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Variation and Plasticity of Skeletal Color in the Zebra Coral *Oulastrea crispata*

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ABSTRACT—The reef-building scleractinian coral *Oulastrea crispata* (Lamarck) has a conspicuous black skeleton. Skeletal pigments of *O. crispata* from 10 sites in Japan were measured quantitatively to compare geographic variation in skeletal color of the species. The level of pigment concentration varied from place to place, but showed no consistent pattern with the geographic distribution. Cross transplantation experiment between two of the sites showed that the skeletal color is changeable in both directions rather than being a genetically fixed trait at each site. Although environmental factor(s) controlling skeletal pigmentation has not been specified, it is assumed that light and sea water temperature are not involved in skeletal pigmentation.

INTRODUCTION

The reef-building coral *Oulastrea crispata* (Faviidae, Scleractinia) is relatively small, usually less than 10 cm in diameter with an encrusting or massive growth form. This species is usually distributed in turbid environment forming a monospecific assemblage where other corals are seldom found. This species is distributed from tropical regions of the Pacific Ocean to temperate region of central Japan. *O. crispata* is found in sub-tidal biotopes (very rarely >12m depth, Veron 1992). The northernmost record of any zooxanthellate coral was reported for this species from Sado Island in Japan Sea, 38°4'N, 138°14'E (Honma and Kitami, 1978). Furthermore, this species is found at a shore region around the east side of Noto Peninsula, on the west coast of Japan mainland, where sea water temperature reaches 7°C and air temperature falls below freezing point in winter (Yajima *et al.*, 1986). Additionally, this species is unique among scleractinian corals because it has conspicuous black skeleton, hence the common name called “zebra coral”. The columellae, coenosteum and parts of the septa are colored black. Kawaguti and Sakumoto (1954) obtained pigmented acid-insoluble matter from decalcified skeletons of *O. crispata*, and found that the major absorption peak was 405 nm. These skeletal pigments consist of small particles (0.5 μm in diameter) as determined by transmission electron microscopy (Kawaguti, 1985). However until now, quantitative measurements of skeletal color, and comparison of skeletal coloration with respect to geographic distribution have not been conducted. To compare skeletal coloration in this coral, quantitative measurements of skeletal color were carried out on specimens collected from different sites in Japan. Furthermore, cross transplantation was done to determine if skeletal color is a genetically fixed trait or not.

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MATERIALS AND METHODS

Specimens of the coral *Oulastrea crispata* were collected from 10 sites in Japan (Fig. 1) to include different environmental conditions such as clear/turbid, warm/cold waters. Ibaruma (IBA, 24°30'N, 124°16'E), Ishigaki Island, warm and turbid water. Sesoko Island (SES, 26°38'N, 127°52'E), where Sesoko Station of University of the Ryukyus is located, warm and clear water. Shioya (SHI, 26°39'N, 128°06'E), where corals are distributed near an estuary, warm and turbid water. Chichi-jima (CHI, 27°05'N, 142°11'E), Ogasawara Islands, clear water. Kagoshima (KAG, 31°32'N, 130°33'E), near the Kagoshima Port, turbid water. Tomioka (TOM, 32°30'N, 130°02'E), Amakusa Islands, polluted and considerably turbid water. Aitsu (AIT, 32°30'N, 130°25'E), Amakusa Islands, turbid water. Seto (SET, 33°41'N, 135° 22'E), Kii Peninsula, turbid water. Ubara (UBA, 35°07'N, 140°16'E), Bousou Peninsula, clear water. Akasaki (AKA, 37°20'N, 137°15'E), Noto Peninsula, near the northern limit of zooxanthellate corals (this species), turbid and cold water. All specimens (ranged from 2.5 to 7 cm in mean diameter) were collected at a depth 1m or less at low tide except CHI samples collected from 0.5 m to 5 m deep. Coral soft tissue was removed with a solution of 1% sodium hypochlorite, and then the coral skeleton (central region of the corallum including more than 5 corallites was used) was pulverized in a stainless steel mortar. About 0.1 g of skeletal powder was decalcified with a solution of 20% acetic acid. Acid-insoluble matter was obtained as a black residue and concentrated by centrifugation for 10 min at 2,000 rpm, solubilized with 0.2 ml of tissue solubilizer Soluen-350 (0.5 M quaternary ammonium hydroxide in toluen, Packard, Connecticut) for 1 day at room temperature or 2 hr at 50°C, and diluted with an adequate volume of toluen and centrifuged. All the skeletal pigment was in the supernatant, and the pellet was white. Absorption spectra determined by a spectrophotometer (UV-160, Shimadzu, Kyoto) showed a major absorption peak at 405 nm and 4 small subpeaks (Fig. 2). Thus, concentration of skeletal pigment was measured at 405 nm by spectrophotometer (100–10, Hitachi, Tokyo).

