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Sex Differentiation of the Larval Newt Composed of Heterosexual Halves

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It is generally accepted that the genetic constitutions relating to sex is already established in fertilized eggs of various forms of animals.

On the problem of sex differentiation of gonads in later stages of development, many investigations have been performed in the fields of embryology and endocrinology. However, the study of the mechanism how the genetic difference related to sex is realized during the ontogeny is still opened to question.

As a possible clue for solving the question, it is worth while studying the stability of genetic sex during the early embryonic development. If so, it may surely be a way to settle the problem to trace the sexuality which will appear in the later development of embryos whose lateral halves are heterosexually constructed.

In the present study, the embryos with heterosexual halves were experimentally produced, and their later development was investigated, particularly regarding the sex development of the gonad.

Material and method

The early gastrulae of *Triturus pyrrhogaster* (Okada and Ichikawa's stage 12b) were employed. The method of operation is schematically illustrated in Textfig. 1. Two early gastrulae of almost the same size were picked out at random from a stock of materials. Both of them were bilaterally separated through the centre of blastpore using a hair needle, and the four hemispheres thus obtained were exchanged each other and set together again; so that the two early gastrulae with heterogenous halves were newly produced; one is designated *a* and another is designated *b* in the present paper.

The embryos of exactly the same stage of development were cut off in the similar way and their halves were recombined immediately after the operation without exchange and these embryos were reared as the control.

The experimental animals and the controls were reared up to the completion of metamorphosis and then they were fixed in Boin's fluid, and their urogenital organs were excised and offered to histological observations. The sections were cut in 10 μ thick and serially observed.

Results

The experimental took about 145 days on the average to finish metamophosis, and 32 out of 162 experimental animals were successfully collected as shown in Table 1.

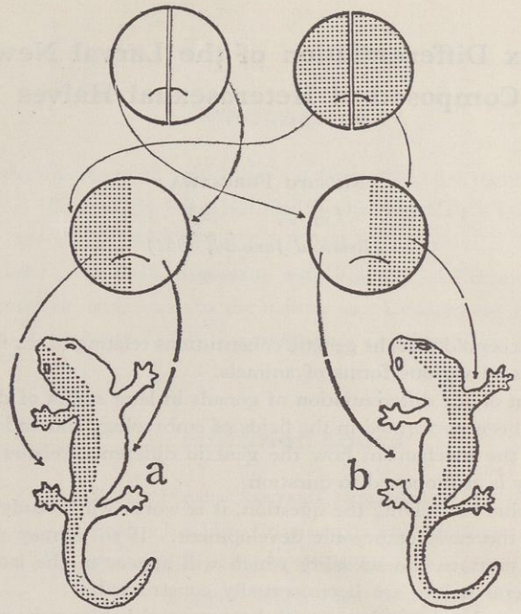

Fig. 1. Operation scheme.

Table 1. Number of experimentals.

Total no. of exp.	Lost	Preserved
162 (81 pairs)	130	32: 12 not in pairs, 5 in pairs (♂ 5, ♀ 4, female-type 23)

Abbreviations: ♂; specimen having normal embryonic testis, ♀; specimen having normal embryonic ovary, female-type; specimen having modified gonad of ovarian type.

No appreciable external differences between intact animals and the experimental were observed (Plate 1, Fig. 1 and 2). Generally speaking, the structure of all organs and tissues other than gonads and gonoducts in the experimental were not significantly different from those of the control.

Developmental patterns of the gonads of the cases which both of the experimental pairs were survived and preserved were shown in Table 2. The number of the paired specimen is 5 in total as seen in Table 2. The left gonad of specimen *a* and the right gonad of specimen *b* were derived from the very same egg, i.e., they are genetically equivalent, and so with the right gonad of *a* and the left gonad of *b*. As one can expect, these relationships can be evidenced histologically in the gonad of four paired specimens; histogenesis of the genetically equivalent gonads of the pair are closely resemble, as seen representatively between the gonads of specimen 82-a and 82-b in Fig 2, B.

Table 2. Sex differentiation of gonads in experimentals preserved in pairs.

Specimen	Left gonad		Right gonad	
	Diff	Mt	Diff	Mt
74-a	Ts	+	*Ts*	+
74-b	Ov	+++	Ov	++
75-a	*Ov*	−	*Ov*	−
75-b	*Ov*	−	*Ov?*	++
77-a	*Ov*	+	*Ov?*	−
77-b	*Vs*	+++	*Ov*	+
82-a	*Ov?*	+++	*Ov*	++
82-b	*Ov*	+	*Ov?*	++
83-a	Ov	−	*Ov?*	++
83-b	*Ov?*	++	*Ov*	−

Abbreviations: Diff; sex differentiation, Mt; mesonephric tubule-like structure differentiated in the gonad, Ov; normal embryonic ovary, *Ov*; ovarian-like gonad with oocytes, *Ov?*; ovarian-like gonad without oocytes, Ts; normal embryonic testis, *Ts*; affected testis, *Vs*; vestigial gonad, +, ++, +++; grade of the formation of mesonephric tubule-like structures, −; complete absence.

