an Edinger projection apparatus or under the microscope with the aid of a camera lucida in the same magnification, in case the boundary of the tubules was not shown clearly by the Edinger projection apparatus. The area occupied by the intertubular tissue was reckoned by counting the squares, and the area occupied by the spermatic tissue could be easily calculated by subtracting the area of the intertubular tissue from the area of the whole field using the formula πr^2. Thus the percentages of these two tissues were known. Then by multiplying the corresponding percentages by the average weight of the paired testes of each month, the absolute value of the corresponding tissues in one individual was obtained, assuming that the density of these two tissues is approximately the same.

III DIFFERENCE IN THE REPRODUCTIVE ORGANS OF BUFO BUFO AND RANA NIGROMACULATA

The reproductive organs of Bufo bufo are different from those of Rana nigromaculata chiefly in that in the former species a marked hermaphroditic condition is shown. There is a pair of Bidder's organs attached to the anterior ends of the functional gonads of both males and females although in the latter they may be much degenerated or have wholly disappeared in the adult. The rudimentary oviducts in the male Bufo were also much more developed than in the male Rana. Further, the testes of Bufo bufo are long and cylindrical, while those of Rana are bean-shaped.

IV SEASONAL TEMPERATURE VARIATIONS AND ACTIVITIES OF BUFO BUFO AND RANA NIGROMACULATA

By comparing the seasonal activities of Bufo bufo and Rana nigromaculata (Table I) with the temperature variations (Table II), it is shown that Bufo bufo begins its hibernation at a higher temperature, around 65 °F, and appears in the spring at a lower temperature, around 50 °F; while for Rana nigromaculata these temperatures are around 60 °F and 55 °F respectively. In other words, Bufo bufo hibernates earlier and comes out earlier, while Rana nigromaculata hibernates later and comes out later. Aside from this specific difference,

TABLE I.

Seasonal activities of Bufo bufo and Rana nigromaculata

Dates	Bufo bufo		Rana nigromaculata	
1937 Mar. 10	in shallow water	♂ 94 ♀ 12—eggs not laid		
Apr. 5	"	♂ 20 ♀ 10- {8 eggs not laid, 2 eggs laid}		
Apr. 8		♂ 61 ♀ 31- {13 eggs not laid, 4 eggs in oviducts, 14 eggs laid}	in shallow water	♂ 76 ♀ 21- {15 eggs not laid, 5 eggs in oviducts, 1 eggs laid}
Apr. 9		♂ 19 ♀ 15- {7 eggs not laid, 8 eggs laid}		
Apr. 13		♂ 10 ♀ 12- {8 eggs not laid, 4 eggs laid}		
Apr. 29			"	♂ 53 ♀ 10- {4 eggs not laid, 1 eggs in oviducts, 5 eggs laid}
May. 13	at water bank, catching insects	♂ 42 ♀ 28—eggs all laid		
May 27			at water bank, catching insects	♂ 9 ♀ 49- {2 eggs not laid, 4 eggs in oviducts, 43 eggs laid}
Sept. 25	some go down water to hibernate			
Oct. 15			majority bury into soil	
1938 Jan. 1	under water	♂ 38 ♀ 29—eggs not laid		
Feb. 26		♂ 40 ♀ 27—eggs not laid		
Mar. 20	begin egg-laying			
Mar. 31			1st croaking heard in Yuan Min Yuan	
Apr. 2		♂ 65 ♀ 21—eggs not laid		
Apr. 3			begin egg-laying	
May 1	catching small animals at water bank			
May 8				♂ 45 ♀ 36- {9 eggs not laid, 6 eggs in oviducts, 21 eggs laid}
June 1				♂ 35 ♀ 81- {1 eggs in oviducts, 80 eggs laid}

TABLE II.

Temperature variations in Peking from February 1937 to July 1938. Records are from the Observatory, Yenching University, and in Fahrenheit.

Dates	Max.	Min.	Mean	Dates	Max.	Min.	Mean	Dates	Max.	Min.	Mean
1937, Feb. 1—7	40	20	30	Aug. 2—8	88	78	83	Jan. 22—28	31	13	22
8—14	30	17	24	9—15	92	78	85	29—Feb. 4	31	14	23
15—21	42	21	34	16—22	89	76	83	Feb. 5—11	34	18	26
22—28	44	25	40	23—29	84	69	77	12—18	40	21	31
Mar. 1—7	44	29	37	30—Sept. 5	78	64	71	19—25	44	21	33
8—14	46	28	37	Sept. 6—12	84	65	75	26—Mar. 4	51	31	41
15—21	50	31	42	13—19	81	61	71	Mar. 5—11	37	23	30
22—28	42	27	35	20—26	82	63	73	12—18	52	29	41
29—Apr. 4	54	36	45	27—Oct. 3	71	54	63	19—25	58	40	49
Apr. 5—11	67	42	55	Oct. 4—10	71	56	64	26—Apr. 1	60	43	52
12—18	58	42	50	11—17	62	41	52	Apr. 2—8	72	50	61
19—25	63	46	55	18—24	72	45	59	9—15	72	45	59
26—May 2	76	53	65	25—31	64	46	55	16—22	75	50	63
May 3—9	82	53	68	Nov. 1—7	61	40	51	23—29	77	50	64
10—16	86	61	74	8—14	59	35	47	30—May 6	78	55	67
17—23	83	59	71	15—21	40	32	36	May 7—13	76	53	65
24—30	79	54	67	22—28	40	27	34	14—20	72	55	64
31—June 6	87	66	77	29—Dec. 4	37	22	30	21—27	73	60	67
June 7—13	82	65	78	Dec. 5—10	40	23	32	28—June 3	82	65	74
21—27	83	70	77	11—17	34	23	29	June 4—10	79	65	72
28—July 4	86	70	78	18—24	27	13	20	11—17	88	67	78
July 5—11	87	68	78	25—31	31	14	23	18—24	88	70	79
12—18	92	72	82	1938, Jan. 1—7	25	11	18	25—July 1	87	70	79
19—25	93	76	85	8—14	49	13	31				
26—Aug. 1	90	74	82	15—21	29	13	21				

the importance of the temperature in affecting the activities can be seen by the fact that in the spring of 1938, since the temperature got warmer earlier than in 1937, Bufo bufo started its egg-laying earlier and ended it earlier.

As to the length of the breeding period of the two species, it is found that in Rana nigromaculata the breeding period covers about two months, while that of Bufo bufo lasts only half a month.

The preponderance of the male toads and frogs in the mating season is also worth noticing. This is because males are much more active than the females during the mating season and come out earlier and in greater numbers. Liu ('31) has also found that in Rana nigromaculata and Bufo raddei, the males always appear first in the breeding season and increase in number very rapidly.

V AGE AND BODY SIZE

During the course of the experiment, the writers made measurements of the average body length (snout to vent) of the adult mature individuals in an effort to eliminate the error of mixing the younger immature individuals with the mature ones. The results are shown in Table III.

TABLE III.

Average body length of adult Bufo bufo and Rana nigromaculata

	Sexes	Average body length	Number of individuals measured
Bufo bufo	male	92.1	163
	female	98.4	115
Rana nigromaculata	male	64.2	168
	female	72.3	178

The table shows that the males are always smaller than the females by a little less than 1 cm. and the mature toad is much larger than the frog.

For the relation of age to body length or the growth rate, various methods have been used by different writers. Force ('33) studied the age of attainment of sexual maturity in the leopard frog, Rana pipiens, by means of measuring a large number of frogs at different times of the year and comparing the modes of the curves. Hamilton ('34) studied the rate of growth of the toad, Bufo americanus, by means of repeated measuring and releasing of the toads confined in a garden. This is really the most reliable way because in the method used by Force error may come from wrong interpretation. The method used by the writers was to collect at random a large number of individuals at one time within a few days. After their body length had been measured, the external secondary sex-characters as well as the condition of the gonads of a number of individuals of different sizes was examined. Then frequency polygons were constructed to see the relation of the mode and the condition of the gonads and secondary sex-characters observed so that they could be grouped into different years. However, there was one unavoidable difficulty, because

although the animals were collected at random, yet the larger ones were more apt to be collected, so that multimodal polygons indicating one year's difference were not necessarily obtained. In that case, the final judgment must depend on the observation of the condition of the gonads and the secondary sex-characters. The results are shown in figs. A, B, C, D, and Table IV.

TABLE IV.

The condition of the gonads and some secondary sex-characters in the first three years of development of Bufo bufo and Rana nigromaculata. (Sept. 1937)

	Bufo		Rana	
	male	female	male	female
1st year	no nuptial pads, externally sex not distinguishable, testes small	ovaries small and cream-colored	no nuptial pads or vocal sacs, externally sex not distinguishable, testes very small	ovaries small and cream-colored
2nd year	nuptial pads present, testes more developed, sperm active	ovaries immature, small black ova present	nuptial pads present, vocal sacs small, sperm active	ovaries immature ova larger than 1st year
3rd year	testes larger and mature	ovaries mature, eggs ready to be laid next spring	vocal sacs well-developed, testes large and mature	ovaries mature, eggs ready to be laid next spring

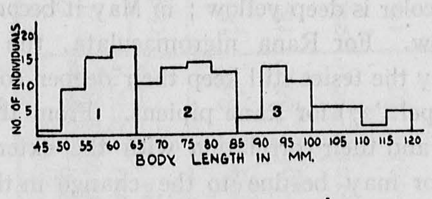

Fig. A. Frequency polygons of body length from the measurement of 132 female Bufo bufo collected in October 1937. The number in each polygon represents the age.

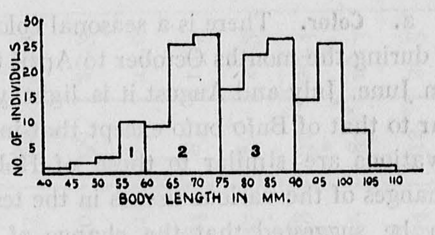

Fig. B. Frequency polygons of body length from the measurement of 190 male Bufo bufo collected in October 1937.

The results obtained from the frequency polygons and other observations show that it takes three years for both Bufo bufo and Rana nigromaculata to become fully mature.

The first year toads and frogs in these measurements made in September or October are actually six months old; the second year, $1\frac{1}{2}$ years old; the third year, $2\frac{1}{2}$ years old. This result is similar to that observed by Hamilton ('34) for Bufo americanus, and Force ('33) for Rana pipiens. It shows also that Bufo bufo has a quicker growth rate in spite of the fact that the newly metamorphosed young of Bufo measures only 18 mm. in body length, while that of Rana nigromaculata measures 24 mm.

