Karyotypes and Nucleolar Organizer Regions in Two *Abudefduf* Fishes (Pomacentridae, Perciformes)

Akinori Takai

Abstract

Karyotypes and nucleolar organizer regions (NORs) were studied in two *Abudefduf* species (Pomacentridae, Perciformes), *Abudefduf bengalensis* and *A. septemfasciatus*. These species had the same chromosome number, 2n = 48, and similar karyotypes consisting of one pair of metacentric, one pair of submetacentric, and twenty two pairs of acrocentric chromosomes. The fundamental number was 52. NORs detected by the Ag-NOR staining (Ag-NORs) were located in the short arms of a pair of submetacentric chromosomes in both species, but the chromosomal locations of Ag-NORs were different; interstitial regions in *A. bengalensis* and terminal regions in *A. septemfasciatus*. Based on the present and previous studies, karyotypic differentiation and phylogenetic relationships among *Abudefduf* species are discussed. It has been suggested that studied karyotypes of *Abudefduf* species have been mainly differentiated by inversions in the NOR-bearing chromosomes. The present study revealed evidence of karyotypic differentiation involving paracentric inversion, which is difficult to elucidate without G-banding or similar analyses.

Key words: karyotype, NORs, paracentric inversion, Pomacentridae, phylogenetic relationship

1. Introduction

The family Pomacentridae of the order Perciformes comprising 28 genera with about 348 species, is a large group of small fish, commonly known as damselfish, which is widely distributed in tropical seas of the world[1].

Cytogenetic investigations have been performed in over 35 species[2-13]. In these results, pomacentrid fishes are characterized by the chromosome number varying from 27 to 48 and the fundamental number (NF) ranging from 48 to 86, and it has been suggested that karyotypes of pomacentrids have diversified from the ancestral karyotypes consisting of 48 acrocentric chromosomes toward an increase of NF value and decrease of chromosome number[4, 6]. For a more detailed analysis, nucleolar organizer regions (NORs) detected by the Ag-NOR staining (Ag-NORs) have been investigated in most species shown above[4-13]. The studies using Ag-NOR analysis have elucidated data enhancing the understanding of karyotypic evolution and phylogenetic relationships.

The present study focused on karyotypes and NORs in two *Abudefduf* species of Pomacentridae, *A. bengalensis* "Tenjiku-suzumedai" and *A. septemfasciatus* "Shichisen-suzumedai."

2. Materials and methods

A. bengalensis (one specimen) and *A. septemfasciatus* (one specimen) from a fish dealer in Osaka, Japan, were used for this study.

Chromosome preparations were made according to the direct method using kidney tissue[4]. Kidney tissue was removed, placed on a petri dish with about 0.5 ml of culture medium (e.g., Eagle's MEM), and crushed with flat-headed tweezers. After about 4 ml of the medium was added, pipetting was performed to liberate the cells, and then tissue fragments were removed. The obtained cells were treated with 0.1 μg/ml colchicine for 2 h, hypotonized with 0.068 M KCl for 20 min, and fixed with a freshly prepared mixture of acetic acid and methyl alcohol (1:3). The cell suspension was placed on clean slides, air-dried, and stained with 2% Giemsa solution diluted with 1/15 M phosphate buffered saline (pH 6.8).

Following microscopic observations, the slides were destained with 70% alcohol, and then Ag-staining was performed according to the one-step method of Howell and Black[14].

Conventional and silver staining chromosomes on the same metaphase plates were analyzed according to the chromosome nomenclature established by Levan et al. [15]. For the calculation of fundamental number (NF),

two were assigned to meta- and submetacentrics and one to subtelo- and acrocentrics.

