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Karyotypes and Localization of Constitutive Heterochromatin on the Mitotic and Meiotic Chromosomes in *Cynops pyrrhogaster*

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Abstract : Karyotypes of male and female Japanese newts, *Cynops pyrrhogaster*, were studied by the preparation of conventional Giemsa stain and by C-staining technique. Twelve pairs of somatic chromosomes consisted of eight metacentric and four submetacentric homologues. Both male and female karyotypes have identical morphology and no manifestation of heteromorphism of chromosomes defined as a sex-specific pair. Meiotic chromosomes were also examined in male individuals together with their gut tracts. All bivalents at prometaphase of first meiotic division indicated the interstitial chiasmata which were terminalized on all chromosome arms. No end-to-end paired bivalent was seen.

C-banding appeared most intense in the pericentric region of either both chromosome arms or on a single arm of submetacentric chromosomes. Centric heterochromatin was faintly detected in a few chromosomes. The banding pattern of mitotic and meiotic chromosomes of *C. pyrrhogaster* revealed the dissimilarity of localization of constitutive heterochromatin with related European *Triturus*, whose karyotypes were quite similar each other.

Introduction

Cynops pyrrhogaster, one of the most common Urodela in Japan, is only a species of Salamandridae distributed in main islands ; Honshu, Shikoku and Kyushu. Chromosomes of the newt have been observed by only a few workers in early years (Iriki 1932, Sato 1932, Kawamura and Utsunomiya 1957). Because of having difficulty of getting mitotic cells from urodelan tissues and blood and of chromosome preparations, published data on accurate karyograms were concerned only limited species until recent years.

Technical improvements by Kezer and Sessions (1979) and Schmid *et al.* (1979) made it possible to obtain numerous mitotic cells from the urodelan gut tract and that facilitated the application of the differential staining techniques to the chromosomes. Among a series of chromosomal banding techniques, C-staining method (Pardue and Gall 1970) has been most widely and frequently applied in various specimens. This method is known to reveal chromosome regions composed of highly repetitive DNA sequences which are cytologically called constitutive heterochromatin. Development of the technique to detect the number and position of the specific bands on the chromosomes greatly facilitates the identification of individual chromosome and matching of the homologues in the karyotype with greater precision. In addition, the comparison of the specific band pattern on corresponding chromosomes between related species with similar karyotypes improved cytotaxonomic investigation.

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The chromosome study of common Japanese newt is of interesting because of its wide distribution over the country and having several geographic varieties in external features. As a preliminary report, we will present more precise karyotypes of both sexes and clarify the distribution of centromeric heterochromatin on the chromosomes by C-staining method. The banding pattern produced along the mitotic chromosomes can be compared with that of European newts, genus *Triturus*, published by Nardi *et al.* (1973), Schmid *et al.* (1979), and Ragghianti *et al.* (1980), for testing the rate of differentiation between these karyotypes for recognizing the systematic and phylogenetic position of the Japanese newt species.

Materials and Methods

Male and female newts, *Cynops* (former *Triturus*) *pyrrhogaster* BOIE were collected in the vicinity of Matsue, Shimane-ken. Animals were kept in the laboratory at room temperature and fed beef liver before being used in experiments.