It is a noteworthy fact that all the gonads of specimens 75, 77, 82 and 83 are to be placed under the category of ovarian differentiation after the histologic observation, with the exception of the vestigial left gonad of the specimen 77-b which contains comparatively small number of germ cells (Plate 1, Fig. 7). Between specimens 74-a and 74-b a quite different condition is observable, namely, in 74-a the testicular differentiation occurs in each gonad, whereas both gonads of the corresponding specimen 74-b are apparently differentiating into ovaries (Textfig. 2, C and Plate 1, Fig. 9, 10).

Table 3. Sex differentiation of gonads in experimentals.

Specimen	Left gonad		Right gonad		Specimen	Left gonad		Right gonad	
	Diff	Mt	Diff	Mt		Diff	Mt	Diff	Mt
19-b	Ts	−	Ts	−	58-a	Ov	−	*Ov*	−
36-b	Ts	−	Ts	−	80-a	*Ov*	+	Ov	−
53-a	Ts	−	Ts	−	16-a	*Ov*	−	*Ov*	−
62-a	Ts	++	Ts	++	29-a	Ov	−	*Ov*	−
64-a	Ts	−	Ts	+++	10-a	*Ov*	−	Ov	−
					57-b	*Ov?*	−	Ov	−
5-b	Ov	−	Ov	−	9-b	*Ov?*	−	*Ov*	−
37-b	Ov	−	Ov	−	63-a	*Ov?*	−	*Ov*	−
52-a	Ov	−	Ov	−	51-a	*Ov?*	−	*Ov?*	−
59-b	Ov	−	Ov	−	66-b	*Ov?*	−	*Ov?*	++
					55-b	*Vs*	−	*Ov*	−
30-a	Ov	−	*Ov*	−	68-b	*Vs*	−	*Ov*	−

For abbreviations, see Table 2.

The other 22 specimens lost the pairing ones in their later development. The results observed in these cases are summarized in Table 3. In short, the individual which reveals the ovarian differentiation is as a whole considerably preponderant in number compared with the one which develops the testicular differentiation.

Another noteworthy fact about the result obtained would be that the ovarial and the testicular gonads never co-exist in one individual at the time of metamorphosis. No cases were found in which the sexuality of developed gonads differs according to the side.

The gonads of the control which completed metamorphosis developed normally and their sex distribution shows basic 1:1 ratio.

It should be added here that a certain anormal histogenic development is observed among the experimental, i.e. the gonad with mesonephric tubule-like structure in its hilum region can be found out in 19 out of 32 specimens (Table 2, 3 and Plate 1, Fig. 6). The site occupied by such abnormally developed tissues corresponds to the region of ordinary ovarian cavity or the gonadal rete. This fact seems to deserve a special attention.

Discussion

The majority of the experimental which were placed under the category of female differentiation in the present experiment carries modified gonads of ovarian type (Textfig. 2, B and Plate 1, Fig. 3; *cf*. Fig. 5). These ovarian type gonads, however, are not always the same in their fundamental structure depending on the side, though they are quite ovarial in nature. The representative example can be seen in specimen 83-b, namely, in this specimen, the right gonad is completely ovarial with developed oocytes and the left side gonad is of the primordial one having a little ovarial cavity developed in central region (Textfig. 2, A). Moreover, there are cases in which the right gonad is definitely differentiating into an ovary, whereas the left one remains vestigial, as seen in specimens 77-b, 55-b and 68-b (*cf*. Plate 1, Fig. 7, 8).

Considering on the aforesaid facts in contrast to the ordinary ovarial development in which the gonads of both sides develop in same degree, the author is inclined to have the opinion that the almost all above-stated specimens are derived from the gastrulae composed of heterosexual halves.

After this opinion, it may be considered that although the embryos possess the prospective fate provided for certain sex differentiation at the early stage of gastrula, they are not yet completely determined and still have competence in regard to sex to be modified by the influence exerted from the embryos of the different genetic constitution, if they are happened to be combined with the latter ones.

