Comparative coral cytogenetics

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Abstract There is little information available on the chromosomes of scleractinian corals. Until now, the chromosome numbers for only 29 of the 800 species of reef corals have been published, which represents less than 4%. Furthermore, only 6 genera (of 110) in 4 families (of 18) have been examined. The data available show a considerable variation in diploid chromosome numbers within the genus *Acropora*, whereas a diploid chromosome number of 28 is conserved across 4 scleractinian families and may represent the basic chromosome number of this group. Here we present data regarding chromosome numbers and morphologies in six species of scleractinian corals. While the chromosome sizes of *Favia pallida* were suitable for karyotyping (1 to 5 µm in length), those of *Galaxea fascicularis*, *Acropora millepora*, *A. spathulata*, *A. papillare* and *A. nasuta* were too small (less than 1 µm in length) for identification and pairing. *F. pallida* had 14 pairs of metacentric to submetacentric chromosomes; diploid chromosome number estimates for the other species were 26 for *G. fascicularis* and *A. papillare*, 28 for *A. millepora* and *A. spathulata*, and 40 for *A. nasuta*.

Keywords *Acropora*, *Favia*, *Galaxea*, karyotype, chromosome number, polyploidy

Introduction

The number of chromosomes per cell is invariant for most species. Considerable information can be obtained by examining karyotypes: for example, sex determination depends in many cases on the presence or absence of sex chromosomes that may be morphologically differentiated. Comparing chromosomes in different species is also useful when examining evolutionary relationships. In plants, such as wheat, hybridization leads to network-like, or reticulate, phylogenies that can be examined through karyotyping.

Wijsman and Wijsman-Best (1973) first examined coral chromosomes by using somatic tissue from adult colonies. They observed numerous small chromosomes, but were not able to count them. Following the discovery by Harrison et al. (1984) that the majority of coral species reproduce by releasing gametes into the water followed by external fertilization and development, Heyward (1985) used the rapidly dividing cells of such embryos to obtain karyotypes for *Goniopora lobata*, *Lobophyllia hemprichii*, *Montipora digitata* and *M. dilatata*. All four species, representing the 3 families Poritidae, Mussidae and Acroporidae, had 14 pairs of metacentric to submetacentric chromosomes following the nomenclature of Levan et al. (1964). Ten years later Kenyon (1997) observed 28 chromosomes in 16 species of *Acropora*, as well as in *Montipora verrucosa*, *M. spumosa* and *Fungia scutaria*; however, 6 other species of *Acropora* had disparate chromosome numbers of 24, 30 (2 species), 42, 48 and 54. These results indicate that there is considerable variation in chromosome numbers in at least some groups of scleractinian corals and that comparative cytogenetics may shed light on the systematics and evolution of these organisms.

Until now, the chromosomes numbers of only 29 of approximately 800 reef coral species have been determined, representing 6 genera (of 110) in 4 families (of 18). Here we present new data on two coral species common in the Indo-Pacific, *Favia pallida* and *Galaxea fascicularis*, belonging to two families for which no chromosomal data were previously available (Faviidae and Oculinidae). Additionally, four species of *Acropora* were investigated, one of which is very common in Australia (*A. nasuta*). Three others (*A. millepora*, *A. spathulata*, *A. papillare*) present morphological similarities suggesting they may be related by polyploidization or hybridization, an hypothesis that we wished to examine in light of potential differences in their chromosome numbers.

Materials and Methods

Fertilization experiments were conducted at the Orpheus Island Marine Station (James Cook University, Australia) from December 3rd to 6th, 2001. Six species of corals were collected from the reefs near the island and kept in running seawater until spawning: *Favia pallida* Dana 1846, *Galaxea fascicularis* Linnaeus 1767,

Acropora millepora Ehrenberg 1834, *A. spathulata* Brook 1891, *A. papillare* Latypov 1992 and *A. nasuta* Dana 1846. For each species, gametes from three to six colonies were mixed in a large bowl of seawater to maximize fertilization success by avoiding self-incompatibility. Following Kenyon (1997), 10-hour old embryos received a 2-hour 0.02% colchicine treatment, followed by a 20-minute hypotonization in a mix of seawater and tap water (2:1). Embryos were then fixed using three changes of freshly mixed solution of absolute ethanol, glacial acetic acid and distilled water (2:1:1).