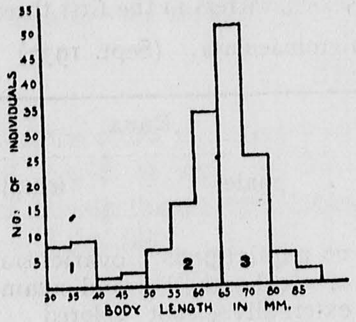

Fig. C. Frequency polygons of body length from the measurement of 169 female Rana nigromaculata collected in September 1937.

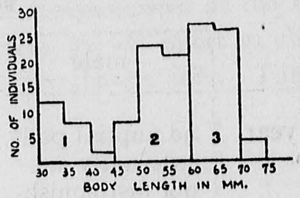

Fig. D. Frequency polygons of body length from the measurement of 132 male Rana nigromaculata collected in September 1937.

VI SEASONAL VARIATIONS IN THE REPRODUCTIVE ORGANS

A. MALE

1. PRIMARY SEX-ORGANS—THE TESTES

a. **Color.** There is a seasonal color variation in the testes of both species. For Bufo bufo, during the months October to April the color is deep yellow; in May it becomes lighter and in June, July and August it is light yellow. For Rana nigromaculata, the change is similar to that of Bufo bufo except that in May the testes still keep their deeper color. These observations are similar to those of Holzappel ('37) for Rana pipiens. From the study of the changes of the various tissues in the testes and their correlation with the external color, it can be suggested that the change of color may be due to the change in the relative amount and nature of the spermatic aud the intertubular tissue. When the intertubular tissue is less the testes become lighter in color and vice versa owing to the fact that the intertubular tissue contains interstitial cells which have much fat (Humphrey,'21) and these fatty cells may account for the change of color.

b. **Quantitative changes.** The testes undergo conspicuous seasonal changes in their size, weight and volume as seen from Tables V and VI for Bufo bufo and Rana nigromaculata respectively.

TABLE V.

Quantitative measurements of the testes and fatbodies of Bufo bufo.

	No. of males measured	Body length (snout to vent) Range in mm.	Average body length in mm.	Average length of testes in mm. Right	Left	Mean	Average width of testes in mm. Right	Left	Mean	Average weight of paired testes in gm.	Average volume of testes in cc.	Weight of testes / Volume of testes	Average weight of paired fat bodies in gm.
Feb.	10	83-89	87	18.2	17.6	17.9	2.3	2.4	2.4	0.162	0.16	1.01	0.091
	13	93-99	*96	18.2	19.3	18.8	2.9	2.9	2.9	0.244	0.24	1.02	0.365
Mar.	7	94-101	*98	20.6	18.9	19.8	2.8	2.7	2.8	0.241	0.23	1.05	0.025
Apr.	18	93-101	*97	19.4	18.0	18.7	2.4	2.4	2.4	0.184	0.17	1.08	0.091
May	7	86-92	88	16.4	15.7	16.1	2.3	2.3	2.3	0.132	0.13	1.02	0.037
June	17	81-93	86	18.7	17.2	18.0	2.7	2.7	2.7	0.208	0.18	1.16	0.127
	8	95-104	*99	22.3	22.4	22.4	3.6	3.6	3.6	0.408	0.39	1.05	0.098
Aug.	5	83-91	88	21.6	20.0	20.8	2.9	3.2	3.1	0.297	0.27	1.10	1.715
Sept.	5	83-84	83	19.4	19.2	19.3	2.6	2.7	2.7	0.234	0.23	1.02	0.489
Oct.	3	84-87	86	20.5	19.3	19.9	2.7	2.6	2.7	0.195	0.19	1.03	0.327
	12	88-98	*92	20.4	20.2	20.3	3.0	3.0	3.0	0.280	0.28	1.00	0.348
Dec.	6	81-86	83	18.3	17.8	18.1	2.3	2.3	2.3	0.157	0.15	1.05	0.105
	9	88-94	*92	20.3	18.9	19.6	2.5	2.6	2.6	0.210	0.20	1.05	0.130

*Groups of larger body size.

TABLE VI

Quantitative measurements of the testes and fatbodies of Rana nigromaculata

	No. of males measured	Body length (snout to vent) Range in mm.	Average body Length in mm.	Average length of testes in mm.			Average width of testes in mm.			Average weight of paired testes in gm.	Average volume of testes in cc.	Weight of testes / Volume of testes	Average weight of paired fatbodies in gm.
				Rignt	Left	Mean	Right	Left	Mean				
Feb.	7	60—64	62	4.9	4.9	4.9	2.2	2.2	2.2	0.029	0.027	1.07	0.032
Apr.	13	60—65	62	4.5	4.6	4.6	1.3	2.2	2.3	0.031	0.030	1.03	0.031
	8	66—70	68	5.7	5.6	5.7	2.4	2.4	2.4	*0.042	0.041	1.02	0.037
May	13	60—64	62	4.2	4.3	4.3	2.0	2.0	2.0	0.021	0.021	1.00	0.007
June	9	60—63	62	4.6	4.6	4.6	2.1	2.0	2.1	0.027	0.026	1.04	0.008
	7	56—64	59	3.9	3.8	3.9	1.8	1.9	1.9	*0.015	0.015	1.00	0.095
July	9	61—64	63	3.7	3.8	3.8	1.9	1.9	1.9	0.019	0.017	1.12	0.127
	4	67—70	69	4.9	4.7	4.8	2.3	2.4	2.4	*0.035	0.033	1.06	0.118
Aug.	11	61—66	63	5.3	5.0	5.2	2.5	2.5	2.5	0.039	0.037	1.06	0.207
	9	66—68	67	4.7	4.7	4.7	2.4	2.3	2.4	*0.036	0.033	1.09	0.352
Sept.	7	60—64	62	5.0	4.4	4.7	2.7	2.7	2.7	0.033	0.031	1.06	0.174
	8	65—69	66	5.1	5.3	5.2	2.5	2.5	2.5	*0.047	0.044	1.07	0.375
Oct.	6	60—65	63	4.6	4.7	4.7	2.3	2.3	2.3	0.033	0.032	1.03	0.188
Dec.	9	60—65	62	5.1	5.1	5.1	2.3	2.3	2.3	0.033	0.032	1.03	0.070

* Values not used in calculating the quantities of the intertubular and the spermatic tissues

TABLE VII

Histological changes in the testes of Bufo bufo and Rana nigromaculata

	Bufo bufo				Rana nigromacultata			
	Spermatogonia	Spermatocytes	Spermatids	Spermatozoa	Spermatogonia	Spermatocytes	Spermatids	Spermatozoa
Feb.	few, beginning of multiplication	none	some	many, near periphery	few, resting stage	very few	some	many, near periphery
Mar.	few, multiplication	none	few	,,				
Apr.	,,	few, early maturation	few	less, near periphery	beginning of multiplication	none	some	,,
May	more, multiplication	,,	few	less	more, multiplication	early maturation	few	fewer, near periphery
June	many, active multiplication	many, active maturation, many synizetic figures	few	less, some near periphery, some near center	many, active multiplication	many, active maturation, synizetic figures abundant	few	few, near center
July	less	many, early synizetic figures abundant	more	fewer	less	many, active maturation	more	more, near center
Aug.	few	less, all stages of maturation, lumen larger	many	many, near periphery	few	many, early synizetic figures abundant, lumen larger	many	many, some at center, some near periphery
Sept.	few	many, all stages of maturation	many	many, being pushed to center	few	more, all stages of maturation	many	,,
Oct.	few	less	some	many, near periphery	very few	fewer, but active maturation	some	many, near periphery
Dec.	few	few	some	,,	very few	very few	some	,,

As to the size, the measurements of the left and the right testes are separated to see whether there is any bilateral asymmetry present. However, from Tables V and VI it shows that there is no definite difference in the size of the testes of the two sides. When the length of the testis of one side is shorter than the other, the width of the same side is usually increased so that the actual volume of the two sides is nearly the same. Concerning the seasonal changes, both length and width vary in the same direction, that is to say, when the length increases, the width also increases. The greatest size is attained during the months of July and August for Bufo bufo and in August and September for Rana nigromaculata; the minimal size is found at the end of the mating period which for Rana nigromaculata is about two months later than for Bufo bufo.

As to the weight, they show the same fluctuation as in size. The increase of the density when the testes grow larger in the summer shows that the testes are then really more solid than at other periods and this will be proved by histological sections.

c. **Histological changes.** The histological changes are summarized in Table VII. Spermatogenesis is the most active from June to September consisting of two spermatogenetic waves. The multiplication of spermatogonia starts about February for Bufo bufo and April for Rana nigromaculata. In August the testes already contain a number of spermatozoa.

As to the "intertubular tissue", the term means the entire mass of tissue in between the tubules, consisting of nerves, blood vessels and connective tissue cells. The first two of course do not undergo change, while the connective tissue may change in number and shape of cells and may be modified into "interstitial cells". Champy ('08) observed that in Rana esculenta the interstitial cells undergo degeneration and become connective tissue cells during July. Aron ('26) also found the same phenomenon, that the size and number of the interstitial cells was minimal in August and September. For our Chinese Bufo bufo and Rana nigronaculata, because of the lack of special fixation and staining it is rather

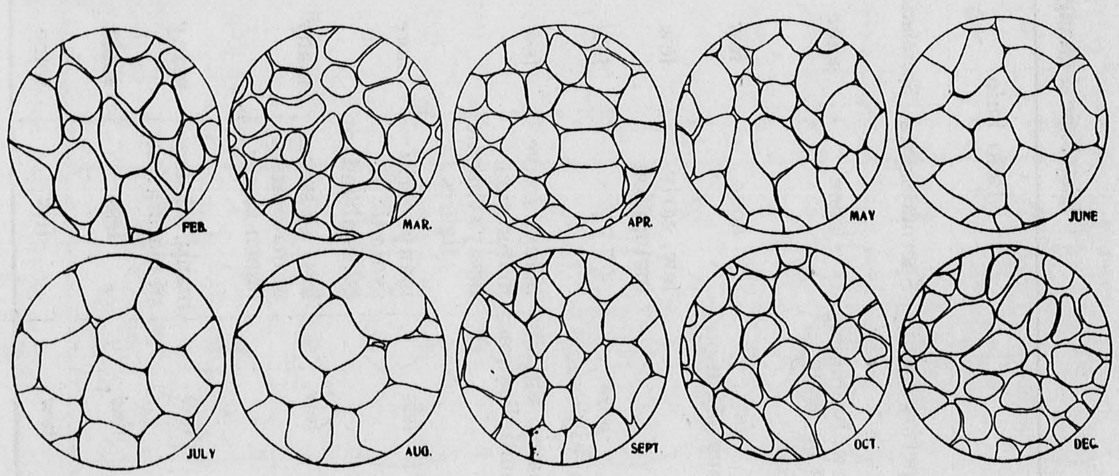

Fig. E. Relative area occupied by the intertubular and the spermatic tissues of Bufo bufo in different months.