To observe initial deposition of skeletal pigment, small corals consisting of a single polyp (not a part of dead colony) were collected from SHI on 8 March 1997, where many small newly settled corals were distributed.

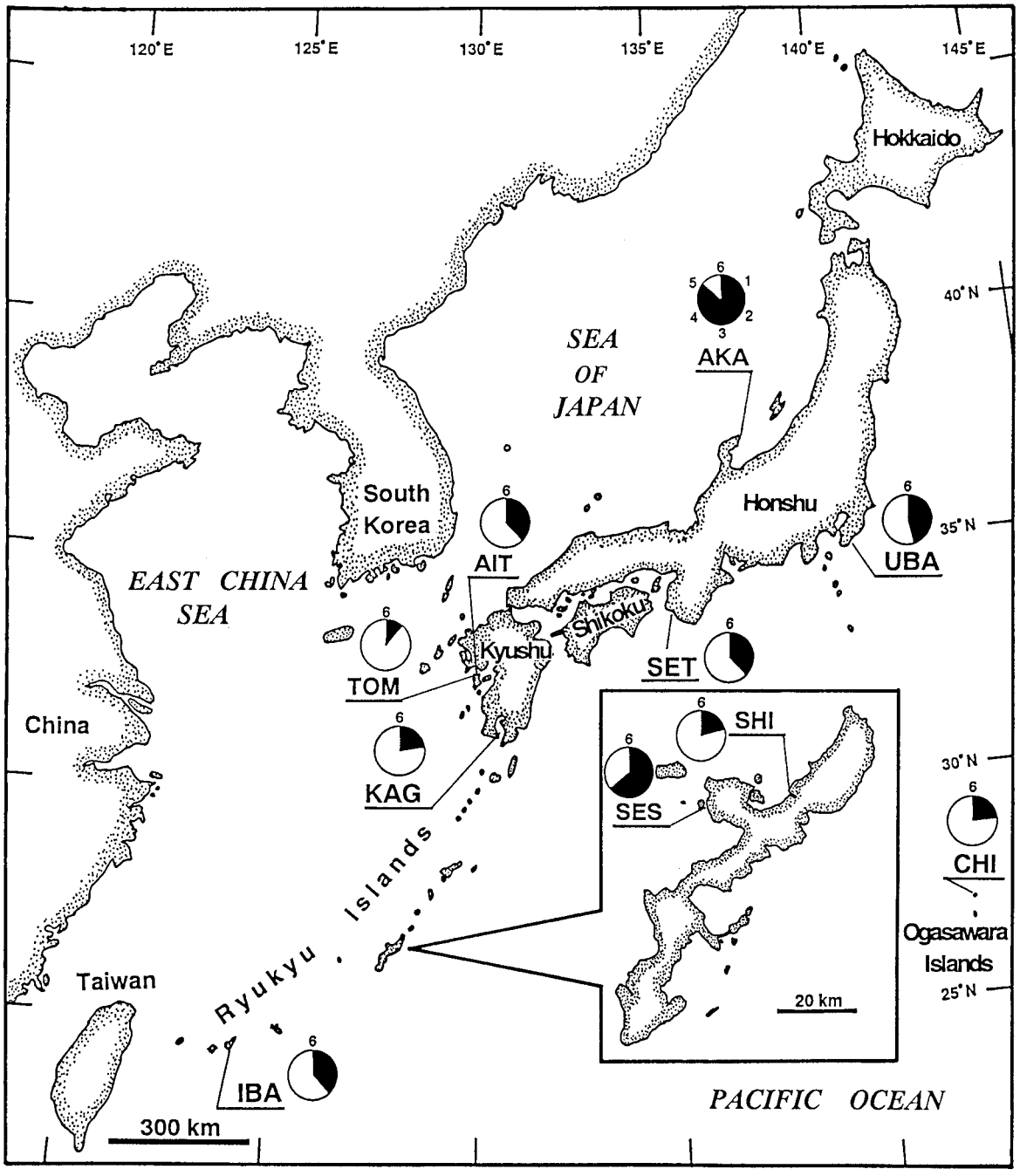

Fig. 1. A map showing the sampling locations of the coral *Oulastrea crispata*. Transplantation experiment was conducted between SES and SHI. Pie charts show the value of skeletal pigments (mg pigment / g skeleton).

Cross transplantation was carried out between SES and SHI to see whether local environmental conditions affect skeletal color. Skeletal color of SES specimens was more blackish than that of SHI. On 3 May, 1992, 13 colonies from each site were split into two halves using a chisel and a hammer to prepare clonemates in the field. One half of the corallum was returned to the site of origin by fixing it to rock substratum with underwater epoxy resin, and the other half was transplanted to the other site and attached in the same way. After 1 year (on 5 May 1993), all transplanted corals were collected again and the skeletal color of the two clonemates was compared visually because the one year skeletal growth was superficial to study quantitative comparison.

In order to determine the effect of light quantity on the skeleton color, based on the result of cross transplantation between SES and SHI (see results), seven SHI specimens were transferred to SES (Sesoko Island) on 8 November 