For light microscopic observations of the chromosomes, fixed embryos were soaked in diethyl ether for 4-24 hours in order to remove intra-cellular lipids, then returned to the fixative and transferred to a droplet of 2% lacto-aceto-orcein on a glass slide for staining. Best results were obtained with 5 minutes of staining followed by a few seconds of destaining in lactic acid. Stained embryos were squashed between a slide and a coverslip, sealed with transparent nail polish and observed using an Olympus BX50 optical microscope located at the Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus. Digital pictures were taken using a DP50 Olympus camera connected to the microscope. Alternatively, fixed embryos were permeabilized for a few hours in a solution of 0.1% Triton X100, then soaked for 30 minutes in DAPI, a DNA-specific fluorescent dye. Chromosomes were observed under UV using a karyotyping workstation equipped with a Zeiss Axioplan microscope and the GENUS software from Applied Imaging, located at the Muséum National d'Histoire Naturelle in Paris.

Results

Chromosomes of *Favia pallida* varied from 1 to 5 µm in length in their most condensed state (Fig. 1). Chromosomes were easier to observe using DAPI than lacto-aceto-orcein; however, more morphological details could be seen using lacto-aceto-orcein than DAPI. This species was found to possess 14 pairs of metacentric to submetacentric chromosomes.

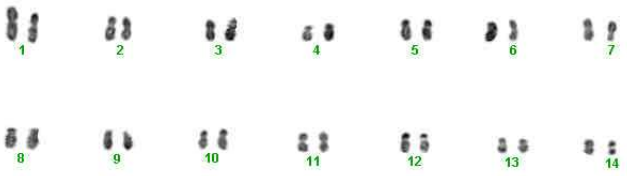

Fig. 1. Karyotype of *Favia pallida* (28 chromosomes).

Chromosomes of *Galaxea fascicularis* (Fig. 2) and the four species of *Acropora* examined (Fig. 3-6) were very small, i.e. less than 1 µm in length in their most condensed state. As a result, chromosome pairs could not be identified and we resorted to counting the numbers of chromosomes in several chromosome plates from several embryos. Lacto-aceto-orcein staining of the DNA yielded easier chromosome counts than DAPI staining because small particles of background fluorescence were difficult to distinguish from bona fide chromosomes using the latter technique. No differences in chromosome numbers were observed between embryos of the same species. Estimate diploid numbers were 26 for *G. fascicularis* and *A. papillare*, 28 for *A. millepora* and *A. spathulata*, and 40 for *A. nasuta*. The chromosome number we found for *A. millepora* was the same as previously determined by Kenyon (1997).

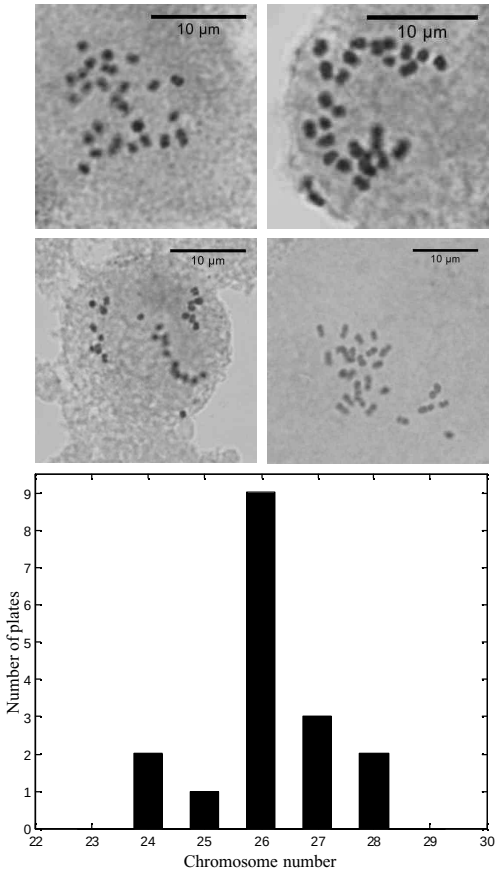

Fig. 2. Four examples of chromosome spreads obtained for *Galaxea fascicularis* (26 chromosomes) and histogram of the chromosome numbers observed in different metaphase plates.

Discussion

The data reported here have increased our knowledge of chromosome numbers in corals to 34 species. As only 4 families had been studied previously, the present study represents an increase in familial coverage of 50% with two families examined for the first time. However, chromosome numbers and morphologies for a vast number of species, genera and whole families of scleractinian corals remain undetermined.

We found 40 chromosomes in *A. nasuta*, which is the first time such a chromosome number is reported for any coral. Based on Kenyon's (1997) Figure 3, a chromosome number of 40 may result from the complete loss of a chromosome pair or the fusion of two pairs of chromosomes in a triploid Acroporid (2n = 42) such as *A. valida*, but there may be other explanations.