hard to distinguish the ordinary connective tissue cells from the interstitial cells. However, it has been found that the cells (either connective tissue cells or interstitial cells) in between the wall of the tubules decrease in number and have flattened nuclei in the summer testes (those lying in the intersection may be less flattened) (Plate, figs. 1 and 2), while in winter and spring the number is more and the nuclei are oval (Plate, figs. 3 and 4).

d. **Quantity of intertubular and spermatic tissue.** The quantity of the intertubular and the spermatic tissue has been calculated from the ratio in a representative field (figs. E and F) and the total weight of each month and are tabulated in Table VIII. Curves are also constructed to show the relationship of these two tissues (figs. G and H). They show that the intertubular and the spermatic tissue vary almost inversely, i.e., when the spermatic tissue is at the minimum the intertubular tissue will be at the maximum. For Bufo bufo the spermatic tissue is at its lowest quantity during April due to the discharging of the spermatozoa and is at its highest quantity in August, while the intertubular tissue is greatest at the end of March, i.e., at the height of the mating season, and lowest during the summer. If these curves are compared with those constructed by Oslund ('28) from Champy's ('13) data for Bufo vulgaris of Europe, they are found to be quite different. The mating period as shown from the chart of Oslund is at the end of February. After the mating the interstitial tissue increases rapidly until June, then it goes down to its minimal quantity in September and October. In November it increases a little again and keeps the same amount to the middle of March. The spermatic tissue is nearly in the reverse condition. The mating period for the Chinese Bufo

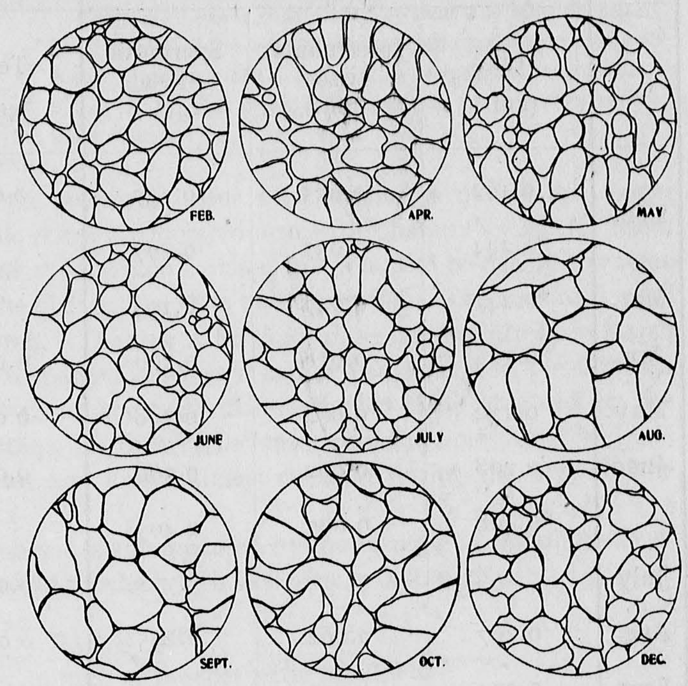

Fig. F. Relative area occupied by the intertubular and the spermatic tissues of Rana nigromaculata in different months.

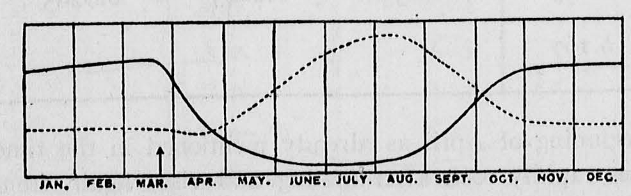

Fig. G. Curves constructed to show the relationship of the quantity of the intertubular and the spermatic tissue of Bufo bufo. Solid line represents intertubular tissue, broken line represents spermatic tissue, arrow represents mating.

TABLE VIII

Seasonal variations of the quantities of the intertubular and the spermatic tissues of Bufo bufo and Rana nigromaculata

	Bufo bufo			Rana nigromaculata		
	Testes (gm.)	Intertubular tissue (gm.)	Spermatic tissue (gm.)	Testes (gm.)	Intertubular tissue (gm.)	Spermatic tissue (gm.)
Feb.	0.162	0.025	0.137	0 029	0.0042	0.0248
	*0.244	0.037	0.207			
Mar.	*0.241	0.047	0.194			
Apr.	*0.184	0.018	0.166	0.031	0.0054	0.0256
May.	0.132	0.004	0.128	0.021	0 0034	0.0176
June	0.208	0.002	0.206	0.027	0.0042	0.0228
	*0.408	0.005	0.403			
July				0.019	0.0022	0.0168
Aug.	0.297	0.002	0.295	0.039	0.0016	0.0374
Sept.	0.234	0.011	0.223	0.033	0.0019	0.0311
Oct.	0.195	0.028	0.167	0.033	0.0034	0.0296
	*0.280	0.041	0.239			
Dec.	0.157	0.032	0.125	0.033	0.0045	0.0285
	*0.210	0.043	0.167			

bufo is from the end of March to the beginning of April as already mentioned in the time table. The intertubular tissue decreases rapidly soon after mating and rises again from September. Climatic difference might be one of the factors causing this discrepancy, but in Oslund's chart the interstitial cells increase two months after the mating period. This seems too long a period. Since the quantitative method was first introduced by Bascom ('25) for

* From group of larger body size.

studying the testes, Champy's result must have been merely by estimation. Therefore inaccuracy is unavoidable and the difference may be due to this.

For Rana nigromaculata the spermatic tissue decreases quite rapidly from April to July, then is followed by a rapid growth in July and August and then gradually falls down again. The intertubular tissue decreases from May to August and increases gradually to its maximum value in April. The longer period of decreasing of the spermatic tissue after mating is due to the longer breeding period of this species. If this result is compared also with Champy's ('13) for Rana temporaria of Europe the changes of the spermatic tissue are found to be nearly the same and the difference is simply due to the difference in the mating period. The mating period of Rana temporaria is in early March. The spermatic tissue decreases rapidly from March to May, then it rises gradually until September whereas it makes a slight decrease in October. But the changes in the interstitial tissue are quite different from those obtained by the writers. The interstitial tissue of Rana temporaria increases rapidly from April to June, then it drops down rapidly to August and keeps almost constant during the rest of the months.

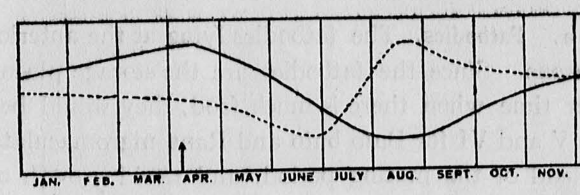

Fig. H. Curves constructed to show the relationship of the quantity of the intertubular and the spermatic tissue of Rana nigromaculata. Solid line represents intertubular tissue, broken line represents spermatic tissue, arrow represents mating.

The increase of the spermatic tissue is accompanied by the increase in the diameter of the tubules as found by the average of 30 tubules which is shown in Table IX.

TABLE IX

Average diameter in mm. of the spermatic tubules of Bufo bufo and Rana nigromaculata.

	Bufo bufo	Rana nigro.
Feb.	0.24	0.19
Mar.	0.23	
Apr.	0.25	0.19
May.	0.23	0.18
June.	0.27	0.19
July	0.35	0.22
Aug.	0.40	0.28
Sept.	0.24	0.25
Oct.	0.23	0.24
Dec.	0.21	0.22

2. ACCESSORY SEX-ORGANS

a. Fatbodies. The fatbodies lying at the anterior ends of the gonads also change with the seasons. Since the fatbodies are the storage place for food, it was expected that in the summer time when there is much food, they would be larger. The average values given in Tables V and VI for Bufo bufo and Rana nigromaculata show that the fatbodies are smallest at the end of the mating period, and rapid growth occurs in July and August, and from September they become smaller again owing to the lack of food and its consumption for the manufacture of the reproductive products.

As to the histological changes when the fatbody is large, each fat cell contains a large fat drop with the nucleus squeezed to the periphery and indistinct. On the other hand, when the fatbody becomes small, each fat cell contains only a drop of fat and the nucleus becomes oval. During the transitional period the cells lying at the inner part of the fatbody contain larger or smaller fat drops than the outer ones. This is because the larger blood vessels run in the middle of the fatbody and thus fat is deposited or consumed first among the inner cells.

b. Bidder's organ (Bufo bufo). This organ has already been described by Ting and Boring ('39). It does not degenerate much in the male adult. It varies in size and shape individually and there is no definite correlation found with the seasons.

c. Ureters. The ureters of Bufo bufo measure about one mm. in diameter at the posterior part of the kidneys of the adult male and do not undergo changes in size and cell structure as those found in Rana nigromaculata. In Rana nigromaculata the ureters measure about 0.8 mm. in diameter at the part posterior to the kidneys in ordinary months, but increase to 2.0 mm, during mating for the temporary storage of spermatozoa. As to their histological change, the epithelial lining is thick and folded with closely packed columnar cells in ordinary condition, but appears thin, with cuboidal cells and without folds when they are enlarged.

d. Rudimentary oviducts (♂ Bufo bufo). The rudimentary oviducts in the adult males measure from 0.8 mm. to 1.5 mm. in the largest diameter. Individual variation is great and no definite seasonal change has been observed. The difference in the larger and smaller ones lies in the condition of the glandular layer which may be better developed.

e. Secondary sex-characters—the nuptial pads. The nuptial pads of Bufo bufo are different from those of Rana nigromaculata morphologically. They have been considered by some writers as indicators of the sex-hormone. The pads of Bufo bufo contain black pigmented papillae in certain months and occur at the mesial surface of the 1st (rudimentary) 2nd, 3rd and 4th fore-limb digits. The pigment is found deposited in the dead epidermal layer of the papillae. But the pads of Rana nigromaculata consist mainly of acinous glands having granular cytoplasm and of minute papillae instead of being deeply pigmented as in Bufo bufo and occur only at the mesial surface of the 2nd fore-limb digits. The presence of the glandular structures makes the pads vary in size conspicuously during the seasons. The seasonal variations are shown in Table X. For Bufo bufo the papillae and pigment appear gradually from September to April (Plate, fig 5). Thence they degenerate gradually and almost disappear in

TABLE X.
Seasonal changes in the nuptial pads of Bufo bufo and Rana nigromaculata

	Bufo bufo		Rana nigromaculata	
	Appearance	Histological Condition	Appearance	Histological Condition
Jan.	black and thick	papillae prominent, pointed and deeply pigmented		
Feb.			gray swollen	glands oval, full of granules, lumen small, nuclei near the proximal end and irregular in shape, papillae many
Mar.	,,	,,		
Apr.	,,	,,	,,	,,
May	deep gray, less thick	papillae less pointed and less pigmented	gray, less swollen	glands somewhat emptied, lumen larger, cell wall conspicuous, nuclei oval at center of cells, papillae still present.
June	light gray	papillae almost disappear, contain a very little pigment	less gray little swollen	glands more emptied, deformed, papillae more blunt
July	,,	,,	very light gray, little swollen	glands smaller, papillae inconspicuous
Aug.	a little darker	papillae short and blunt, more pigmented, nuclei of epidermis oval-shaped, cells seemed active	,,	,,
Sept.	more dark and thicker	papillae less blunt, more pigmented	more gray, more swollen	glands larger, containing more granules, nuclei near the proximal end, papillae more conspicuous
Oct.	black and thick	papillae prominent, pointed and deeply pigmented, nuclei less oval, cells seemed less active	gray, more swollen	glands oval, full of granules, nuclei irregular in shape, papillae present
Dec.	,,	,,	gray, swollen	glands more full of granules, lumen small

June and July (Plate, fig. 6). For Rana nigromaculata the glands increase gradually from September to their largest size in April (Plate, fig. 7) thence they become smaller and smaller after the mating (Plate, fig. 8). The papillae undergo similar changes. These changes are parallel with those of the intertubular tissue.

