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Genetic polymorphism of the adult medusae invading an electric power station and wild polyps of *Aurelia aurita* in Wakasa Bay, Japan

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A large quantity of the jellyfish, *Aurelia aurita*, invade cooling water systems and cause serious problems at several electric power stations in Japan. In the present study, we examined intra-species genetic variation of *A. aurita* in Wakasa Bay, Japan in order to estimate the original polyp habitat of the adult medusae invading electric power stations. Total DNA was extracted from the adult medusae and the wild polyps, and polymerase chain reaction (PCR) was performed using the specific primers for amplification of nuclear internal transcribed spacer one (ITS-1) and mitochondrial cytochrome oxidase *c* subunit 1 (CO1). Then the DNA sequences of the PCR products were compared. The results showed genetic polymorphism of *A. aurita* in Wakasa Bay and locally specific frequency of each haplotype. The haplotype frequency, especially in CO1, of the adults collected at one of the power stations in Wakasa Bay was similar to that of the polyp colonies at harbours in the embayed area, not at another harbour in the western entrance of the bay. The polymorphic analysis is, therefore, thought to be useful for the determination of original polyp habitat as source of the adult medusae in relatively limited regions such as Wakasa Bay.

INTRODUCTION

The cosmopolitan jellyfish *Aurelia* is another fouling animal in addition to several sessile marine organisms invading electric power stations. A large quantity of the jellyfish, *Aurelia aurita*, sometimes invade the cooling water systems and cause serious problems for the operation of power stations, and some measures have been taken against these invasions (Marks & Cargo, 1974; Marks, 1975). In this jellyfish, fertilized eggs develop to swimming planula larvae, and then settle and metamorphose to polyps on the substrata. The polyps asexually proliferate by budding and constitute colonies on suitable substrata. Moreover, the polyp asexually (by strobilation) releases a number of free-swimming ephyrae which develop into medusae. Few ecological investigations on wild polyp colonies of *Aurelia* in natural habitats have been carried out, in contrast to many investigations on the field ecology of ephyrae and adult medusae in some areas (Yasuda, 1970, 1974, 1975, 1976; Toyokawa et al., 1997). Therefore, correlation between wild polyp habitats and the adult medusae invading electric power stations have not been elucidated.

On the other hand, taxonomy in the genus *Aurelia* has not been established because of their ecological and morphological plasticity (Gershwin, 2001). Recently, molecular studies on worldwide speciation of the jellyfish have been developed, and DNA sequence data indicated the need to revise the taxonomy (Dawson & Jacobs, 2001; Schroth et al., 2002). Management strategies for natural populations can often benefit from knowledge about the movement patterns of organisms from their natal sites and the magnitudes of realized gene flow that such dispersal entails. Molecular markers can assist greatly in these analyses, and the findings often have implications for applied problems (Avise, 2004).

In the present study, we examined the intra-species genetic variation of *A. aurita* along the western coast of Wakasa Bay in the central part of Japan in order to estimate the original polyp habitat of the adult medusae invading an electric power station located on the coast.

MATERIALS AND METHODS

Animals and sample preparation

Adult medusae invading the power station A in Wakasa Bay on 20 July, 2002 and on 9 July, 2003 were collected with a landing net. Twelve individuals in 2002 and 50 in 2003, respectively, were selected at random for analyses. After cleaning with seawater, bell margin tissues with tentacles were biopsied and preserved in 99.5% ethanol. By SCUBA diving, very large and dense colonies of wild polyps of *Aurelia* were observed on the shadowed surfaces (around the depths of 0.2–2 m) of quays or pontoons in three harbours B, C and D. The wild polyps were collected from five to 13 points in each harbour.

Several polyps inside the area of 400 cm² were collected in each point, transferred to the laboratory and cultured. Three to nine individual polyps were selected at random from each point, and each selected polyp was kept in a Petri dish one by one. Each polyp was clonally cultured by feeding twice a week with *Artemia* larvae. When the polyp asexually proliferated to more than ten individuals by budding in the Petri dish, they were preserved in 99.5% ethanol for DNA preparation.

DNA extraction, amplification and sequencing

DNA was extracted from all specimens using Easy DNA Kit (Invitrogen). DNA templates (40 ng) were subjected to PCR in 20 µl total volume of 1 x PCR buffer, containing 4 pmol of dNTP mixture, 10 pmol of each primer and 1 unit of Taq polymerase (Gene *Taq*, Nippon Gene). Cycling profile on a GeneAmp PCR System 2700 (Applied Biosystems) thermal cycler was 35 cycles of 15 s at 95°C, 15 s at 55°C (for ITS-1) or 48°C (for CO1), and 30 s at 72°C, followed by a final extension step at 72°C for 7 min. The following primer pairs were used for amplification of fragments of nuclear internal transcribed spacer one (ITS-1) and mitochondrial cytochrome oxidase *c* subunit 1 (CO1), respectively (Folmer et al., 1994; Dawson & Jacobs, 2001). Primers for ITS are jfITS1-5f = 5'–ggtttccgtaggtgaacctgcggaaggatc–3' and jfITS1-3r = 5'–cgcacgagccgagtgatccaccttagaag–3'. Primers for CO1 are HCO2198 = 5'–taaacttcagggt gaccaaaaaatca–3' and AaCO1i-L = 5'–gcccgtyy taataggrgggtttgg–3'. Amplifications were confirmed by standard submarine gel electrophoresis using 1% agarose/TBE gels, stained with ethidium bromide.