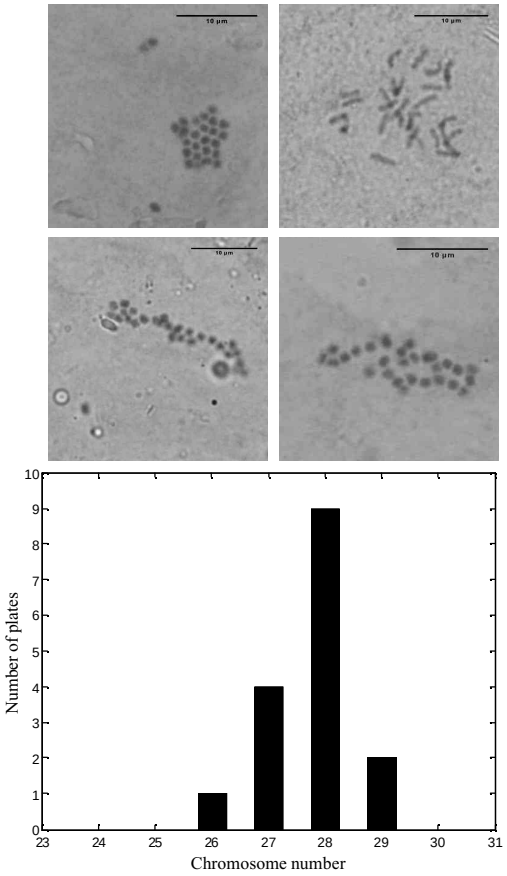

Fig. 3. Four examples of chromosome spreads obtained for *Acropora millepora* (28 chromosomes) and histogram of the chromosome numbers observed in different metaphase plates.

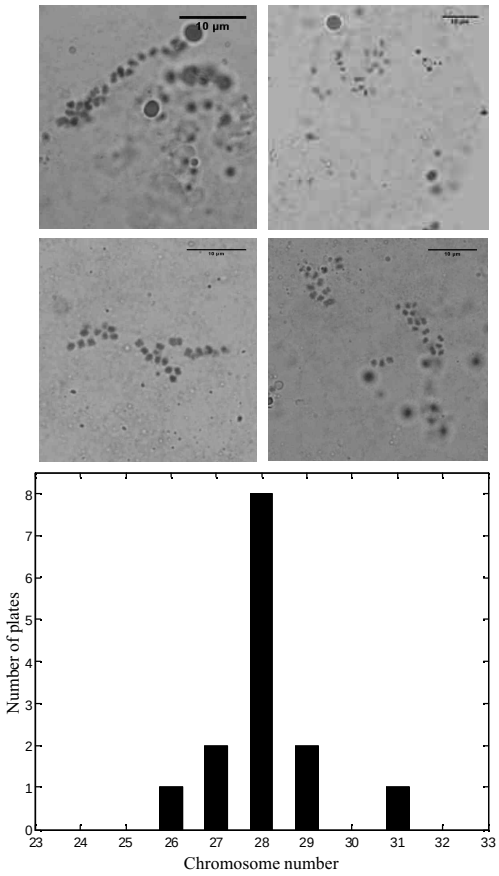

Fig. 4. Four examples of chromosome spreads obtained for *Acropora spathulata* (28 chromosomes) and histogram of the chromosome numbers observed in different metaphase plates.

We found 14 pairs of metacentric to submetacentric chromosomes in *Favia pallida,* which is similar to karyotypes published by Heyward (1985) for four other species. Such a karyotype appears widespread among scleractinian corals since it has been observed in 4 genera belonging to 4 different families: *Montipora* (Acroporidae), *Favia* (Favidae), *Lobophyllia* (Mussidae) and *Goniopora* (Poritidae). Twenty-eight chromosomes were also reported for *Fungia scutaria* (Fungiidae) and 16 *Acropora* species by Kenyon (1997), but no details on chromosome morphology were given. We report here a similar chromosome number for one additional *Acropora* species, *A. spathulata*. Since a chromosome number of 28 is found in both the "robust" and "complex" clades of corals (Romano and Palumbi 1996; Cuif et al. 2003), it is probably ancestral among the Scleractinia. It is, however, not ancestral for Hexacorallia, since a diploid number of 32 chromosomes has been described for two species of sea anemones (Fukui 1993, 1996), and 30 chromosomes for two species of *Hydra* (Rahat et al. 1985). Twenty-six chromosomes, as observed in *Galaxea fascicularis* and *A. papillare*, could be derived from a primitive chromosome number of 28 by fusion of two chromosome pairs or complete loss of one pair of chromosomes. A more thorough examination of chromosome morphologies will allow us to test these hypotheses in the future.