B. FEMALE

PRIMARY SEX-ORGANS—THE OVARIES

a. General appearance. The general appearance of the ovaries changes because of the continuous development of the ova and ovulation. Ovaries with large ova as in winter and before the spawning are black in Bufo bufo and deep gray in Rana nigromaculata. But after the ovulation they become reddish gray containing small light yellowish ova and some pigmented ones in Bufo bufo, or yellowish in Rana nigromaculata. In the growing period they are light gray in color and gradually become deeper again as the ova get mature.

b. Quantitative changes. The quantitative changes in the ovaries are summarized in Tables XI and XII together with the changes of the oviducts. They show that the ovaries

TABLE XI

Quantitative changes in the ovaries, oviducts and fatbodies of Bufo bufo

	No. of females measured	Body Length (snout to vent) Range in mm.	Average length in mm. (Snout to vent)	Paired ovaries			Paired oviducts			Average weight of paired fatbodies in gm.
				weight in gm.	volume in cc.	weight/volume	weight in gm.	volume in cc.	weight/volume	
Feb.	10	95—103	99	23.46	21.41	1.09	5.17	4.70	1.10	0.111
Apr.	*10	95—103	99	1.20	1.12	1.07	1.47	1.39	1.06	0.049
May	12	95—102	99	0.97	0.93	1.04	1.14	1.08	1.05	0.043
June	6	86—94	91	0.94	0.92	1.02	0.44	0.44	1.00	0.019
July	3	92—109	101	4.06	3.67	1.11	0.84	0.80	1.05	1.337
Aug.	8	92—100	97	9.17	8.31	1.10	1.95	1.79	1.09	3.886
	4	102—108	105	15.60	14.13	1.10	3.29	3.06	1.08	2.403
Sept.	4	93—99	95	16.27	14.65	1.11	4.27	3.73	1.14	0.408
Oct.	8	93 102	97	23.20	21.14	1.09	5.05	4.67	1.08	0.090
Dec.	10	93—102	97	22.43	20.39	1.10	5.12	4.54	1.13	0.096

* Eggs laid

TABLE XII.

Quantitative changes in the ovaries, oviducts and fatbodies of Rana nigromaculata

	No. of females measured	Range in mm.	Average Length in mm. (snout to vent)	Paired ovaries			Paired oviducts			Average weight of paired fat bodies in gm.	Remarks
				weight in gm.	volume in cc.	weight/volume	weight in gm.	volume in cc.	weight/volume		
Feb.	6	61—80	69	4.63	4.20	1.12	1.72	1.54	1.12	0.036	
Apr.	4	66—75	71	4.88	4.50	1.08	2.12	1.95	1.08	0.011	eggs not laid
	8	68—76	72	0.44	0.42	1.05	0.53	0.51	1.04	0.023	eggs laid
May	4	67—71	68	4.14	3.75	1.10	1.58	1.40	1.13	0.009	eggs not laid
	9	67—73	69	0.46	0.43	1.07	0.31	0.29	1.07	0.008	eggs laid
	20	67—82	78	1.57	1.50	1.05	1.55	1.47	1.05	0.106	eggs laid
June	11	68—72	70	0.71	0.67	1.06	0.35	0.33	1.06	0.032	
	6	63—80	75	1.25	1.20	1.04	0.77	0.72	1.07	0.189	
July	4	77—79	78	1.04	0.97	1.07	0.47	0.45	1.05	0.105	
Aug.	9	63—73	68	0.53	0.49	1.08	0.20	0.19	1.05	0.418	
	5	73—81	78	2.28	2.05	1.11	0.81	0.74	1.09	0.750	
Sept.	22	70—78	73	3.61	3.27	1.10	1.38	1.25	1.10	0.256	
	9	78—82	79	4.71	4.26	1.11	1.88	1.69	1.11	0.409	
Oct.	7	67—75	71	3.61	3.23	1.11	1.24	1.10	1.13	0.077	
Dec.	8	65—71	69	3.88	3.53	1.10	1.13	1.13	1.10	6.049	

are heaviest just before ovulation and are lightest after it. Rapid growth is found in July, August and September and after that only a slight increase is noticed until the eggs are laid. The density also shows the same tendency (Tables XI and XII), lowest after egg-laying and highest just before ovulation.

c. **Histological changes.** The histological changes in the ovaries are summarized in Table XIII. It shows that small ova can be found throughout the year, but they increase

TABLE XIII
Histological changes in the ovaries of Bufo bufo and Rana nigromaculata

	Bufo bufo	Rana nigomaculata
Feb.	Large ova* 1.35 mm. in diameter, few small pigmented degenerating ova	Large ova 1.40 mm. in diameter, few small ova
Mar.	Large ova 1.40 mm. in diameter, few small ova, many oogonia, few degenerating ova	Large ova 1.45 mm. in diameter, few oogonia, few small ova and few pigmented degenerating ova
Apr.	(Eggs laid) More pigmented degenerating ova, few small ova 0. 1 mm. in diameter, formation of the analogous corpora lutea in the ruptured egg spaces	(Eggs laid) Many black degenerating ova, presence of large egg spaces filled with "luteal cells", many small ova 0.30 mm. in diameter.
May	More pigmented ova, more growing ova 0 42 mm. in diameter, egg spaces smaller	Some large ova 0.45 mm. in diameter, many small ova 0.56 mm. in diameter, egg spaces smaller, fewer pigmented degenerating eggs, oogonia actively multiplying
June	Fewer pigmented ova, more small ova, larger ova 0.52 mm. in diameter, egg spaces almost disappear	Ova 0.70 mm. in diameter, "corpora lutea" almost disappeared, a very few pigmented eggs
July	No pigmented ova, few small ova, larger ova 0.70 mm. in diameter	
Aug.	Few small ova, large ova 0.84 mm. in diameter	Ova 0.98 mm. in diameter, few pigmented ova
Sept.	Few small ova, large ova 1.12 mm. in diameter	Ova 1.18 mm. in diameter
Oct.	Presence of few small pigmented ova, few oogonia and small ova, large ova 1.26 mm. in diameter	Ova 1.27 mm. in diameter, few small ova
Dec.	Multiplication of oogonia in groups, large ova 1.30 mm. in diameter	Ova 1.36 mm. in diameter, few small ova

* Here ova is used to include also the oocytes

in number greatly after ovulation. Multiplication of the oogonia may start in the winter. The black pigmented ova are really degenerating ones incapable of further development. Since the formation of the ova is not all at the same time, the size of all developing ova will not be the same. But owing to rapid growth of all the ova in summer, the difference becomes slight. During the winter season the smaller ova will grow to full-size, while the larger full-grown ones remain unchanged. The appearance of many black pigmented ova after the spawning indicates that the immature ova usually undergo degeneration during the breeding season. After the ova have been extruded, the follicle cells multiply rapidly nearly filling all the empty spaces occupied by the original ova (fig. I). They are analogous to the cells of the corpora lutea. They gradually degenerate so that the spaces become smaller and finally disappear.

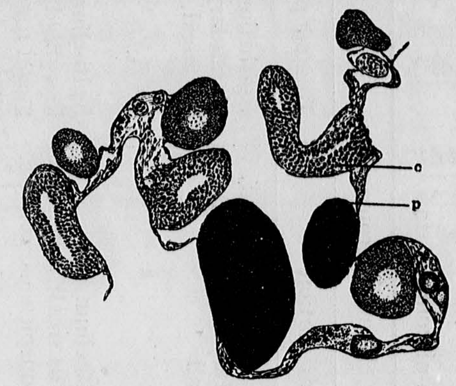

Fig. I. Section of a portion of ovary of Bufo bufo in April showing the "corpora lutea" (c) and the pigmented degenerating ova (p).

2. ACCESSORY SEX-ORGANS

a. **Fatbodies.** The changes of the fatbodies in the female are shown also in Tables XI and XII together with the changes of the ovaries and oviducts. They undergo changes similar to those in the male, lowest in weight during the spawning season, increasing rapidly in the summer months expecially in July and August, and then getting lighter again toward the winter because of the lack of food and the consumption of the stored material for the growth of the ova.

b. **Bidder's Organ (Bufo bufo).** The Bidder's organ does not undergo cyclic changes in normal individuals although normal ova may be found in this organ in some cases and develop as those in the ovaries. The discussion of these cases has already been published by Ting and Boring ('39).

c. **Oviducts.** The oviducts of frogs and toads are differentiated into two portions, the upper tubular and the lower dilated portion. The tubular portion consists of four layers: the outer peritoneal covering, outer adventitia of connective tissue and thin muscularis of circular smooth muscle cells, the glandular layer and the inner ciliated columnar epithelium containing many goblet cells. The lower dilated portion lacks the glandular layer.

The general color of the oviducts is cream-white before the spawning. But after that they become yellowish by pouring out the secretion. They gradually resume a cream-white color again as they grow larger.

