CHROMOSOME OBSERVATION ON A BRACKISH-WATER AMPHIPOD,
EOGAMMARUS KYGI (DERJAVIN)

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汽水性端脚類の1種トゲオヨコエビ *Eogammarus Kygi* (DERJAVIN) の
染色体数と染色体観察法

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At present, fewer reports on chromosome numbers have been known on gamma-ridean Amphipoda, Crustacea, compared to vast taxonomical work of this group in the world.

They have amounted to under twenty papers referred to NIYAMA (1950, 1959), LOP (1989), and SALEMA and HEINO (1990). When we compare the gap between both fields of the studies, further information on the former thesis would be necessary to develop the genetic studies on this big crustacean group.

The previous methods of chromosome study on amphipods have used paraffin preparations (NIYAMA, 1950, 1959, ORIAN and CALLAN, 1957) and the next step of improving the applied air-drying technique (SALEMA and HEINO, 1990). The latter is still considered as adequate to observe such materials. Our modified technique using frame-drying applied from our previous report on a sea urchin (YAMANAKA *et al.*, 1989) would be more successful than the above mentioned methods, though it was essentially similar to HASEGAWA's try (1981) on several crustaceans. For an example of applying our method, a brackish water amphipod, *Eogammarus kygi* (DERJAVIN) which is commonly distributed in Hokkaido district, northern Japan was used.

Chromosome numbers of the species were also studied comparative to that of the other gammaridean species reported, but determination of the karyotype was unsuccessful because of either the condition of the materials or of the species.

Fig. 1. Male of *Eogammarus kygi*. t. testis, d. ductus deferens.

Material and Method

More than 100 individuals of *Eogammarus kygi*, "Togeo Yokoebi" in Japanese, having a body length of 9–17 mm were collected from a small inlet (Temp. 16°C, Salinity 30.30‰) in the inner site, Ubaranai area of Notori Lake (N 44°03', E144°09') opening on to the Okhotsk Sea, Abashiri City, eastern Hokkaido, Japan. The living males were treated as following procedure which was modified from the method for sea urchin(YAMANAKA *et al.*, 1989) due to active cell divisions and less content of fatty acids of this species.

- 1) 0.01 ml of 0.1% colchicine was injected from the posterior dorsum into frontal pleon of the animal and kept in sea water including colchicine as 0.02% for 5 hours.
- 2) The animal surgically operated at the dorsal part, was kept in 0.01% colchicine solution of distilled water for 30 minutes.
- 3) Two or three drops of Carnoy fluid were added to the operated on part for fixation. Then, the gonadal part was quickly taken out and put into Carnoy fluid with tweezers, and fixed for 1 hour.
- 4) The fixed gonadal part was put into warmed acetic acid for 10 minutes.
- 5) After that, it was placed on a clean slide and a drop of distilled water was added, then the tissue was separated to several parts with tweezers.

- 6) One or two drops of Carnoy fluid were added to the suspension of the tissue, and it was dried indirectly on the underside of the slide by an alcohol burner.
- 7) After confirmation of complete desiccation, the tissue was stained with a 3% solution of Giemsa (Merck Co.) by phosphate buffer (Iatron Lab. Inc., Rm 102-2, 1/15M, pH 6.8) for 15 minutes.
- 8) Microscope observing was done after washing with tap water.

Results and Discussion

After many tries for preparation of the observing chromosomes, we used testis of the animal because it was easy to obtain several phases of cell division every times. The advantages of the frame-dry method which we used in this study are: 1) availability of clear shapes and sizes of chromosomes, 2) adaptability of several staining methods such as the Banding and others, 3) being able to defend exfoliation of tissues. In this work, longer time for hypotonic treatment than in the case of sea urchin (YAMANAKA *et al.*, 1989) was necessary because of the slow permeation of the material.

In the procedure, reagents and their concentrations are almost the same as that of HASEGAWA (1981), but the treatment of materials is different from his method. Although he separated the gonadal part from the body of the animal and treated hypotonicity and others after that, we tried hypotonic treatment and fixation on the surgically operated part of the animal to prevent the loss of tissues. Subsequently, he broke the tissue into smaller pieces in Carnoy fluid, but we separated it instead, in distilled water to protect it from desiccation and to make it easy to break by using tendered tissue. This fine modification was consequently favorable to treat smaller animals.

In our observation of the spermatogonia of the testis, fine somatic and meiotic metaphases were obtained. At first meiotic metaphase, all of the group was bivalent chromosomes. Chromosome numbers found at somatic division of the spermatogonia were $2n=56$ and $n=28$ at first meiotic metaphase. In its composition, metacentric chromosomes were observed in high ratio as shown in Fig. 2. Small circular chromosomes seen in the outer side of the group at the somatic division (Fig. 2A) are supernumerary chromosomes and looked like B-chromosomes. The average size of chromosomes at somatic division was $1.6\mu\text{m}$.