It is difficult to count coral chromosomes on squash preparations because they fall at the lower end of the size range typical of animal chromosomes. Well-spread chromosomes are easier to count, but these counts are not reliable because chromosomes may be displaced to or from neighboring cells. Methodological improvements are needed before chromosome numbers become widely used in coral systematics. Improvements can be made using fluorescent antibodies directed towards coral histones; precise three-dimensional pictures of the chromosomes could then be obtained using a confocal laser fluorescent microscope. Fast and reliable chromosome counts could also be provided by the use of flow cytometry, which is often used to quantify chromosome numbers in animal and plant nuclei and is able to distinguish differences of less than one chromosome. Alternatively, the technique of fluorescence *in-situ* hybridization using fluorescent DNA probes targeting specific sequences would allow researchers to draw homologies between the chromosomes of different coral species, yielding a wealth of new taxonomical information. Such techniques would also allow us to easily check whether species with unusually high chromosome numbers evolved by repeated chromosomal fissions, autopolyploidy or allopolyploidy.

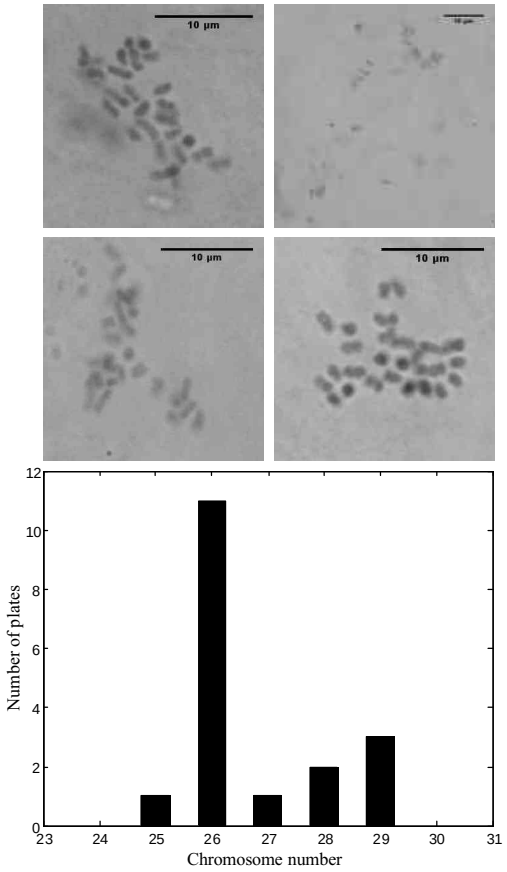

Fig. 5. Four examples of chromosome spreads obtained for *Acropora papillare* (26 chromosomes) and histogram of the chromosome numbers observed in different metaphase plates.

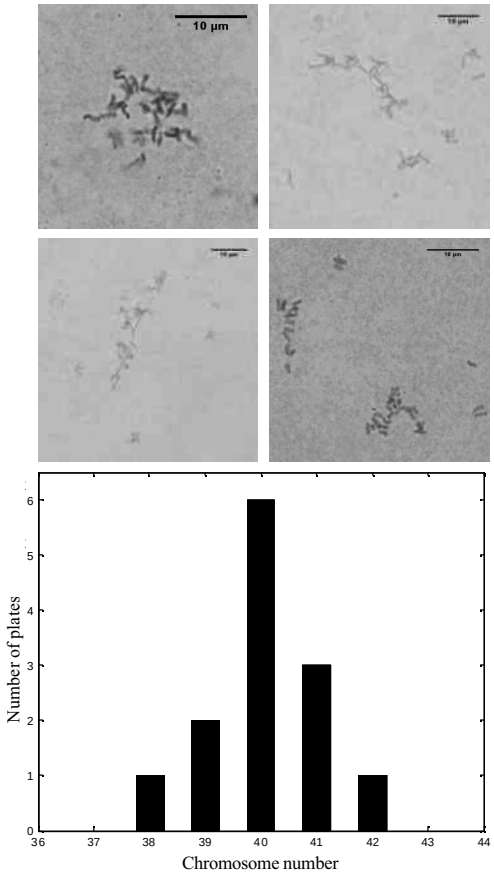

Fig. 6. Four examples of chromosome spreads obtained for *Acropora nasuta* (40 chromosomes) and histogram of the chromosome numbers observed in different metaphase plates.

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