The quantitative changes are shown in Tables XI and XII. The oviducts have the greatest weight just before ovulation and are lightest one or two months after. The continuous lowering after the ovulation can be explained by the fact that the glandular secretion may still be poured out after the passage of the shed ovarian tissue. Rapid growth is found in

TABLE XIV

Histological changes in the oviducts of Bufo bufo and Rana nigromaculata

	Bufo bufo		Rana nigromaculata	
	Tubular portion	Dilated portion	Tubular portion	Dilated portion
Feb.	Epithelium stretched, many goblet cells, glands large, full of granules, nuclei very small	Epithelium folded, thick, glandular, nuclei not clear	Epithelium glandular stretched, glands large, full of granules, nuclei very small	Epithelium thick glandular, nuclei flattened
Mar.	”	”		
Apr.	(Eggs laid) Epithelium much folded due to the contraction of glands, fewer goblet cells, glands have less granules, nuclei oval at base of cell	Epithelium thin, stretched, less glandular, nuclei round or oval	(Eggs laid) Epithelium less glandular, glands small, nuclei round, lumen conspicuous, cell wall clear	Epithelium thin, less glandular, nuclei round or oval
May	”	”	”	”
June	Epithelium slightly stretched, gland more granules, nuclei star-shaped	Epithelium thin, nuclei still conspicuous	Epithelium more glandular, glands slightly larger, more granules	Epithelium more glandular
July			”	”
Aug	Epithelium more stretched, glands more granules, nuclei very small at base	Epithelium folded thicker and more glandular, nuclei not conspicuous	Epithelium glandular and stretched, glands larger	Epithelium thicker, more glandular, nuclei small
Sept.	Epithelium much stretched, glands full of glanules, nuclei very small	”	”	”
Oct.	”	Epithelium thick, others same as above	Glands much larger containing many granules, nuclei small	Epithelium thick nuclei flattened
Dec.	”	”	”	”

July, August and September and later on the growth becomes slow until the spawning. The changes of the oviducts are comparable to these of the ovaries.

As to the density, it is found also in Tables XI and XII that the lowest density occurs when the oviducts are lightest. The similarity of the change of the density and the oviducts indicates that it is the change of the glandular layer which is reponsible for the change of the density, and the glandular secretion is really the heaviest material in the oviducts.

The histological changes in the oviduct are summarized in Table XIV. It is found that the oviduct is more glandular before egg-laying. But after the secretory granules are poured out as the eggs are laid, the glands of the oviduct become smaller and the epithelial layer becomes also thinner. They recover quickly during July, August and September, and nearly reach their full size before hibernation.

VII A COMPARISON OF THE PHYSICAL BEHAVIOR OF THE OVIDUCTS OF BUFO BUFO, RANA NIGROMACULATA AND RANA TEMPORARIA CHENSINENSIS.

Oviducts of toads and frogs possess great power of absorbing water because of the presence of the "colloid granules" (named by Neumann) in the mucous glands. For this reason the oviducts of Rana temporaria (the Siberian woodfrog) have been utilized by people of the northeastern provinces of China for making soup and are sold in the Chinese drug stores everywhere as nutritious medicine. Therefore the writers decided to make a study of the physical behavior in water as well as the seasonal variations of the oviducts of Bufo bufo and Rana nigromaculata and to compare them with those of Rana temporaria to see why the Chinese people have selected only Rana temporaria and not the other species of Amphibia for this puropose.

Dry Rana temporaria were bought from the Chinese drug store of Peking. It costs 10 cents for one animal. The body color is dark on the back and light brown on the belly. The average weight is about 18 gm. From the condition of the eggs in the ovaries, the frogs must have been caught just before the hibernation (about the end of September). Since the stomachs contained very little food material, we can guess that they must have been stored up for a few days before they were killed so as to empty the food material from the stomach and the intestine. The method of killing seems to be quite cruel, that is, to pierce through the orbits of the eyes and mouth cavities with a string and then let them dry, since no other injury was found in the body.

The method of determination of the absorbing power of the oviduct is as follows: After the oviducts and eggs were taken out from the abdomen, the oviducts were weighed, while the egg clumps were put in water so that they could be separated and counted. The dry condition of the oviducts was in clumps and had a translucent light brown color. But as they were soaked in water, they gradually swelled up and became of a whitish color but still remained in pieces. It took about a whole day for the oviducts to swell up completely. They were filtered in a funnel lined with wet cotton gauze whose weight had already been determined. When the water was drained off, they were weighed together. Thus, the weight of the swollen oviducts was known. The result is shown in Table XV which shows that the average weight of oviducts is about 2.88 gm. and they can take about 40 times their dried volume of water.

TABLE XV

The Absorptive power of the oviducts of Rana temporaria and the number of eggs in the ovaries

Body length in mm.	Dry weight of oviducts in gm.	Weight of oviducts after the absorption of water in gm.	Weight of water absorbed per gm. of dry material	Number of eggs in the ovaries
83	2.13	87.5	41.1	1975
85	3.50	167.1	47.7	1579
85	3.40	145.5	42.8	1993
80	1.81			1709
92	3.47	124.2	35.8	
82	2. 5			
81	2.43			
90	3.23			
91	3.77			
Mean 86	2.88		41.9	1814

For the seasonal variation of the oviducts of Bufo bufo and Rana nigromaculata, samples of the oviducts were dried each month. The absorbing power was determined by the same method as that in Rana temporaria and is shown in Table XVI. The dry oviduct of Bufo bufo in the winter months is slightly yellowish in color while that of Rana nigromaculata is more transparent. The absorbing power of both species is at the minimum after the eggs are laid. Then it gradually increases until it reaches the maximum before spawning. However, if the absorbing power of Bufo bufo is compared with that of Rana nigromaculata, the former is found to be much lower in each corresponding month. But if the latter is compared with Rana temporaria, it is approximately the same. In addition to these facts, it was found also that the water in which the oviducts of Rana nigromaculata were soaked would become slightly turbid, whereas that of Rana temporaria remained clear. What is more strange is what occurred in Bufo bufo: the surrounding water simply became gelatinous containing small pieces of dissociated tissue. Therefore it could not be filtered

TABLE XVI

A comparison of the absorptive power of water in the oviducts of Bufo bufo and Rana nigromaculata for each month

	Bufo bufo						Rana nigromaculata			
	Weight of dry sample of oviducts in gm,	Weight after the absorption of water	Weight of water absorbed by 1 gm. of dry sample	Weight when the diffused portion was poured out	Weight of water absorbed by 1 gm. of dry sample after the diffused portion was poured out	Remarks	Weight of dry sample of oviduct in gm.	Weight after the absorption of water	Weight of water absorbed by 1 gm. of dry sample	Remarks
Jan.	4.06	*164.0	40.4	35.7	8.8	*diffused				
Feb.	5.12	*190.2	37.1	50.7	9.9	*diffused	2.64	101.0	38.2	
Apr.							4.34	181.9	41.9	Eggs not laid
	2.28	14.5	6.3			eggs laid	0.48	2.03	4.5	Eggs laid
May	2.71	16.9	6.2				1.23	7.05	5.7	
June	0.13	1.03	7.9							
July	0.18	1.70	9.5				0.30	3.53	11.8	
Aug.	0.91	11.54	12.7				0.79	14.97	18.1	
Sept.							1.57	59.7	38.0	
Oct.	5.12	*169.9	33.2	51.8	10.1	*diffused	2.40	97.0	40.4	
Dec.							2.70	106.1	39.3	

completely. If this gelatinous part was washed away, a much smaller weight would be left (see Table XVI). These phenomena may be due to the difference in the chemical composition and the pH value of the "colloid granules" in these three species.

Concerning the dry weight of the oviducts of these three species, from Tables XVI and XVII it is shown that the oviducts of Rana temporaria are the heaviest, Bufo bufo the second and Rana nigromaculata the lightest. In regard to the number of eggs, Bufo bufo has the greatest number, Rana nigromaculata has less, while Rana temporaria has the least. Each egg of Rana temporaria therefore has more jelly than either of the other species.

In conclusion, so far as the physical behavior of the oviducts is concerned, neither the oviducts of Bufo bufo nor of Rana nigromaculata can take the place of Rana temporaria for soup making because of the fact that the oviducts of the former have less absorbing power and possess a peculiar behavior in water, while the oviducts of the latter have too small an amount of jelly.

TABLE XVII

The weight of the oviducts of Bufo bufo and Rana nigromaculata and the number of eggs in the ovaries

		Body length in mm.	Weight of one pair of ovaries in gm.	Number of eggs in the ovaries	Weight of one pair of oviducts in gm.	Weight of dry oviducts in gm.
Bufo bufo	Feb.	98	32.675	13,700		2.227
		105	40.873	15,500		2.538
	Mean			14,600		2.383
Rana nigro-maculata	Feb.	62	4.140	2,274		0.412
	Apr.	74	5.192	2,864	1.424	
		77	5.462	2,487	2.256	0.710
	Mean			2,542		0.561

VIII DISCUSSION

From the above observations, it is shown that the sexual organs of both Bufo bufo and Rana nigromaculata undergo a continuous series of variations throughout the year although the change is small during the hibernation season. It shows also that temperature and food are the two main factors governing the seasonal changes of the organs.

The effect of the temperature on the sexual cycle of toads and frogs can be seen clearly from the fact of the dependence of the spring appearance and mating period on the temperature. Besides, the writers have subjected toads and frogs from winter to summer to cold temperature. The reproductive organs and secondary sex-characters remained the same as those found just before mating. Histologically, the testes showed abundance of intertubular tissue and spermatozoa. For other animals, Turner ('19) has noticed that the expulsion of spermatozoa in perch is affected by the temperature of the water and Craig-Bennett ('31) has also found that the temperature may condition the spermatogenesis of the stickleback (Gasterosteus). Well ('35) after his study of the sexual cycle of the annual-breeding rodent (Citellus) suggested that temperature is probably one factor governing the sexual rhythm.

The rapid growth of the fatbodies and ova in the summer shows definitely that food is also an important factor. Champy ('26) has found that the change in amount of nutrition may disturb the sexual character, also Rugh ('35) has observed that frogs which had been kept at ordinary laboratory temperature without food would rapidly consume the fatbodies and even the eggs within the ovaries.

In regard to the endocrine character of the gonads, the controlling effect of the testis hormone on the development of the male accessory sex-organs and secondary sex-characters has generally been accepted. However, the specific source of the testis hormone has been widely discussed and opinions differ. Bouin and Ancel ('03, '04) suggested that it comes from the interstitial cells. They are supported by Steinach ('20), Lipschütz ('24), Courrier ('22, '27), Aron ('24, '26) and Benoit ('29). On the other hand, germinal or Sertoli tissue has been suggested by others, such as, Stieve ('19), Humphrey ('21), Champy ('22, '23) and Orban ('29). In spite of this controversy, in mammals and birds it has been definitely proved that the accessory sex-organs will function to a maximum degree without the presence of any cells of the germinal epithelium by Bouin and Ancel ('03), Moore ('24-'28), Domm ('27), Gray ('30), and Jeffries ('31). But this does not mean that the same tissue is responsible for the secretion of a hormone in other groups of animals.