Humphrey (1933) demonstrated clearly using the tail-bud stage embryo of an anuran species, *Rana sylvatica*, that the intermediate mesoderm when orthotopically transplanted into another embryo of opposite sex contributes not only to form a gonadal medulla itself, but also is able to decide the direction of the sex differentiation of the gonad in accordance with the genetic sex of the donor. In other words, these facts appear to indicate that there already exist some qualitative differences related to sex between male intermediate mesoderm and female one at the tail-bud stage of development in anuran species. In the field of cytology, Ishizaki and Kosin (1960) pointed out that "sex-chromatin" of chick embryo, that is cytologically regarded as the manifestation of sex dimorphism or sex difference, first appears and is discriminated at the time of gastrulation.

These findings together with the present result may involve an important significance on the problem of determination and differentiation of sex in ontogeny. In conclusion, the author may insist that the potency of sex differentiation is still considerably labile at the gastrula stage; in other words, the determination of sex is not so well fixed at the said developmental stage.

Based on the histologic observation of the gonad developed in the experimental,

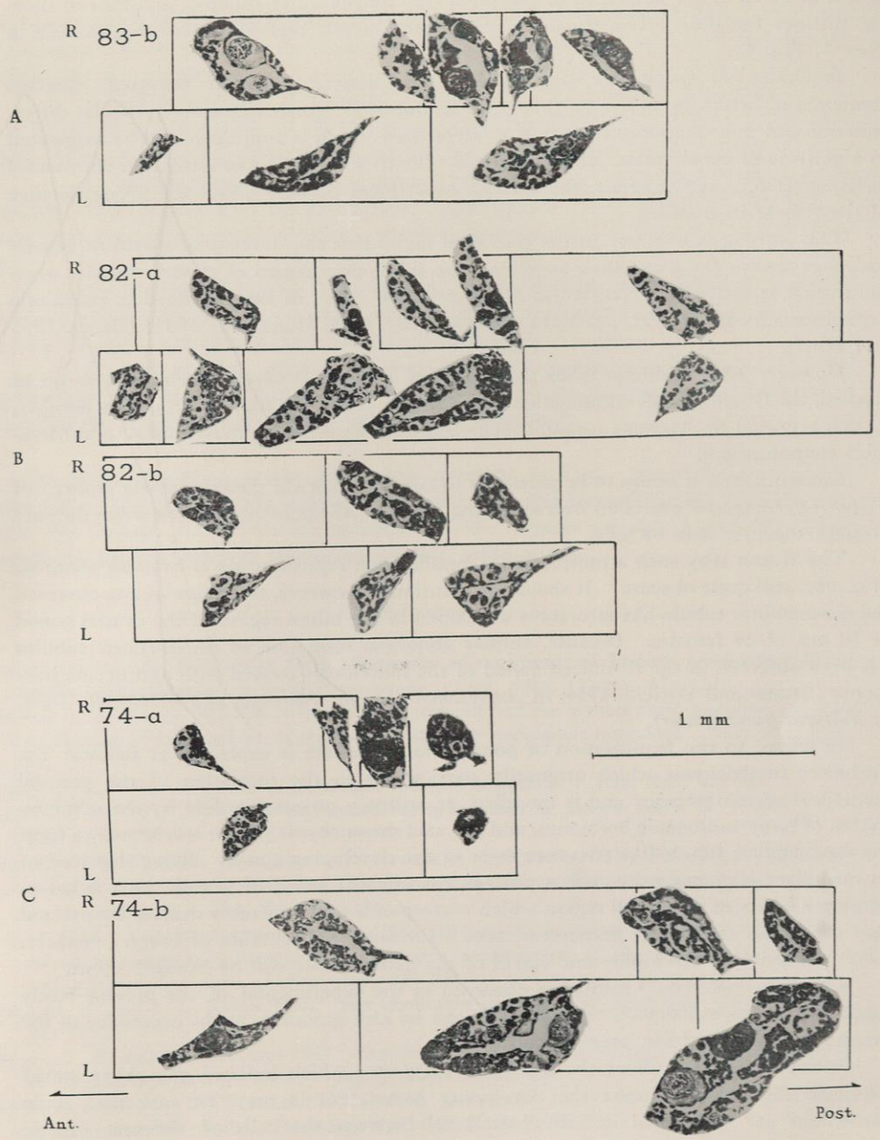

Fig. 2. Diagrammatic illustrations of the gonad in the experimental. Detailed explanation in text.
Ant: anterior, Post: posterior, R: right side, L: left side, A: specimen 83-b, B: specimen 82-a and 82-b, C: specimen 74-a and 74-b.

27 out of 32 cases are judged to be classified into female differentiation, and four of them are distinct females. The remaining five cases are of male differentiation as seen in Plate 1, Fig. 4.