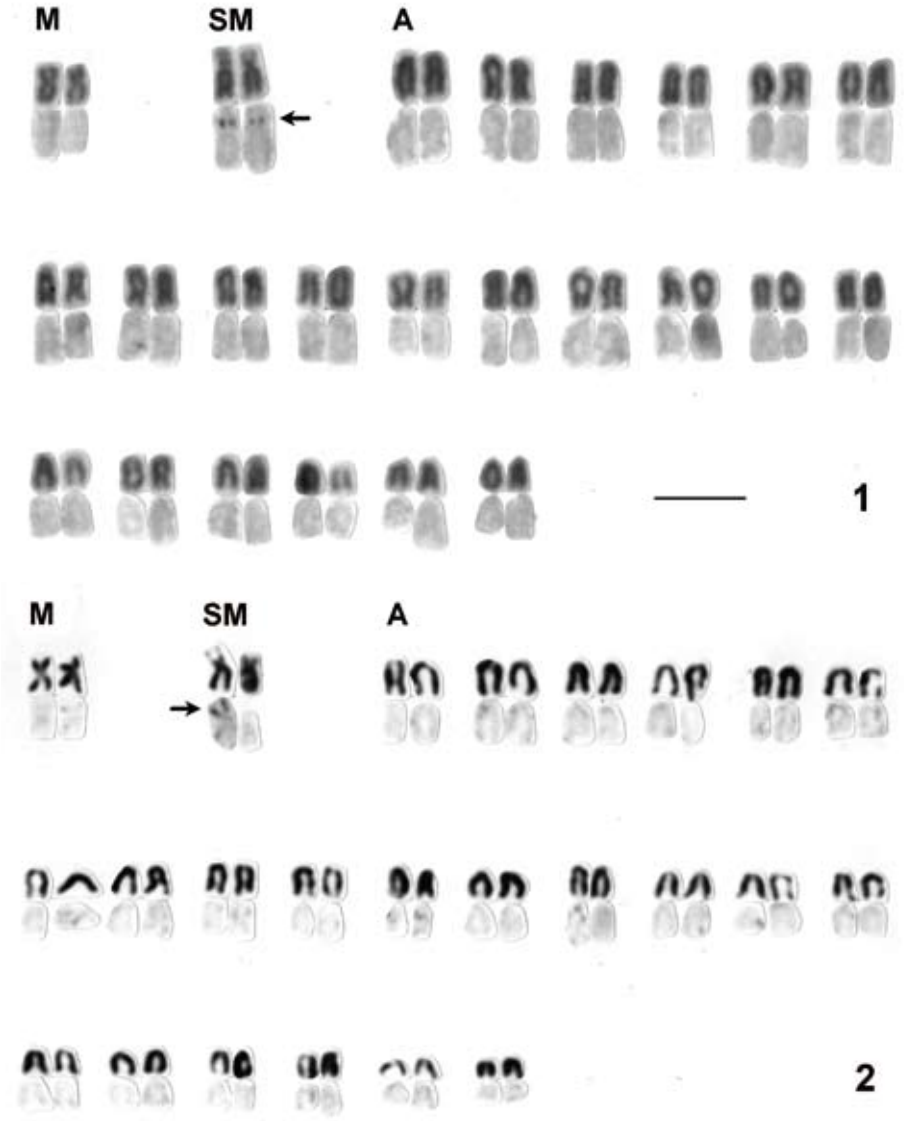
3. Results

The chromosome number of *A. bengalensis* was 2n = 48, and the karyotype consisted of one pair of metacentric (M), one pair of submetacentric (SM), and twenty-two pairs of acrocentric (A) chromosomes (2n = 48, 2M + 2SM + 44A) (Figure 1). The fundamental number was 52. Ag-NORs were located at interstitial regions in the short arms of a pair of SM chromosomes. The Ag-NORs appeared as small dot-like forms in faintly stained secondary constrictions (Figure 1).

The chromosome number of *A. septemfasciatus* was 2n = 48, and the karyotype consisted of one pair of M, one pair of SM, and twenty-two pairs of A chromosomes (2n = 48, 2M + 2SM + 44A) (Figure 2). The fundamental number was 52. The Ag-positive NORs were located at terminal regions in the short arms of a pair of SM chromosomes. In all cells (n = 8), Ag-NORs were observed as stick-like forms in faintly stained secondary constrictions in only one member of the NOR-bearing homologous pair (Figure 2).