For salientia Aron (26) has correlated the change of the nuptial pads with that of the interstitial cells in Rana esculenta. The results obtained by the writers in Bufo bufo and Rana nigromaculata show that the amount of the intertubular cells is correlated with the development of the nuptial pads; while the amount of the spermatic tissue is the greatest when the nuptial pads are the least developed.

Champy ('13) failed to correlate the relationship between the development of the interstitial cells and the development of the secondary sex-characters in anurans. Since Champy's work came before the introduction of the quantitative method for such problems by Bascom in 1925, his data for the different months must have been merely estimated. The inaccuracy of this method may be one of the factors leading to the different results. But from the results shown in this paper there is a distinct possibility that the source of the testis hormone lies in the intertubular tissue, although the writers are not ready to commit themselves to this definite statement until more data are collected by the use of the quantitative method in the study of other Amphibia.

IX. SUMMARY

There is a seasonal cycle of changes in the sexual organs of Bufo bufo and Rana nigromaculata. They can be summarized as follows :—

1. Primary sex-organs—the testes and the ovaries. The testes change in color, size, weight and histological structure. The color changes are from deep yellow in winter to light yellow in summer. The size and weight are smallest after mating and largest during the late summer. The spermatogenesis is most active during the summer and early autumn. Abundance of spermatozoa is already found in winter preparatory to mating The amount of the intertubular tissue is smallest in late summer when the spermatic tissue is greatest, and greatest during the mating period, when the secondary sex-characters are well-developed. The spermatic tissue is smallest just after the mating because the reproductive cells have been discharged.

The ovaries are dark-colored and largest just before ovulation, but become lighter in color and smallest when the eggs are laid. The formation of the ova is the most active after the spawning and rapid growth is found in summer and early autumn.

2. Accessory sex-organs—fatbodies, oviducts, ureters, Bidder's organ and rudimentary oviducts. The fatbodies of both males and females are lightest in weight during the mating season, but increase rapidly during the summer when food is abundant. They decrease rapidly again during the autumn, as the reproductive cells are being actively formed.

The oviducts are heaviest just before ovulation. After the spawning they naturally become lightest and rapid growth follows in summer and early autumn.

The ureters change only in male Rana nigromaculata. The region behind the kidneys is enlarged during the mating period for the temporary storage of the spermatozoa.

No seasonal changes are found in Bidder's organ or the rudimentary oviducts of Bufo bufo.

3. Secondary sex-characters—the nuptial pads. The nuptial pads begin to develop in the autumn and attain full development just before the mating period. In summer they are much degenerated. The changes in the nuptial pads are parallel to the changes in the amount of the intertubular tissue.

The main difference in the sexual cycle of Bufo bufo and Rana nigromaculata lies in the fact that the changes in the latter are always a little later.

4. From the seasonal point of view, in the spring the sexual activities are the most active when mating and ovulation occur. The sexual organs are the most developed before the sexual products are discharged. But after that all the organs collapse. In the summer and early antumn rapid growth follows. The gonads become active in manufacturing new

germ-cells and the accessory sex-organs make rapid reparation and growth, while the secondary sex-characters degenerate. But in the late autumn and winter, growth of the gonads and the accessory sex-organs is retarded, whereas the secondary sex-characters make their appearance again.

5. Other observations connected with this problem are included, such as, the body size of the mature individuals, the age of attaining maturity, the physical behavior of the oviducts, and the main factors affecting the sexual cycle.

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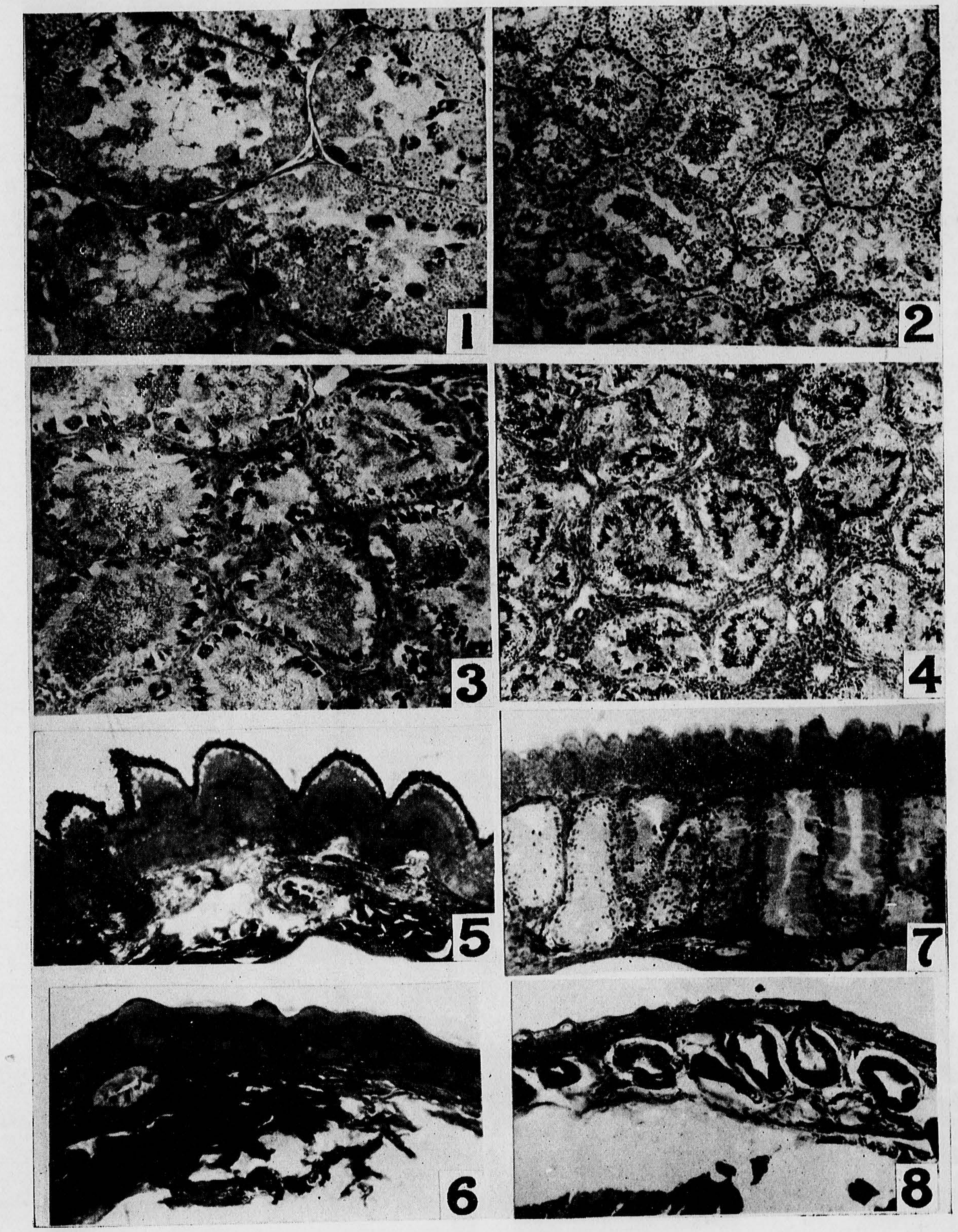
EXPLANATION OF PLATE

(Photomicrographs, 80X)

Fig. 1. Cross section of the testis of Bufo bufo in August.
Fig. 2. Cross section of the testis of Rana nigromaculata in August.
Fig. 3. Cross section of the testis of Bufo bufo in March.
Fig. 4. Cross section of the testis of Rana nigromaculata in April.
Fig. 5. Cross section of the nuptial pad of Bufo bufo in January.
Fig. 6. Cross section of the nuptial pad of Bufo bufo in June.
Fig. 7. Cross section of the nuptial pad of Rana nigromaculata in April.
Fig. 8. Cross section of the nuptial pad of Rana nigromaculata in August.



Ting and Boring, Reproductive Cycle in Chinese Toad and Pondfrog.

see mimeograph material file for extra copies (distributed Apr. '46)

FIVE AND A HALF MONTHS

IN A JAPANESE PRISON

A Personal Narrative

by

Stephen Tsai

Comptroller of Yenching University, Peiping, China.

Foreword

This is a moving story. It is regrettable that it cannot be made available to a wider public than will be reached in a mimeographed form. One reason for the delay in its appearance has been the effort, unsuccessful, to find for it a more satisfactory medium of publication.

Those who have a chance to read it will again be impressed by what has been witnessed to so many times in recent years: the powers of endurance which the human body can exhibit and the victory of mind and spirit over physical pain and mental anguish. Stephen Tsai emerged from his ordeal a bigger and a better man than he was before - and all who have known him as a boy at Yali, in Changsha, as a student at Yale, or as a member of the Yenching faculty know that such a statement means a great deal.

Mr. Tsai's story in this form was abbreviated by Miss Grace Boynton of the Yenching faculty from a longer narrative written especially for an American college classmate, Capt. George S. Franklin.

FIVE AND A HALF MONTHS

IN A JAPANESE PRISON

By Stephen Tsai, Comptroller of Yenching University

In May, 1941, Mr. Tsai was in America upon University business. His return to North China was planned for a sailing upon the SS President Pierce, but shortly before it was due to leave he was notified that the ship had been cancelled and that if he returned to Asia he must travel on a Japanese liner.

"The idea of sailing on a Japanese ship had never come into my mind before and it was anything but pleasant. But the pull of home was very strong... with the international situation in the Pacific getting more critical every day. Inasmuch as I was going to Japanese occupied territory to be at the mercy of the invaders in any case, why then any hesitation about taking a Japanese boat?... After consultation with friends and the New York office, I decided to go, but I lay awake most of the night before I reached San Francisco, feeling no small degree of uneasiness and misgiving."

A Premonition of Trouble

The journey passed without much difficulty until the arrival at the Chien Men Station in Peking.

"As the train slowly pulled into the station, I looked out of my window to see if anyone was there to meet me, and when I saw no one I knew, I sensed that all was not well. ...I waited until the train was almost empty before I called for a porter and began handing him my bags. Then I suddenly saw Dr. Stuart hurrying toward me. I was so delighted that I dropped my things and waved frantically as I rushed forward to meet him. He was looking stern and disconcerted, which was in great contrast to his usual cheerful confidence, and he told me to get out of the station as quickly as possible. He also told me what to say in case I was questioned in any way. Without further words I went back to the train where I had left the porter. There a group of Japanese, three men and two women stopped me, and one of them spoke to me very excitedly. I could not understand what it was all about, and did not know what to do. Fortunately at this juncture, Hsiao, Dr. Stuart's Japanese interpreter came to my aid. He talked to the Japanese and explained to me that his grievance was that I had made insulting remarks about one of the women who, he said, was his sister, and had been on my train. I was accused of having said that she was pregnant. As she was a virgin this was not to be borne. Hsiao asked in what language I had made this remark. The man said in Japanese. Hsiao made good use of this absurdity because I do not know any Japanese. Then he advised me to make an apology in order to get out of the mess, and I bowed to the group in good Japanese style.