Until now, the reported chromosome numbers of amphipods range from $n=24$ to $n=28$ without exceptional observations (NIHAYAMA, 1950, 1959; LOP, 1989; SALEMMMA and HEINO, 1990). Species of *Echinogammarus bevilloni*-group which are freshwater inhabitants and belong to the Gammaridae family, are the same as *Eogammarus* have and almost the same range between $n=25$ and $n=28$ (LOP, 1989). Therefore,

Fig. 2. Chromosomes in spermatogonia of *Eogammarus kygi*. (A). Metaphase plate of the somatic division, $2n=56$. (B). 1st meiotic metaphase plate, $n=28$. s. supernumerary chromosomes.

Fig. 3. Occurrence of *Eogammarus kygi* in Abashiri district, eastern Hokkaido.

most of the species included systematically had the same groups as expected, having about these numbers of chromosomes, except some extraordinary cases of zygoty in *Echinogammarus calvus* (LOP, 1987) and polyploidy in *Pontoporeia affinis* (SALEMMA, 1984) $n=26$ in *E. berilloni* group is postulated as the ancestral number of it, and is also usually found in the related genus *Gammarus* (SALEMMA, 1984; LOP, 1989). $n=28$ was recorded in *E. navaensis* for the first time for gammarids (LOP, 1989), but this karyotypic diversification has not been explained though, considering the morphological examination. Our 2nd record in gammarids, $n=28$ of *Eorammarus kygi*, has also no relating information on the same genus-group. Actually, *Eogammarus* has nine related genera belonging to *Anisogammarus*-group (BARNALD and BARNALD, 1983 a) and has the widest distribution in the group from South China to California through the cold north Pacific region. It needs numerous works on karyology and morphology in this wide area to solve the implication of the chromosome numbers.

Observing the plates of the somatic division in Fig. 2, there is no clear differentiation between each chromosome. Nevertheless, the big systematic scale of Gammaridea can presume the evolution to $n=28$ from $n=26$ or something by Robertsonian fragmentation-fusion.

The existence of sex-chromosomes was not confirmed from the plates in Fig. 2. A few examples of the presence of X-Y chromosomes have been reported in *Gam-*

marus chevreauxi and *Anisogammarus annandalei* (NIYAMA, 1950), and in *Ecchinogammarus calvus* (LOP, 1987). At present, these are thought to be rare cases of gammaridean amphipods.

Eogammarus kygi was obtained from several locations of marine and fresh-waters in Aug. 1991 as shown in Fig. 3 by us and others, in which the population at fresh-water zone of the Onnebetsu River demonstrated the numerical density, 48–136 individuals/m², and the biomass, 1.0–5.4 g/m² (T. AASAMI, personal comm.). These figures do not mean high productivity or high reproductive rate of the species, considering examples of marine gammarids, and *Corophium uenoi* distributed in an inlet of the Tokyo Bay which revealed 72,000 ind./m² (KUWABARA, 1986a), and *C. insidiosum* in Yokohama harbor of 40,200 ind./m² (KUWABARA, 1986b). *Eogammarus kygi* is a common euryhaline distributed in a wide variation of the environment as intertidal, brackish and riparian, and considered to have no peculiar life history. Its subspecies and geographical variety have not been recorded, though the genus includes nine species which are diversified morphologically and geographically (BARNALD and BARNALD, 1983b). *E. kygi* is therefore thought to be a stable species adapting to wide range of salinities. In the related species, many euryhaline are known in distributive and physiological studies i. e. *Gammarus duebeni*, *G. locusta*, *G. zaddachi*, and *G. oceanicus* (LOCKWOOD, 1976). But *G. pulex* has n=26 chromosomes, any useful information concerning the solving of the diversity of chromosomes by influences of less-physiological adaptability or environmental stress are unknown.

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要 約

汽水域に生息するトゲオヨコエビ (*Eogammarus kygi*) の雄成体の精巣より染色体を観察した。コルヒチン処理の後、背部を切開して生体のまま組織を低張処理することにより組織の喪失を防ぎ、本種のような体長約 9-17 mm のごく小型の生物でもフレームドライ法によって明瞭な核板を得ることができた。染色体数は $2n=56$, $n=28$ でこれとは別に微小染色体 (B-chromosome) が存在した。染色体の大きさは平均 $16\mu\text{m}$ でほとんどの染色体が中部着糸型染色体であった。また、第一成熟分裂中期像では全ての染色体が二価染色体を形成し、性染色体の存在は認められなかった。本種の染色体数について、Gammaridae の近縁グループおよび同属近似種の染色体数、地理的分布との関係を検討した。