1997. Specimens were split into halves and one half was reared for 215 days in a shaded aquarium (about 15% of natural sunlight) covered with a black nylon net. Similar treatment was done for SES specimens (n=8). The other half was washed with a solution of 1% sodium hypochlorite as a control.

RESULTS

Treatment by a 1% solution of sodium hypochlorite over

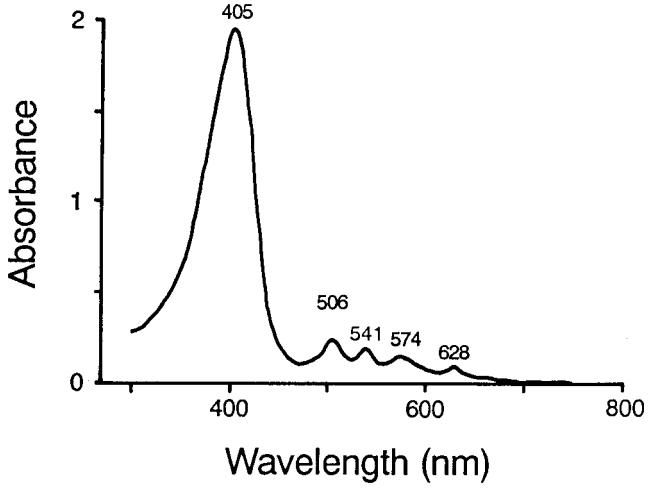

Fig. 2. Absorption spectrum of skeletal pigments of the coral *Oulastrea crispata*. Acid-insoluble skeletal pigments were solubilized with Soluen.

a 3 day period to remove coral soft tissues did not cause bleaching of the colored skeleton. The tissue solubilizer Soluen completely solubilized the pigment as brown solution. Absorption spectra in a Soluen solution of skeletal pigments from the 10 sites in Japan were similar with respect to main peak at 405 nm and 4 subpeaks at 506, 541, 574 and 628 nm (Fig. 2).

Skeletal color of the coral *Oulastrea crispata* widely varied from nearly white to black (Figs. 3, 4). Coral specimens collected from TOM were almost white, and those from AKA was almost black. This was supported by the results obtained from the quantitative measurements for the skeletal pigment (Fig. 4). The value of skeletal pigment was highest in AKA specimens followed by SES. The color variation for specimens from the same site was small. The differences in the value of skeletal pigment were significant ($p<0.0001$, Kruskal-Wallis test).

