Mitotic chromosomes of freshwater crayfish

*Procambarus (Austrocambarus) llamas* (Villalobos, 1955)

Dr. Lenin Arias-Rodríguez
Dr. Jeane Rimber Indy
M.C. Salomón Páramo-Delgadillo
Dr. Wilfrido M. Contreras-Sánchez
Dr. Carlos A. Álvarez-González
M.C. Ulises Hernández-Vidal
M.C. Alejandro Mcdonal-Vera

Abstract

The karyotype of the Mexican Cambarid freshwater crayfish *Procambarus llamas* was studied by examining the metaphase chromosomes spreads with high chromosome number $2n=120$ obtained from gill tissue of adult specimens. A total of 189 mitotic metaphases were examined and the diploid chromosome number ranged from 98-120 per metaphase with a mode at 120 chromosomes that were represented by 67.7 % of the total counted metaphases. The karyotype was characterized by sixty pairs of monoarms chromosomes and sex chromosome in this species was not identifiable.

Introduction

*Procambarus (Austrocambarus) llamas* is a freshwater crayfish endemic to south-east of Mexico with aquaculture potential. Cytogenetics study of crustacean are relatively few because their chromosome numbers are large, chromosome sizes are small, and most of them are metacentric and submetacentric which makes them difficult to differentiate in comparison with the chromosomes of insects and vertebrates. In freshwater crayfish, the highest chromosomes number of 376 has been found in an *Astacid Astacus towbridgii* (Niiyama, 1962). Karyological studies have provided basic information on the number, size, and morphology characters of chromosomes structure that is important to undertake chromosome manipulations essays for aquatic organisms.
To understand the cytogenetic profile of *P. llamasi*, we examined in detailed the structure and number of their chromosome.

**Objective and Reason**

The objective of this study was to investigate on the karyotype of this species since their karyotype analysis has not previously been published. The karyotype analysis is a key for stock improvement by polyploidy manipulation, hybridization and related genetic engineering.

**Material and methods**

Fifteen adult specimens of *P. llamasi* ranging from 5.5-14 cm of total length were used and were incubated in 10 ml transparency bottle contained 1 ml of 1% colchicine solution, aerated for 5 hr. Minced gill tissues were kept in hypotonic treatment in 1.0 % sodium citrate for 2 h at room temperature. The tissues were fixed in 4:1 Carnoy’s solution (methanol: acetic acid). It was then centrifuged at 3000 rpm for 10 min, the cell pellet re-suspended in fresh fixative, and kept at 4°C for 24 h. Then were stained in 10 % Giemsa with phosphate buffer (pH 7) for 20-25 min then air-dried.

Metaphase chromosome slides were prepared from the fixed tissues following Arias-Rodriguez *et al.* (2006) with slightly modification. The well-spread mitotic metaphase spreads were photographed under a microscope (Zeiss Axioistar plus) with a digital camera (Sony cyber shoot 7.2 megapixel). Four of the best mitotic metaphase was karyotyped. The morphometric measurements of chromosomes pictures were conducted with photographic software Photoshop 6.0 (Adobe system). The chromosome pairs were classified following the recommendation of Levan *et al.* (1964).

**Results**

A total of 189 counts were made of chromosomes spreads at metaphase from adult specimens to determine the number of diploid chromosomes. The chromosome counts ranged from 98-120 per
metaphase and all of them were mitotic chromosomes sets. Analysis of these data indicated that the modal chromosome number was $2n=120$, with 67.7 % of the cells examined having this number of chromosomes. The diploid chromosome numbers were considered to be $2n=120$. Sex chromosomes were impossible to differentiate.

The length of long arm was 2.15 to 6.45 µm. All large chromosomes are monoarm which centromeric position determined in telocentric region. Homologous pairs of chromosomes were arranged in decreasing size and centromeric position. The formula proposed for *P. llamasí* was $2n=120$ T.

**Discussion**

In karyotypical studies, rapid growing tissues are required to obtain a large number of chromosomes spreads in metaphase. Several karyological studies on crustacean have been done obtained from adult testis and gonad tissue, for example in freshwater crayfish *Cherax quadricarinatus* has been proved to be an excellent tissue in the karyotype analysis because is not only a source of meiotic metaphases but also a source of mitotic metaphases (Chow et al. 1990) however so far, there is no report on karyotype obtained from gill tissues of freshwater crayfish. An advantage of chromosome preparation from gill tissues over that testis and gonad tissues in crustacean could be suggested and this as very good source of tissues for chromosome analysis.

In this study, we administrated colchicine solution at a dose 1 ml/10 ml freshwater directly to the animal for 5 hr, and obtained very good mitotic metaphase chromosomes spreads. This report is a preliminary report treated animal directly with colchicine solution. Several studies have been reported the differences among colchicine solutions and period of time injected to the animal to arrest the cell division in metaphase. For example, redclaw crayfish *Cherax quadricarinatus* was applied by injecting into intraperitoneal cavity at the base of the first of peleopod of the male at a dose of 2.0µm/g BW with a 5-6 hr maintenance period at 25°C (Tan, 2004). Taken together, these studies suggest that colchicines concentration either injects or direct treatment to the animal to arrest the cell division in metaphase could apply for karyotype analysis, although the colchicines
concentration and maintenance time may vary according to the species.

Conclusion

A total of 189 counts were made of chromosomes spreads at metaphase from adult specimens to determine the number of diploid chromosomes obtained from fifteen adult specimens. The karyotype of *P. llamasi* is shown, by observation of metaphase chromosomes, to be 2n=120. The colchicines concentration either injects or direct treatment to the animal to arrest the cell division in metaphase could apply for karyotype analysis. The karyotype consisted of monoarm large chromosome number and 60 pairs with centromeric position determined in telocentric region. Sex chromosomes were indistinguishable under microscope analysis.

References