"Then we started off, but we were stopped by a Japanese soldier who was patrolling the station. My accuser evidently had appealed to him. A heated discussion developed between Hsiao and my enemy, which lasted more than ten minutes during which the woman wept from mortification, and her 'brother' slapped me in revenge. I took the punishment meekly without attempting to defend myself, realizing the odds were against me. But I found out how difficult it was to be a coward! The soldier evidently considered this enough punishment for me; Hsiao told me to make another bow, which I did, and we then hurried off at top speed to join Dr. Stuart who had been watching the whole humiliating incident with indignation, but who had judged it wisest not to interfere.... I learned later that the party of Japanese boarded the train at Shanhaikuan with the express purpose of getting me into trouble... Why all the bother of a frame-up? Possibly because of the University, and of the fact that I was just returning from an extensive tour in America where I had wide connections. The whole episode illustrates one type of Japanese stupidity."

Old Duties under New Difficulties

Mr. Tsai was no sooner at home on the University campus, than the local Japanese gendarmerie began annoying and alarming visits to his house which they would enter without warning and upon the most trivial pretexts. It seemed best not to leave the campus, and for two months Mr. Tsai remained within the Yenching walls. It seemed that his Japanese visitors were most interested to find out the individual attitudes of the American staff toward American-Japanese relations. They were told just a few days before December 8, 1941, that the Americans at the University were mostly missionaries who deprecated war, and who devoted their time and energy to religion and education. Politics were not of first interest with them. They would carry on with their work until they were forcibly stopped.

The story goes on to narrate the events on the morning of December 8 when the Japanese entered the university, ordered the staff to their homes and the students to their dormitories. Subsequently, the students were dismissed from the campus, and seven members of faculty and nine students who had been locked up in Bashford Hall were taken away to the city in one of the University buses under guard. That was on December 9th.

"As controller of the University, the physical plant was in my charge. I knew it better than anyone else and I could, therefore, be very useful to the new occupants. From the morning of December 8 until I was arrested on December 27, I was busy from early morning to late at night.... I became so physically exhausted that my memory suffered, and I insisted to my wife that it was only Thursday when it was actually Saturday. Knowing the unreasonableness of the Japanese I was very careful in all my dealings, but even so, I got my share of the slapping which was so common that nobody paid much attention to it........

"From the beginning I assumed that it would be only a matter of time before I would join my colleagues in the Peking gendarme prison. I knew I was being kept on the campus because I was useful there. But every day I wore extra warm garments to be ready for the prison cold. On December 27, I was ordered to report to headquarters and was kept waiting there while my house was searched. The party returned from the search with folders of personal correspondence and an armful of Life magazines.... Other members of the faculty were brought into the room where I was waiting. At five o'clock we were taken into the city.

One Kind of "Lebensraum".

"We left the campus in one of the University buses and a large crowd saw us off, among them my wife. I tried to look calm and self-composed, but my effort did not seem to produce any comfort for her. We arrived at the Peking University where the gendarme headquarters were, at about 5.30, and were immediately herded into a room in the basement where we were identified, finger-printed and very carefully searched. We had to give up everything we had with us, even eyeglasses and belts. Some suffered from poor eyesight and begged to retain their glasses but they were refused. After these formalities we were taken to the other part of the basement where the prison was located. We stood in line in the corridor while cells were being assigned. Through one door, I saw two Yenching students and nodded to them. The guard at once ordered me to face the wall.

"I was placed in lucky No. 13. I had to take off my shoes and leave them outside the door before entering the cell. In No. 13, I found a Yenching student, Yao Ke-ying. There were five other inmates in addition. It was supper time and I was given two pieces of steamed bread. Although I had had nothing to eat since early morning, I was not hungry and so I gave my ration to Yao who was famished and glad to get a little extra. He was eager to hear all the news, as he had had no contact with the outside since the 8th. We had to be very alert in listening for the guard since we were not supposed to talk in the cells.

"I suppose a description of our accommodation would be of interest. The cell had a floor space of about 8 feet by 10, with a ceiling 11 feet high. The floor as well as the walls and ceiling were of thick pine planking. The fourth wall facing the corridor was a grille of heavy square pine posts not more than three inches apart. They extended all the way from the floor to the ceiling with a cross beam half way up. The door to the cell was not more than three feet high; one had to crawl to get in or out.

"There was no furniture at all except an enamel spittoon (used as a water pitcher) marked 'Peking University', and a wooden tub with a tin sheet which was for use as a latrine. The bedding varied from one cotton blanket to as many as eight when the other prisoners were released or transferred to other places. Every day at about four o'clock two inmates from each cell were let out to empty the latrine and refill the spittoon. Before the door was opened, a very strong chain which made a clinking sound was placed in front of each cell, and it had a padlock at each end. The two carriers passed the chain around their waists very tightly and clasped the locks. Then they lined up in the corridor with their tubs and were checked over by the guards before the order was given to march. No chances with escapes were taken; the guards carried pistols with fingers on triggers while the procession was outside the building.

"Night soil dumping may not be regarded as a pleasant occupation by people in normal life, but was a coveted opportunity for us. You see, going outside the building not only gave us a chance to limber up after sitting on the floor all day long, but we also got a change of air, even if it could not be called fresh! What was more, it gave us a chance to see friendly sympathetic faces, and we might succeed in getting a whispered word with one of our group. Of course we had to be very wary, for the guards were vigilant. I was very slow and I got caught once and was slapped in the face.

A Modern Use of Old Techniques.

"With our arrival, there were fourteen members of the Yenching faculty in the prison. On the day after we arrived I had my first examination. I had three in all. My examiner was named Onena and he had a rather agreeable face. He could not have been more than thirty, and I think he may have had a high school education. The interpreter was named Sun and said he was a returned student. He was a tall fine-looking young man who might have been taken for one of our Yenching students.

"The business began with having me submit a written statement about my family, education, and past occupations. After that, Onena lectured at length on Sino-Japanese relations. He alternated flattery with threats in his demands that I should respond truthfully to the questions he was about to ask me. The day ended with no important points taken up.

"The next morning right after breakfast, I was called again. My examiner and the interpreter were very businesslike. Several piles of paper were on the table with well-sharpened pencils, and my examiner took from his briefcase a document in duplicate which apparently contained the questions which I was to be asked.

"The first subject was the policy of Yenching University. I said that Yenching was a Christian Institution founded originally by missionaries and that its policy is best described by its motto 'Freedom through Truth, for Service'. Before I could finish the explanation of the motto, the examiner lost his patience. He demanded the real policy which was advocated and practised and not what we pretended on the surface. When I reiterated that Yenching had no other purpose than the imparting of the teachings of Jesus, side by side with instruction in modern science and arts, he became very angry and accused me of hypocrisy. He was convinced that the United States government subsidized Yenching for political reasons. This I emphatically denied.

Worse and More of It.

"The examiner became furious. He sprang to his feet and banged with both fists on the table and called me names. Before I could take in the situation he came over to me, took me by the throat and pulled me out of my seat. He struck me repeatedly with his fists and over my face and body until I lost my balance and fell to the floor. Then he seized me, dragged me up, pinned me to the wall with one hand while he hit me with the other, using my head as if it were a punch ball. Not content with this he took a rope out of a drawer, threatened to tie me up. At this point the interpreter interceded for me, and pleaded for patience on the part of the examiner and for sincerity from me. (I have no doubt that all this had been arranged between them beforehand.) Then we sat down and started again on the subject of Yenching University policy. I repeated what I had already said and fury broke out afresh. The examiner threatened to report me to his superiors and have no more to do with the case himself. I was ordered back to my cell.

"During this pugilistic performance I had remained calm and cool. I did not ask for mercy nor did I flinch, nor had I any desire to attempt to defend myself. I just took it nonchallantly. I felt as if it was not I who was going through the experience; and I realized that my assailant too was not himself but was actually possessed by evil. I therefore felt no desire for revenge against him personally. I had been praying extra hard for physical strength, and the Lord must have heard me and thus fortified me to go through the ordeal without suffering and anguish.

"I returned to my cell, with my face swollen and my eyes blackened and my body generally battered, and was received with sympathy by my cell mates. I began to feel the pain and discomfort of my condition, but spiritually I was undisturbed.

Gentler Methods but the Same Purpose

"The next morning, I was called up again. My examiner was now solicitous about my hurts and called a medical attendant to see to them. Perhaps he was ashamed of himself when he saw the state I was in, but more probably he was acting in accordance with the Japanese custom of alternating kindness with cruelty.

"We started over again from the beginning and I made the same statement for the third time. He did not stage an outburst as he had before, but wrote down what I said. From then on the examination was carried on in a fairly decent manner. It was clear that the Japanese believed that the university was not so much an institution of liberal education, as it was a propaganda unit intended to foster pro-American sentiment, and to inculcate dangerous democratic ideology which is in direct opposition to 'the kingly way' of the Orient. My examiner urged me to understand the dangers of democracy and liberalism. It was a startling revelation to me that the institutions and ideals which we cherish are as vicious and abominable in their eyes as theirs are in ours. We human beings see in the daylight and lose our power of vision at night; but the owl can see best in the dark. The Japanese are owls, politically and socially. Their white is our black, and vice versa."

Mr. Tsai was examined upon many matters relating to the University, especially its finances and his disbursement of funds to students leaving for Free China. He was also interrogated about the activities of Dr. Stuart, and attempts were made to get him to incriminate his colleagues who were imprisoned with him, on the plea that they were betraying him. He was also required to write a statement of his political beliefs. The examiner announced his conclusion that Mr. Tsai had been so "poisoned by American liberalism from an early age" that it was no wonder he was anti-Japanese and pro-American. Finally they questioned him about the activities of organizations and individuals outside the University. In these matters they had very curiously twisted notions. They asked about the "Chinese-American Co-operatives", by which they probably meant the China-Industrial Co-operatives. Such distortions of facts did not reflect much credit upon the Japanese Intelligence organization.

Old Friends in Strange Surroundings

Mr. Tsai's case was completed by the end of December, and the report was a document 30 pages long which was signed by examiner and interpreter, and was fingerprinted by Mr. Tsai. He found that examinations of his colleagues were proceeding in much the same way. The students involved were kept to the last for attention, but their cases were quickly disposed of and they were freed on January 20th. The torture of other cell mates who were brought in on miscellaneous charges is described and them Mr. Tsai goes on:

"Fairness compels me to state that we Yenching prisoners received special treatment. In the first place we were not tortured, and secondly we were allowed to receive packages from our families twice a week. At first only tid-bits were sent in, but as time went on, our wives got good sized packages to us. Once I got a bottle of milk, another time tomato juice. I divided my food into daily portions to add to what the prison provided and sometimes I shared with fellow prisoners who had been tortured or who were sick.