4. Discussion

According to Nelson[1], Pomacentridae includes four subfamilies, Amphyprioninae, Chrominae,



Figures 1 and 2

Conventional and silver staining (lower row) karyotypes of (1) *Abudefduf bengalensis* (2n = 48, NF = 52) and (2) *A. septemfasciatus* (2n = 48, NF = 52). M, metacentric; SM, submetacentric; A, acrocentric. Arrows indicate silver stained NORs. Bar scale represents 5 μm.

Table 1. Cytogenetic data of *Abudefduf* species

Species	CN[1]	NF[2]	Karyotype[3]	NORs[4]	References
A. vaigiensis	48	52	2M + 2SM + 44A	2, SM, T	Takai & Ojima[4)]
A. sexfasciatus	48	50	2M + 46A	2, A, I	Takai & Ojima[4)]
A. saxatilis	48	52	2M + 2SM + 44A	2, SM, T	Brum et al.[8)]
A. saxatilis	48	52	2M + 2SM + 44A	2, SM, T	Molina et al.[9)]
A. bengalensis	48	52	2M + 2SM + 44A	2, SM, I	This study
A. septemfasciatus	48	52	2M + 2SM + 44A	2, SM, T	This study
A. notatus	48	50	2M + 2ST + 44A	—	Arai & Inoue[2)]
A. sordidus	48	52	2M + 2SM + 2ST + 42A	—	Arai & Inoue[2)]

1. CN: chromosome number, 2. NF: fundamental number, 3. M: metacentric, SM: submetacentric, ST: subtelocentric, A: acrocentric chromosomes, 4. The number of NORs, NOR-bearing chromosome and NOR location (T: terminal region, I: interstitial region)

Lepidozyginae and Pomacentrinae. The genus *Abudefduf* belongs to Pomacentrinae, which is the largest subfamily with 24 genera.

In the genus *Abudefduf*, five species have been cytogenetically studied[2, 4, 7, 9)]. These species had the same chromosome number of 2n = 48 and similar karyotypes with low NF values from 50 to 52 (Table 1). Arai and Inoue[2)] reported karyotypes with no NOR data of two species, *A. notatus* "Iso-suzumedai" and *A. sordidus* "Shima-suzumedai". Takai and Ojima[4)] reported that two different species, *A. vaigiensis* "Oyabiccha" and *A. sexfasciatus* "Rokusen-suzumedai" had different karyotypes and NOR-bearing chromosomes, and the difference of the karyotypes was derived from that of the NOR-bearing chromosomes. In *A. vaigiensis*, the NOR-bearing chromosome was a large submetacentric type, and the NORs were located in the terminal regions of the short arms; however, in *A. sexfasciatus*, the NOR-bearing chromosome was a large acrocentric type, and the NORs were located in the pericentromeric regions of the long arms. This difference can be induced by a pericentric inversion in the NOR-bearing chromosome.

Brum et al.[7)] and Molina et al.[9)] reported the karyotype and NORs of *A. saxatilis*, which were the same as those of *A. vaigiensis* (Table 1). In the present study, *A. septemfasciatus* had the same karyotype and NORs as those of *A. vaigiensis*. Thus, there were no clear differences in the karyotypic composition and NOR-bearing chromosomes among the three species, *A. vaigiensis, A. saxatilis*, and *A. septemfasciatus*. Therefore, these three species are classified as Group A in the present paper. In A. bengalensis, the karyotype showed the same composition as Group A, but the NORs were located at different chromosomal regions from Group A (interstitial regions of the short arms in *A. bengalensis* and terminal regions of the short arms in Group A). This difference can be induced by a paracentric inversion in the NOR-chromosome.

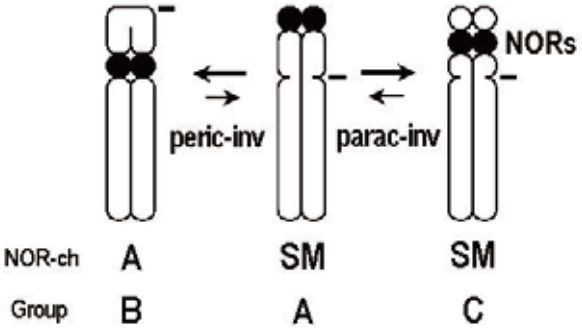


Figure 3.
NOR-bearing chromosomes and their relationships in five *Abudefduf* species. Arrows indicate a direction of karyotypic changes. Thick-line arrows show a more probable direction than thin-line arrows. NOR-ch: NOR-bearing chromosome, A: acrocentric, SM: submetacentric, Group A: *A. vaigiensis, A. saxatilis, A. septemfasciatus*, Group B: *A. sexfasciatus*, Group C: *A. bengalensis*, peric-inv: pericentric inversion, parac-inv: paracentric inversion.Bars indicate centromeric regions.