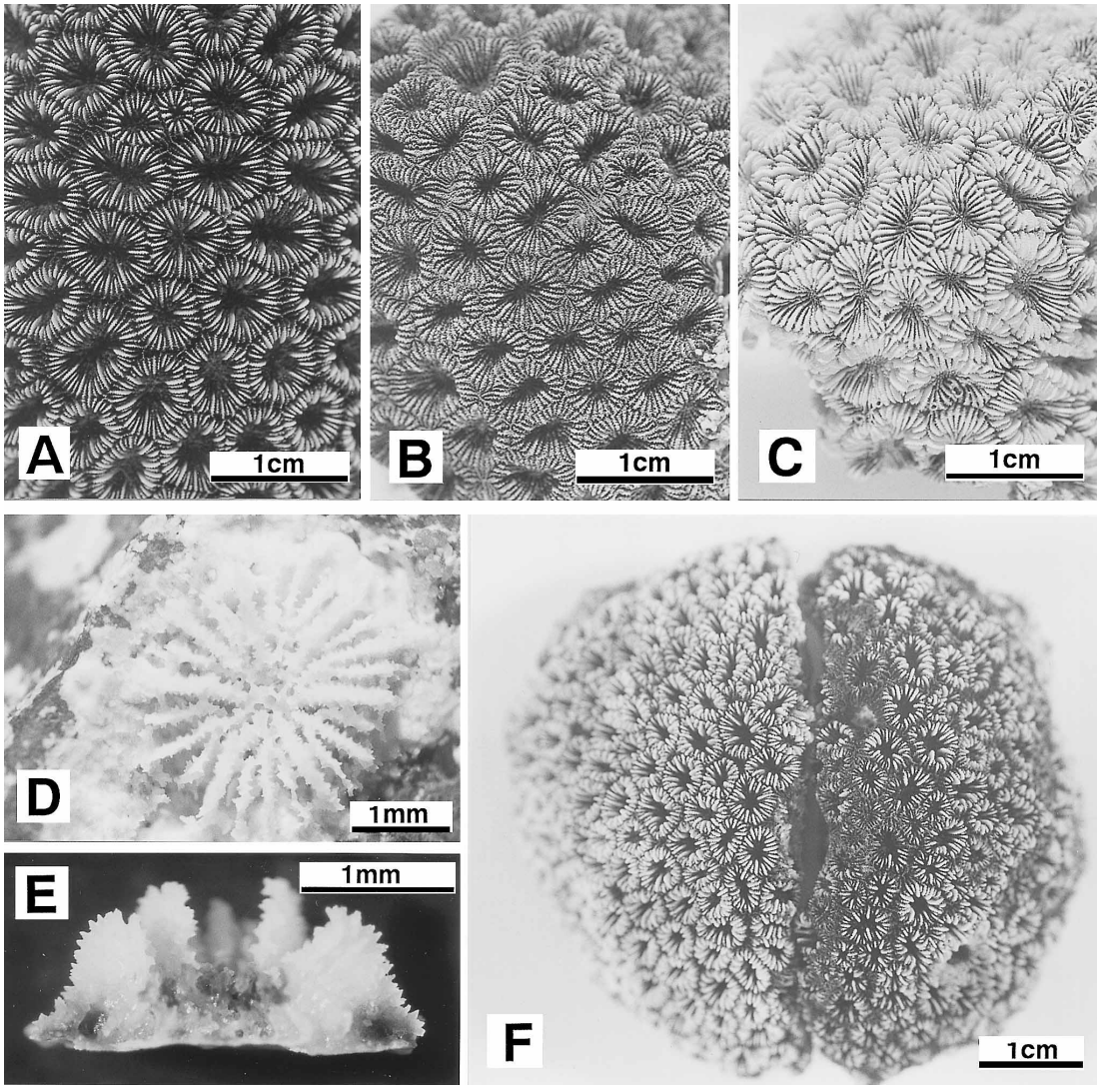

Fig. 3. Bleached and dried skeletons of *Oulastrea crispata*. A, Sesoko Island (SES). B, Akasaki (AKA). C, Tomioka (TOM). D, A young corallite on a substratum of calcareous alga showing a white skeleton. E, Lateral view of a fractured corallite. Columella, basal plate and lower portion of septa are pigmented black. F, Two halves of a SES corallum 1 year after transplantation. The left half of which was transplanted to SHI and the right half was a control that was returned to SES after splitting it.