"We had a weekly bath on Saturday. There was only one tub of water. Everyone was supposed to wash in that, and there were seventy or eighty of us. By the time the last few tens got their chance, the water was rather dense. The Japanese prisoners were given first chance, in the true spirit of 'cooperation and co-prosperity'. I am used to cold water baths, so I never went into that coagulating mess."

January passed without a verdict. On February 7, Mr. Tsai was given pencil and paper to write a composition on "My present frame of mind". The Japanese custom is to get a prisoner to write a confession and statement of penitence before his release. But the Yenching group did not oblige. Mr. Tsai merely said his thoughts were occupied with the hope of rejoining his family by Chinese New Year. The result of this was that the whole group was removed (in handcuffs) to a military prison. This took place on February 10th.

The Companionship of Misery.

"The reception at the prison was frigid in more ways than one. The temperature on the day we arrived was far below freezing, and the prison cells were well ventilated and unheated. We were obliged to strip naked and wait for our clothes and persons to be minutely searched, and the exposure to the intense cold was a serious matter for the more elderly among us. ... Anything not regarded as clothing was taken away from us, including garters, belts and handkerchiefs. I could do without a belt but the dealing with a fluent nose minus a handkerchief caused me no end of misery. Fortunately, I had two pairs of socks and was able to exchange one pair for a handkerchief. ...

"We were all put together in one room with a wooden floor. It was arctic cold. We were told to sit down on reed mats with our legs crossed and our faces to the wall. This was impossible for the elderly ones and they were finally allowed to lean against the wall after pleading sickness. The food consisted of two pieces of wo wo t'ou (ordinarily a course unleavened sort of corn bread) made of a nondescript mixture of black and white material with a good deal of sand, and a bowl of salt water for each person served twice a day. The only way to eat the wo wo t'ou was to swallow it bit by bit without chewing. The moment I tried to masticate, my teeth hurt.

"But in spite of all our hardships we were happier than before, because we were together. In the intense cold at night we huddled together to keep warm. Some of us had been only ordinary acquaintances, but this suffering gave us a new bond and developed an intimacy which could not have happened under any other circumstances. We talked to one another, furtively, of course, and exchanged notes on our experiences thus far. One of us would watch for the guard while the others talked. We had our common worship together. Among the group there were one Roman Catholic, eight professed Protestants, and two free thinkers. We prayed for strength to endure, for God's mercy on us, and for our early deliverance".

But instead of deliverance the group was separated and each one suffered solitary confinement.

"The term 'solitary confinement' does not convey the complete idea of our situation. Beside being alone and sitting in a prescribed position, we were not allowed to stand up or to walk about the cell to limber up the cramps we endured; we just had to sit there facing the wall from 6 A.M. to 9 P.M. - fifteen hours a day. The hours seemed to increase in length as the day progressed because we got weaker with each hour of sitting. The pain in my back became excruciating; I tried to relax my muscles by placing a rolled blanket under me on the back of my legs. But it gave little relief. When I was sure the guard was not watching I would get up and stretch my limbs vigorously. This sitting down business may not sound serious to you, but I would rather endure some other more dreaded forms of torture. Torture is more painful at the time, but it cannot go on ceaselessly day after day. The sitting was prolonged agony and lasted for weeks. At first I hoped I might get used to it, but apparently muscles and bones are not adaptable after one has passed forty.

<u>Light and Healing in the Darkness.</u>

"In order to keep track of the days and dates I copied Robinson Crusoe on his island. But I had neither knife to cut with, nor wood upon which to make notches. I used a button off my coat to make marks on the cement wall, and so I managed to keep everything straight until I was taken ill in March.......

"In spite of the hardship and loneliness of solitary confinement, it was by far the most valuable time I had in my six months of imprisonment. Now I have had it, I would not like to have missed it for anything. Never in my life had I had so much leisure with so little distraction for retrospection and meditation. As I sat in that little cell, my mind roved far and near - from childhood reminiscences to day dreams of the future. I saw my whole past as clearly as if it were being flashed on a screen in front of me. I became completely detached from actual scenes, and found myself in the position of a spectator. I saw, as

I never had before, the wonderful opportunities I had missed during my formative period. For instance, I would like to have the chance of going to school and college again. As an adult, and a person serving in society, how I wished I could begin all over again; how differently I would speak, act and treat people. I saw how miserably I had failed as a son, husband and father, as brother and friend. How much better I could have served the University in my capacity if I had had more consideration for the views of others, more sympathy for their feelings, more understanding of their wishes. This was not abstract theorizing; I recollected actual instance after instance, and saw how differently I might have dealt with these particular problems. I felt so terribly penitent and remorseful that I was at a loss to know what to do. What could I do, but beg the Lord for forgiveness and redemption?

His response was almost instantaneous, and to me dramatic. All of a sudden I felt that the burden was lifted off me and that I was forgiven. I felt sure that the Lord would grant me the opportunity to redeem myself. The habit of praying was not new to me, especially since the beginning of the present experience, but I never had found its real power of invigoration and of life-giving until then. Thereafter, I no longer felt alone in the cell, nor was I afraid of any harm which might come to me. I was in constant communion with the Lord who was all the time making his protecting arms go around me. I then asked Him never to let me drift away from Him for the rest of my life, and if I should become oblivious to make me go back and think of my days in solitary confinement."

<u>The Climax of the Ordeal.</u>

After two weeks of solitary confinement, Mr. Tsai was taken into the presence of "an elderly general" who treated him civilly and gave him the opportunity to read and correct the report of his examination. He was asked upon this occasion if he realized that in supplying students with funds to escape to Free China he had been guilty of treason. He replied that it was his business to handle University money, and he could not see any difference in this particular item from all the rest of his duty.

"The session lasted over two hours. I had been feeling rather low for several days. When I returned to my cell, I realized that I was sick. I asked the guard to send the nurse who came promptly. He took my temperature. It registered 39.5 centigrade. He ordered me to bed and I was only too thankful to obey. The sitting up had become unbearable.

"That very night I was taken ill. T...., another prisoner, was put into my cell. He was a godsend to me, and I might not have pulled through that severe attack of typhus if I had not had him to look after me. He was a foreman in a coal mine, and had been arrested as a communist leader. He had only a primary education, but his philosophy of life and outlook on things in general were on such a high level that it was uplifting to talk with him. He said he was prepared to stay in prison "for the duration", and was not sorry for it, because he felt sure that he would be a free man when he did get out. I am grateful to him not only for the care he gave me during my illness but also for his elevating spirit.

"For about two weeks I kept up a fever of 40.5 and was delirious most of the time. But I was not completely out of my mind. I remember how, one time, one of my feet fell into the latrine and had to be taken out, because I was too weak to do it myself; also how T.... helped me to beg water from the guard. We were only given two cups of water a day to drink and, with my high fever, I wanted to drink like a camel. I think thirst was my greatest affliction. Most of the guards paid no attention to our entreaties. In contrast to them, the two nurses, especially the younger one, were unforgetably good and humane. The latter gave me water whenever I asked for it, and moreover he gave me two ice-packs a day - one in the morning and one at night - to rest my head while I had the fever. He also gave me several injections and took ny blood for a test. Never once did he forget the ice packs, which saved my life I do believe.......

"By the end of the month my fever was down and although extremely weak I was enough recovered to want a cleaning up. I had not washed my face for a whole month. When the opportunity for a bath came, I staggered out of my cell even though I could hardly stand up and tried to join the procession to the bath house. But I was so drowzy and confused that I followed the wrong group, the one just coming back from the bath, and went into the cell with them. The guard discovered me, and slapped me, and somehow I managed to realize my mistake. I turned back, and caught up with the right group composed of my Yenching colleagues. This was the first time I had seen any of them since we had been segregated six weeks before. Their hair and their beards had grown so that they were unrecognizable. My appearance must have been weird in the extreme - all pallid and emaciated and hairy as I was. I must have lost consciousness in the bath, for the next thing I can remember is finding myself prostrate in my cell perspiring all over......

"While I was ill with a high fever my daily worry was to get enough to drink. Now I was convalescent, hunger became my problem. The two bowls of millet a day were not enough to satisfy nature and to rebuild my emaciated body. I begged for more food with no success. The situation was made worse by the spacing of the meals; one was se ved at eleven, the next at two; I could not keep anything to eat at other times because our cells were searched and all food not eaten had to be given back. We also had a bowl of soup with the millet - soup made of the scraps left by the Japanese prisoners who had good food - rice, noodles and vegetables. I am ashamed of having been so ravenous, but that was how I felt."

Release and Restoration.

While still very weak, Mr. Tsai had another hearing of his case. He asked for better food without success; he also asked for a Bible and the official said he "would think about it."

For a long time after his transference to the military prison, Mr. Tsai received nothing from his family and knew his wife was unable to discover where he was. Later she was able to send him fresh clothes but no food. He found that Britishers were also imprisoned, and comments on their treatment. At last he learned that some Yenching colleagues had been released. This was in May, and he impatiently awaited his turn. The formalities of release were long drawn out, and involved several occasions when it seemed likely that a prison sentence would be imposed in place of the restoration of liberty. But on June 18th the handcuffs were removed and Mr. Tsai signed a paper agreeing not to attempt to leave Peking, not to engage in any subversive activity, not to change his address or travel without permission. He asked to see the nurse who had saved his life and expressed his profound gratitude. And then he met his wife outside the prison and went home. Mr. Tsai adds a postscript of especial interest to Yenching colleagues, here in America, for it describes the conditions under which he lived until the end of the war:

"Our new home was a delapidated old house with a brick floor, paper windows and without plumbing. My wife was very apologetic about it. She could not find anything better at a rental which we could afford to pay. She did not realize that however shabby it might seem to her, it was like a royal palace to me. As to food, she tried to serve the best she could; here too she found my taste was very plebeian. I ate anything and everything set before me; her problem was to keep me from overeating. I had six meals a day for the first three weeks of freedom. My normal weight is 126 pounds, and the first day of my liberation I weighed 105. I gained ten pounds during the first week, and 12 more the second.

At the end of the third, I tipped the scales at 140. My feet were swollen and my stomach protruded. Gradually my appetite became less ravenous and I returned to my normal weight......

The years from 1942 to 1945 when Japan capitulated were a period when we struggled for existence. We had no new clothes, and our principal expenditure was for food. By eating course materials, and sparingly of those, we pulled through somehow. The expedient which helped us the most was selling our things to the junk man. Hundreds of these men passed our door every day beating little drums as they went, and many a day I stood waiting for one in order to get some cash with which to go to market.

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