In almost all specimens which presumably derived from the operated gastrula composed of heterosexual halves, that is, in 23 out of 27 female cases, the testicular differentiation can not take place even in the lateral half which is conjectured to be originated in a gastrula of geneic male, and the gonads of both sides carry out altogether the ovarial differentiation. As the result, the present experiment brought about the preponderance of the female in number.

This finding is contrary to the prevailed belief that the developing gonad of genetic male has always the controlling power on the developing gonad of genetic female, when the former is artifically deposited in a site near the latter, as demonstrated in parabiotic experiments by Burns, 1925; Witschi and McCurdy, 1929; Humphrey, 1931; Chang, 1953 and others.

However, the parabiotic twins derived from *Triturus pyrrhogaster* embryos so far as studied, the female-female combination of the gonad shows the preponderance in number, as first reported by Asayama (unpublished) and later accertained by Amanuma and Miyamori (unpublished).

Consequently, it seems to be pertinent to consider that the genetic female embryo of *Triturus pyrrhogaster* exerts an overwhelming effect on the genetic male one when they are brought together side by side.

The reason why such a morphogenic modification regarding sex is brought about is, of course, still quite obscure. It should be mentioned, however, that there can be observed the mesonephric tubule-like structures developed in the hilum region of the ovarial gonad in 10 out of 24 females. Because, similar abnormal formation of mesonephric tubules has been observed in the feminized gonad of the individuals treated with androgenic hormones (Bruner and Witschi, 1954, in *Ambystoma opacum*; Asayama and Matsuzaki, 1958, in *Triturus pyrrhogaster*).

In regard to the feminization of gonads the latter fact is explained as follows: the medullary mesenchyme which ordinarily participates in the formation of the gonadal medulla is of pluripotency and is modified its ordinary prospective fate by the administration of some androgenic hormones, and the said mesenchyme can be accelerated to form the mesonephric tubule-like structure even in the developing gonad. Being deprived of its medullary component by such a process, the affected gonad of genetic male is led to develop a cavity in its central region which corresponds to the primary ovarian cavity, and thus the gonad as a whole becomes to take a fundamental structure of ovary. Namely, the feminization of the embryonic gonad of the genetic male will be brought about.

The abnormal tubule formation observed in the experimental of the present study suggests that the explanation related above can be also applicable to the occurence of the female type gonad of the present study.

Nothing is known about the nature of the factor which induced the above stated histologic modifications upon the developing gonad, but it may be sure that some interactions are introduced in subcellular level between the cells of different genetic constitutions.

Summary

1. The present study was performed with the purpose of studying the mechanism how the genetic difference regarding sex is realized during the ontogeny. As a clue for analysing the mechanism, the stability of genetic sex during the early embryonic development was studied.

2. Early gastrulae of *Triturus pyrrhogaster* were bilaterally separated and the lateral halves were exchanged; so that the gastrulae with heterogenous halves were produced. The experimental were reared up to the completion of metamorphosis.

3. Eighty-three per cent. of the experimental were placed under the category of female differentiation after the histological observations. In other words, the genetic female half of combined gastrulae exerts an overwhelming effect upon the genetic male half.

4. It was speculated that although the embryos possess the prospective potency providing for definite sex differentiation at the early stage of gastrula, the determination of sex is not so well fixed at the said developmental stage.

5. The possible relation between the feminization of the gonads in the experimental and the presence of the mesonephric tubule-like structures which were formed in the hilum region of these gonads, was discussed.

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Explanation of figures

1. External appearance of the intact newt at the time of metamorphosis. ×2.
2. External appearance of the experimental animal (specimen 83-b), just after the completion of metamorphosis (abdominal view). ×2.
3. A typical modified gonad of ovarian type which can be frequently observed in the experimental, which is presumed to be originated in the heterosexual combination. ×200.
4. Larval testis in specimen 19-b. The gonad developed as well as the larval testis in the intact animal. ×200.
5. Larval ovary with developed oocytes in specimen 5-b. The gonad developed as well as the larval ovary in the intact animal. ×200.
6. Mesonephric tubule-like structure located in the hilum region of the gonad in the experimental animal (specimen 82-a). Arrow indicates the mesonephric tubule-like structure. ×200.
7. The vestigial left gonad of specimen 77-b. Only one germ cell is found out in this section. ×400.
8. The right gonad of the same individual in Fig. 7 (specimen 77-b), showing the ovarial structure with internal ovarial cavity. The gonad shows the tendency to distend over the surface of the fat body. ×400.

9 and 10. The left and the right gonad of specimen 74-a respectively, both of them are differentiating into testes (*cf.* Textfig. 2, C). ×200.