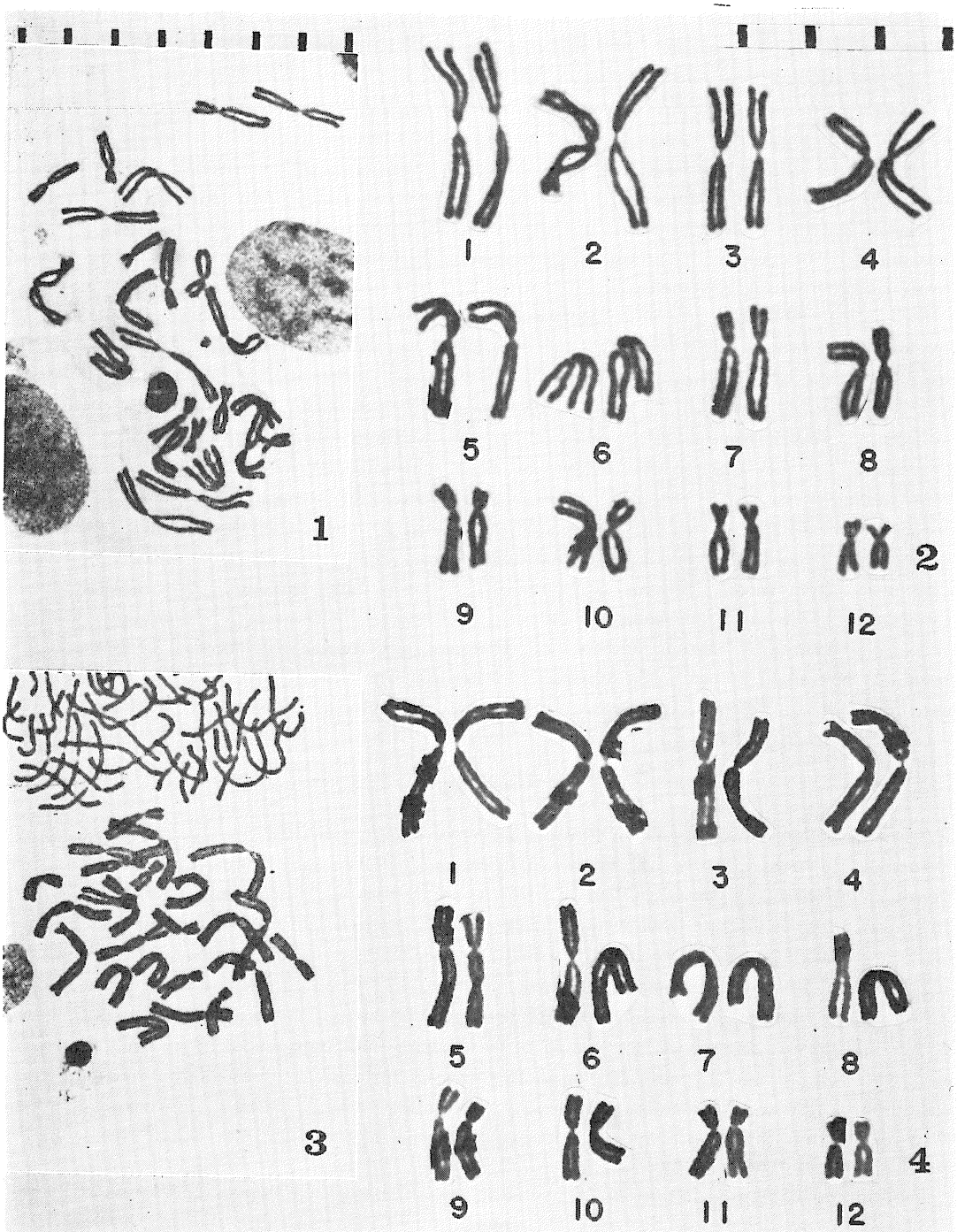
Mitotic chromosomes were obtained mainly from the gut epithelium of adult newts by a method used by Kezer and Sessions (1979). Newts were injected a colchicine solution intraperitoneally at 0.2 mg per gram of body weight. After 48 hours of the colchicine treatment an entire gut tract was removed from the dissected animal and epithelial surface was cleaned in saline solution. The gut tract was transferred into distilled water and was fixed in the acetic-alchohol mixture after 10 minutes of the hypotonic pretreatment. Then a small piece of the specimen was immersed in 45 % acetic acid for a few minutes. Epithelial cells were scraped off on a slide glass from a piece of the tissue with blade edge. Squashed slides were frozen on a block of dry ice and the cover glass was removed to make the preparation permanent.

Meiotic chromosomes were observed in adult male newts during June and August. After colchicine treatment testes were isolated from the animals whose gut tracts were used. Two or three pairs of testicular lobes were gained from an individual and these were fixed in acetic-alcohol mixture. Squash preparation was made by the same technique described above.

Banding patterns in mitotic and meiotic chromosomes were observed by C-staining method of Sumner (1972) for detecting the constitutive heterochromatin, with a minor modification. The slides were, 1) treated in 0.2 N HCl for 30 to 40 minutes at room temperature, 2) incubated in a saturated $Ba(OH)_2$ solution at 50°C for 10 to 30 seconds and 3) briefly washed in 0.1 N HCl, distilled water and absolute ethanol. The preparations were then 4) incubated in 2×SSC at 60°C for 60 minutes and 5) rinsed thoroughly in deionized water. Chromosomes were stained in Gurr's Giemsa solution (2 % ; pH 6.8) for 4 to 5 minutes. The specimens were washed in running water and dried in air.

Results

Both male and female *Cynops pyrrhogaster* possess 24 chromosomes in a somatic cell (Fig. 1 & 3). The diploid number counted coincided with earlier works by Iriki (1932), Sato (1932) and Kawamura and Utsunomiya (1957). Twelve pairs of the complement consisted of 8 pairs of metacentrics and 4 pairs of submetacentric elements. The individual chromosome pair was numbered in order of descending length. As the morphology of most chromosomes in the karyotype were similar each other in relative length and



Figures 1 and 3. Mitotic metaphase chromosomes of *Cynops pyrrhogaster*. The preparation was made by a squash method of the gut epithelium, stained with Giemsa.
1, female. 3, male.