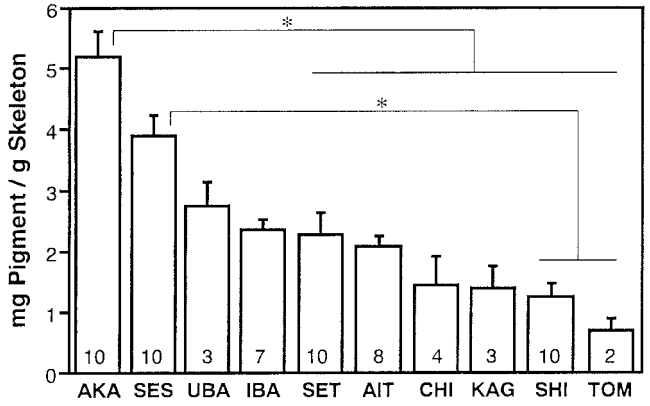

Fig. 4. Comparison of the content of skeletal pigment of *Oulastrea crispata* collected from 10 sites in Japan. Vertical bar represents standard errors. The number in each column is specimens examined. *p<0.05, by nonparametric multiple comparison (Zar, 1996).

In newly settled corals from SHI consisting of a single corallite, pigmentation appeared first as small (about 50 μm diameter) brownish spots, then expanded on skeletal elements including the columella, basal plate or lower portion of septa (Fig. 3E). Corals, consisted of a single corallite, without such pigmentation were also observed (Fig. 3D).

Cross transplantation experiment between SES and SHI caused skeletal color change. Of 52 cut specimens for transplantation (prepared from 26 colonies), 5 specimens were lost from the transplant area during the experiment period, probably due to strong wave action during typhoon season. At the end of the experiment, the numbers of comparable colonies were 10 (from SHI to SES) and 11 (from SES to SHI). Corals that remained attached to the substratum were all alive. One year after transplantation, SHI specimens transplanted to SES showed an increase in the area of black skeleton when compared to control corals returned to SHI. Of ten specimens (from SHI to SES) 7 increased skeletal coloration and other 3 showed no difference ($p<0.01$, Wilcoxon signed rank test). On the other hand, the area of black skeleton was reduced in the SES specimens transplanted to SHI (Fig. 3F). Of eleven specimens (from SES to SHI), 10 reduced skeletal coloration and only 1 specimen showed no difference ($p<0.01$). For the corals whose skeletons changed to black, the colored inner parts of the septa became wider.

In order to observe the effect of light quantity on the skeletal color, 7 corals from SHI were moved to SES. All the SHI corals, reared under dim light at SES for 215 days, showed skeletal growth and the skeletal color changed black ($p<0.01$ Wilcoxon signed rank test). SES specimens treated similarly (n=8) did not show skeletal color change.

DISCUSSION

With the tissue solubilizer Soluen, skeletal pigments were readily solubilized following quantitative analysis of the black skeleton of *Oulastrea crispata*. This reagent has also been used to measure melanin from melanoma cells and hair that were difficult to solubilize for photometric measurement (Oikawa and Nakayasu, 1973).

Cross transplantation experiments showed that the skeletal color of *Oulastrea crispata* is changeable, that is, transplanted corals changed their skeletal color so that they appeared more similar to corals in the area to which they were moved. Although reciprocal transplantation experiments were carried out only between SHI and SES in this study, it is likely that skeletal color of the corals of other sites will also change if transplanted to different sites. Skeletal pigmentation appears to start at the early growth stage after settlement as secondary deposition accompanying calcification. Some environmental factors such as water temperature, salinity, eutrophication, wave action, sedimentation and light intensity including UV radiation may be affecting skeletal color of *O. crispata*. However, it is unlikely that simple latitudinal change in water temperature is the factor since there is no apparent north-south pattern in skeletal pigment (Fig. 1). River run-off may cause complex effects to the corals: decrease in salinity, increase of nutrients and increased sedimentation (shading effect). Considering that SHI specimens living in turbid environment increased black skeleton area when reared at SES under dim light condition, it is unlikely that light has the role controlling skeletal pigmentation. Although the environmental factor(s) influencing skeleton color has not yet been specified, it so that attention must be directed to the physiological role of pigment deposition.

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