Figure 3 shows the relationships of the karyotypes and NOR-bearing chromosomes among five *Abudefduf* species inferred on the basis of the present and previous results. In addition to Group A, *A. sexfasciatus* and *A. bengalensis* are classified as Group B and Group C, respectively. Although it is difficult to determine the original karyotype and NOR location, the relationships of the karyotypes among three groups (Group A, B, C) seems clear, and the karyotypic differences can be explained by inversions in the NOR-bearing chromosomes: peri- and paracentric inversion can

induce the karyotypic differences between Groups A and B, and between Groups A and C, respectively.

Molecular phylogeny[16] showed that five species were closely related and classified as a monophyletic group, but detailed relationships were unclear. However, the most general type of NOR-location in pomacentrid and teleost fishes is that in Group A[4, 5]. Therefore, it is more probable that Group A is original, and Group B and Group C are derived from Group A (A →B, A→C).

As stated above, Pomacentridae have widely ranging NF values, but the NF values of *Abudefduf* species are very low and mostly constant. Therefore, the genus *Abudefduf* was cytogenetically the most conserved group in the Pomacentridae. Further studies will be performed in different species, with detailed chromosome analyses with several types of banding techniques to elucidate the karyotypic differentiation process and phylogenetic relationships. In the present study, only one specimen was analyzed in each species. Therefore, the results should be confirmed by investigations of more specimens.

Large-sized NORs and heteromorphism of NORs between homologous NOR-bearing pairs observed in *A. septemfasciatus* have been reported occasionally in fishes[17]. NORs are highly repetitive sites of rDNA[17]. Therefore, heteromorphism of NORs might easily occur by unequal crossing over. However, heteromorphism of NORs was determined in only one specimen and therefore it is unknown whether this phenomenon is specific or not for this species. Generally, it seems a nonspecific event for this species.

In conclusion, the results of the present study suggest that the karyotypes of *Abudefduf* species have diversified mainly through inversion in NOR-bearing chromosomes. Specifically, this study revealed evidence of karyotypic differentiation involving paracentric inversion, which is difficult to identify without G-banding or similar analyses.

Acknowledgment. I would like to thank Editage (www.editage.jp) for English language editing.