Figures 2 and 4. The same chromosomes arranged in karyotypes. 2, female. 4, male. One section of respective scales on metaphase plates and karyograms indicate 10 micron.

centromere position, the elements could not be grouped (Figs. 2 & 4). Morphology of male and female karyotypes were identical and no heteromorphic pair of chromosomes as sex elements were detected between both karyotypes.

Squash preparations of testes, examined together with a gut tract of the same individual, contained a number of spermatocytes at first and second division. Twelve bivalents showed side-by-side pairing and two chiasmata were generally terminalized so that all bivalents formed ring shape (Fig. 9). No example of end-to-end pairing or of non-synaptic pairing was observed in any meiotic configurations. Identification of the individual bivalent corresponding to the somatic chromosome pair was difficult except the largest and smallest bivalents.

C-banding method applied to urodelan chromosomes visualized specific C-spots in a nucleus and chromosomes (Figs. 5-7). A somatic nucleus showed dark stained granules by C-staining processes and the number of C-spots was almost agreed with those of diploid chromosomes (Fig. 5). The C-banded pattern appeared in characteristic positions on somatic chromosome pairs. Majority of chromosomes showed the constitutive heterochromatin on the pericentiric region of both chromosome arms, except a few pair of submetacentrics which revealed a single pericentric spot on the shorter arm (Fig. 8). Intensity of C-spots was also varied according to each chromosome pair. Beside pericentric C-spots the centric heterochromatin was seen only in two limited pairs of elements. The centric C-spot appeared as a quite faint granule. No interstitial or terminal C-band has been detected.

Meiotic metaphase chromosome of both first and second division showed C-spots on all bivalents and elements of haploid set (Figs 9 & 10). Chromosomes of a primary spermatocyte found to bear 8 or 4 spots on every bivalent, localized at homologous position.

Discussion

Almost decisive karyotypes of European newts were published in these several years according to C-staining methods ; in *Triturus vulgaris*, *T. meridionalis* and *T. italicus* by Nardi *et al.* (1973), in *Natophthalmus* (*Triturus*) *viridescens* by Hutchison and Pardue (1975), in *T. cristatus* by Rudak and Callan (1976), in *T. vulgaris*, *T. alpestris*, and *T. helveticus* by Schmid and Krone (1976) and Schmid *et al.* (1979). and in *T. italicus* by Ragghianti *et al.* (1980). Japanese newts, *Cynops pyrrhogaster*, has been known to have the same chromosome number with animals of genus *Triturus* by earlier workers (Sato 1932, Iriki 1932). However, no precise karyotype of the species has been reported in both sexes. Recently Schmid and Krone (1976) used this species for their studies of the structure and behavior of the acrosomal chromocenter, and illustrated the C-banded karyotype of male *C. pyrrhogaster*. However they did not presented the comparable data which were made by the conventional staining.

Diagrammatic representation of the somatic metaphase chromosomes of *T. vulgaris* and *T. helveticus* by Schmid *et al.* (1979) was interesting to compare with the present results. Ideogram of *T. vulgaris* was identical with that of *C. pyrrhogaster* while *T. helveticus* was found to have two dissimilar pairs, those were chromosome nos. 9 and 12. The dissimilarity were quite little as compared with the identical morphology of large 8 pairs between European species and Japanese species.

The specific C-band pattern of chromosomes so far examined in genus *Triturus* permitted

the recognition of differences between similar karyotypes in closely related species. The present observation revealed the considerable differences between European *Triturus* species and Japanese *Cynops* species, although those karyotypes based on the conventional staining were quite similar each other. Unlike to all the other species that Schmid *et al.* (1979) examined in *Triturus* species, *C. pyrrhogaster* chromosomes possessed a unique C-band

