Karyotypic Study of *Kaloula mediolineata* (Amphibia: Microhylidae)

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Kaloula mediolineata karyotypes were studied from chromosomes in mitotic cell division obtained from the bone marrow of three animals. This study was performed by colchicine hypotonic fixation by the air drying method and using Giemsa stain. The fully mature specimens of *K. mediolineata* weighing approximately 30 grams each were injected with 0.2 mg/ml of colchicine. After two hours, it was found that the cells were in the metaphase stage of mitotic nuclear division. The number of chromosomes was 28 (2n). The chromosomal karyotype revealed that 1, 6, and 7 pairs of chromosomes were acrocentric, metacentric, and submetacentric, respectively. The results obtained are hopefully useful for amphibian categorization and can also be applied in future evolutionary studies.

Key words: Karyotype, *Kaloula mediolineata,* cytogenetics.

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การศึกษาคาริโอไทป์ของอึ่งอ่างก้นขีด (สัตว์สะเทินน้ำสะเทินบก: ไมโครไฮลิดี)

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ได้ศึกษาคาริโอไทป์ของอึ่งอ่างก้นขีด (*Kaloula mediolineata*) จำนวนสามตัวจากเซลล์ในไขกระดูกขณะที่มีการแบ่งเซลล์แบบไมโตติก การศึกษาใช้วิธี โคลชิซิน ไฮโปโทนิก ฟิกเซชัน แล้วย้อมสีด้วยจิมซ่าโดยได้ฉีดโคลชิซิน ความเข้มข้น 0.2 มิลลิกรัม/มิลลิลิตร ให้แก่อึ่งอ่างก้นขีดดังกล่าวซึ่งโตเต็มวัยและมีน้ำหนักตัวละประมาณ 30 กรัม หลังจากนั้น 2 ชั่วโมงได้ตรวจพบเซลล์ที่อยู่ในระยะเมตาเฟสของการแบ่งแบบไมโตติกที่มีโครโมโซม 28 แท่ง (2 ชุด) ประกอบด้วยโครโมโซมแบบอะโครเซนตริก เมตาเซนตริก และสับเมตาเซนตริก จำนวน 1, 6 และ 7 คู่ตามลำดับ ผลที่ได้นี้คาดว่าสามารถนำไปใช้เป็นข้อมูลประกอบในงานจัดลำดับกลุ่มของสิ่งมีชีวิตประเภทนี้และการศึกษาวิวัฒนาการต่อไป

คำสำคัญ คาริโอไทป์ อึ่งอ่างก้นขีด เซลล์พันธุศาสตร์

INTRODUCTION

Different amphibian karyotypes can be distinguished by the shape and number of chromosomes. Information obtained through karyotypes can also be applied to cytotaxonomy, the process of classifying living organisms into groups. Furthermore, related living organisms can represent the evolutionary data called phylogeny, and finally, reproduction also relies on the karyotypes of those living organisms.[1]

Amphibians of the species *Kaloula mediolineata* belong to the family Microhylidae. They are commonly found in the central region of Thailand. The animals are very important for several reasons, including as food for human consumption as an indicator of their ecosystem's health, and as an important factor in balancing the food web in their habitat.[2]

Recently, many ecosystems such as forests have been destroyed, resulting in an alarming reduction in many species, including *Kaloula mediolineata*. Because of this, many research projects have been devoted to studies on the genetic diversity of amphibians in the family Microhylidae. It is hoped that these data will be useful in the amphibian breeding and conservation of remaining genetic resources.[3]

The karyotypes of amphibians have been intensively studied, particularly *Bufo* sp.[4-5] and *Rana* sp.[6-8] However, karyotype studies of *Kaloula* sp. have been rather limited; no karyotype studies have been done on *Kaloula mediolineata*. A previous study reported the number of chromosomes for *Kaloula pulchra* as 28 (2n).[9] It is therefore logical and desirable to perform additional karyotype studies in the genus *Kaloula* for evolutionary information. This study is an important database for breeding, genetic conservation, and evolutionary studies in the *Kaloula* genus.

Figure 1. A fully mature specimen of *Kaloula mediolineata.*

MATERIALS AND METHODS

Fully mature (weighing approximately 30 grams) *Kaloula mediolineata* (Figure 1) were collected from Cha-am, Petchaburi province, Thailand, for this study. Animals were treated with intraperitoneal colchicine injections of approximately 0.1 ml/ 100 grams of the animal weight (0.2% mg/ml) to inhibit cell division during metaphase. After two to three hours the animals were anesthetized with ether. Tissue samples from several limbs were taken and the edge of bone was removed with scissors. Bone marrow samples were then obtained by syringe pressurized injection with dilute 0.075 M potassium chloride.

Bone marrow samples were then minced in the same salt solution and left at 37°C for 15 minutes. The solution was later centrifuged at 2000 rpm for five minutes, most of the supernatant was discarded, and some remained to dissolve the precipitate at a later stage. The precipitates were then homogenized and the freshly prepared cold fixative (methanol:acetic acid in a 3:1 mixture) was added dropwise to the solution so that the total volume was 5 ml.

The solution was then shaken vigorously and recentrifuged. The supernatant was discarded and the fixative was then added. The process was repeated until a white precipitate was obtained and only 1-2 ml of fixative remained in the final sample. Slides were then prepared for chromosomal detection. The precipitates were then evenly resuspended and three separate drops from the resuspended samples were spotted on the slide surface.

Chromosomal samples were distributed and stabilized by passing the slides through a flame. The samples were later stained with 15% Giemsa solution in phosphate buffer (pH 6.8) for 15 minutes before rinsing. The stained chromosomes were then microscopically examined and categorized.[4]

RESULTS AND DISCUSSION

From the karyotypic studies on the bone marrow cells of *Kaloula mediolineata* using the colchicine-hypotonic-fixation air-drying method[4] with Giemsa staining, the number of chromosomes in the somatic cells was found to be 28 (2n) and were observed to be in the metaphase stage of mitotic division. The karyograms obtained from *Kaloula mediolineata* bone marrow, shown in Figure 2, were selected from the most evenly distributed chromosomes with aligned centromeres. The length of the short arm of the chromosomes (L_S), long arms (L_L), and each chromosome (L_T), the relative length (R_L-the ratio between L_T and the summation of the L_T), and the centromeric index (C_I) - calculated from the ratio between L_L and L_T - were later averaged from 5 cells. The standard deviation (SD) and standard error ($S\bar{x}$) were also calculated (data not shown). The karyotypes obtained showed 4 pairs of large chromosomes and 10 pairs of small chromosomes.

The following formula could then be applied to the karyotypic findings

$$(2n = 28) = L_4^{M} + L_4^{Sm} + S_8^{M} + S_{10}^{Sm} + S_2^{Ac}$$

Overall, it may be concluded *that Kaloula mediolineata* karyotypes are asymmetrical and heterogeneous since large and small sizes of chromosomes were visible. This finding agrees with a report by Morescalchi[10] who studied amphibians of the Family Ranidae, a taxon that is believed to be evolutionarily closely related to the Family Microhylidae (in which *Kaloula mediolineata* is included). Morescalchi's karyotypes showed similar metacentric, submetacentric and acrocentric chromosomal characteristics.

Further studies should investigate others frogs' karyotypes in this genus. This will broaden the understanding of the evolutionary relationships of these amphibians.

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Figure 2. a) Mitotic metaphase stage from the bone marrow of *Kaloula mediolineata.*
b) The karyotype of *Kaloula mediolineata.*

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