References

1) Nelson, J. S.: Fishes of the World, 4th Ed. John Wiley and Sons, Inc., New York (2006).
2) Arai, R. and Inoue, M.: Chromosomes of seven species of Pomacentridae and two species of Acanthuridae from Japan. Bull. Natn. Sci. Mus. Tokyo. Ser. A. 2, 73-78 (1976)
3) Ojima, Y. and Kashiwagi, E.: Chromosomal evolution associated with Robertsonian fusion in the genus *Dascyllus* (Chrominae, Pisces). Proc. Japan Acad. 57B, 368-370 (1981)
4) Takai, A. and Ojima, Y.: Comparative studies of karyotypes and distributions of nucleolus organizer regions in pomacentrid fishes. I. Proc. Japan Acad. 63B, 17-20 (1987)
5) Takai, A. and Ojima, Y.: Comparative studies of karyotypes and distributions of nucleolus organizer regions in pomacentrid fish. 2. Cytobios 65, 199-205 (1991)
6) Takai, A. and Ojima, Y.: Chromosome evolution associated with Robertsonian rearrangements in pomacentrid fish (Perciformes). Cytobios 84, 103-110 (1995)
7) Brum, M. J. I., Correa, M. M. O., and Aguilar, C. T.: Karyotype analysis in south Atlantic perciforms *Micropogonias furnieri* and *Abudefduf saxatilis*. Chrom. Sci. 5, 83-89 (2001)
8) Molina, W. F. and Galetti Jr. P. M.: Robertsonian rearrangements in the reef fish *Chromis* (Perciformes, Pomacentridae) involving chromosomes bearing 5s rRNA genes. Genet. Mol. Biol. 25, 373-377 (2002)
9) Molina, W. F. and Galetti Jr. P. M.: Karyotypic changes associated to the dispersive potential on Pomacentridae (Pisces, Perciformes). J. Exp. Mar. Biol. Ecol. 309, 109-119 (2004)
10) Molina, W. F. and Galetti Jr. P. M.: Multiple pericentric inversions and chromosomal divergence in the reef fishes *Stegastes* (Perciformes, Pomacentridae). Genet. Mol. Biol. 27, 543-548 (2004)
11) Kashiwagi, E., Takai, A., and Ojima, Y.: Chromosomal distribution of constitutive heterochromatin and nucleolus organizer regions in four *Dascyllus* fishes (Pomacentridae, Perciformes). Cytologia 70, 345-349 (2005)
12) Takai, A. and Kosuga, S.: Karyotypes and banded chromosomal features in two anemonefishes (Pomacentridae, Perciformes). Chrom. Sci. 10, 71-74 (2007)
13) Takai, A. and Kosuga, S.: Karyotype and banding patterns of an anemonefish, *Amphiprion perideraion* (Pomacentridae, Perciformes). Chrom. Sci. 16, 47-50 (2013)
14) Howell, W. M. and Black, D. A.: Controlled silver-staining of nucleolus organizer regions with a

protective colloidal developer: a 1-step method. Experientia 36, 1014-1015 (1980)
15) Levan, A., Fredga, K. and Sandberg, A. A.: Nomenclature for centromeric position on chromosomes. Hereditas 52, 201-220 (1964)
16) Jang-Liaw, N.-H., Tang, K. L., Hui, C.-F., Shao, K.-T.: Molecular phylogeny of 48 species of damselfishes (Perciformes: Pomacentridae) using 12S mtDNA sequences. Mol. Phyl. Evol. 25, 443-454 (2002)
17) Takai, A. and Ojima, Y.: Some features on nucleolus organizer regions in fish chromosomes. In: Indo-Pacific Fish Biology: Proceedings of the Second International Conference on Indo-Pacific Fishes (T. Uyeno, R. Arai, T. Taniuchi, and K. Matsuura eds.), pp. 899-909, Ichthyological Society of Japan, Tokyo (1986)

Accepted：April 30, 2016
Corresponodence：
Akinori　Takai
Institute of Life and Environmental　Sciences,Osaka Shin-Ai College, 6-2-28 Tsurumi, Tsurumi-ku, Osaka 538-0053, Japan (E-mail: takai@osaka-shinai.ac.jp)

大阪信愛女学院短期大学紀要　50：A1（2016）

スズメダイ科 *Abudefduf* 属魚類 2 種の核型と核小体形成部位

高井　明徳

スズキ目スズメダイ科の *Abudefduf* 属魚類 2 種 *Abudefduf bengalensis*“テンジクスズメダイ”と *A. septemfasciatus*”シチセンスズメダイ”の核型と核小体形成部位 (NORs) を明らかにした。これらの種は同じ染色体数 2n = 48 を有し、核型構成も同じで、1 対のメタセントリック染色体、1 対のサブメタセントリック染色体、22 対のアクロセントリック染色体で構成され、基本数は 52 であった。NORs は共に 1 対のサブメタセントリック染色体の短腕に認められたが、その位置は両種で異なり、*A. bengalensis* では短腕介在部に対し、*A. septemfasciatus* では短腕端部であった。本研究及び従来の研究結果を基に *Abudefduf* 属魚類は主に NOR 染色体の逆位により核型が分化してきたことが示唆された。本研究では、新たな知見として腕内逆位による核型分化の証拠を示した。腕内逆位は、通常 G-band や類似の分析法を使用しないとその発見は困難であるが、本研究では NORs を指標に見出すことができた。