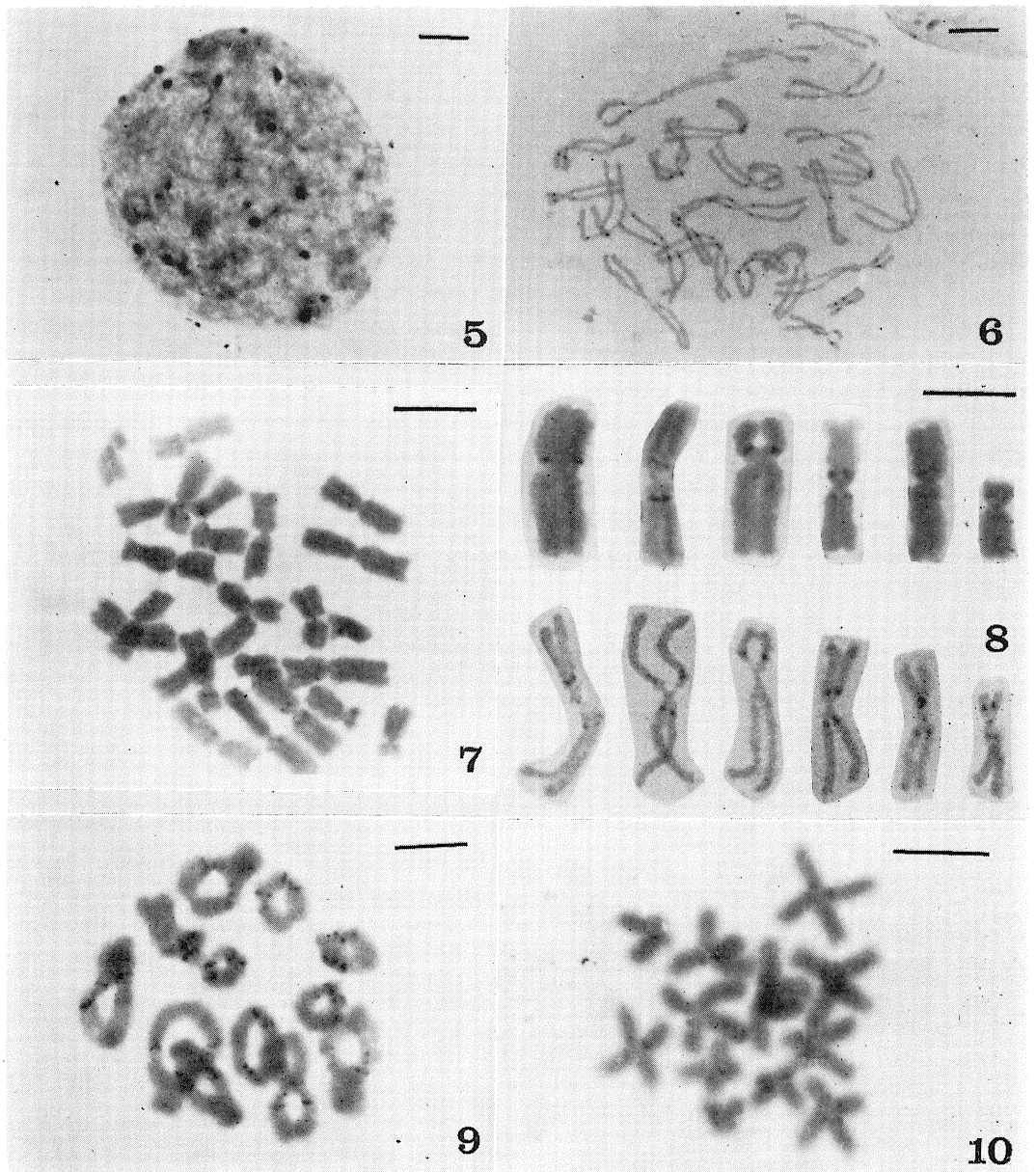


Figure 5. Nucleus of a female gut cell after C-staining processes, showing intensely stained granules. Figures 6 & 7. C-banded chromosomes of female gut cells in the metaphase. Figure 8. An example of C-band patterns of selected chromosomes of male and female cells. Figures 9 & 10. C-banded bivalents of first meiotic division of male and C-banded chromosomes of a haploid set of second division.

pattern ; 12 pairs of chromosomes bore pericentric spots of constitutive heterochromatin but none or quite faint C-spot was appeared in the centromere. In the study of acrosomal chromocenter localization, Schmid and Krone (1976) briefly described on the chromosomal C-band of *C. pyrrhogaster*. The present study supported their results, which indicated no centric heterochromatin on majority of chromosomes except two pairs of chromosomes having a very faint centric C-spot.

Concerning the sex chromosomes in salamandrid caudates (reviewed by Morescalchi 1975), certain authors have shown the presence of heteromorphic sections as regards the length and shape of the loops in the two homologous chromosomes of a lampbrush bivalent in *Triturus cristatus*, *T. marmoratus* and two species of *Pleurodeles* (Callan and Lloyd 1960, Mancino and Nardi 1971). They considered that this phenomenon constituted the proof of the presence of sex chromosomes (female heterogamety). As regards the somatic karyotype analyses, however, it is said that the female line has been very little studied contrary to the male line until quite recent years. Consequently no confirmable datum on the female heterogamety was reported since then.

Obvious evidence of the first definite example of sex specific chromosomes in Amphibia were presented by Schmid *et al.* (1979) by the banding method. Their evidences based on both the C-band pattern of male and female karyotypes and the pairing configuration of meiotic chromosomes in male were explainable by a XX/XY-type of chromosomal sex determination. These features were, however, so far represented only by *T. a. alpestris* and *T. v. vulgaris*. The present study indicated any manifestation of heteromorphism of the chromosomes neither in somatic karyotypes nor in meiotic configurations of male. More comprehensive studies by differential staining technique of chromosomes is required on the Japanese salamandrid species.

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