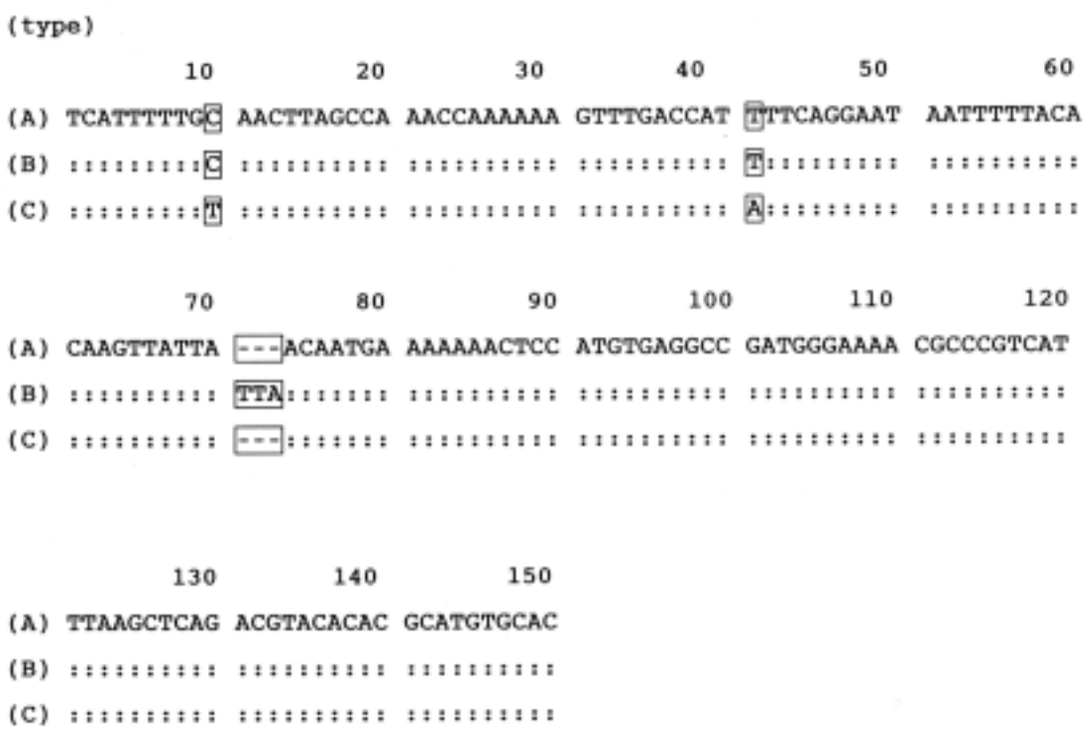

Figure 1. Sequence variation of ITS-1. The partial sequences of nuclear coded ITS-1 gene from the sampled polyps and adult medusae showed three polymorphic sites in 150 bp (10th, 41st and 71st–73rd), and were classified into three sequence types A, B and C. Each individual animal was found to be either homo- or hetero-zygote. All six genetic types (AA, AB, AC, BB, BC or CC) were found in the specimens examined.

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Some PCR products were sequenced directly using ABI PRISM377 automated DNA sequencer. Sequencing of the remaining PCR products was consigned to Simadzu Genomic Research Center. The PCR products were directly sequenced by the dideoxy chain termination method on a RISA-384 automated DNA sequencer (Shimadzu) with Dynamic ET Terminator Cycle Sequencing Kit (Amersham) in accordance with the manufacture's instructions. A sequence homology search was performed using BLAST.

RESULTS

DNA sequence data of the nuclear internal transcribed spacer one (ITS-1) were obtained from six adults and 54 polyps collected in 2002. Those of mitochondrial cytochrome oxidase *c* subunit 1 (CO1) were obtained from five adults and 48 polyps collected in 2002 and 22 adults in 2003.

The partial DNA sequences of ITS-1 showed three polymorphic sites in 150 bp, and were classified into three sequence types A, B and C (Figure 1). Each individual animal was shown to be either homo- or hetero-zygote, and all specimens were grouped into six genetic types (AA, AB, AC, BB, BC and CC). The frequency of ITS-1 sequence types is shown in Figure 2. The adult medusae invading power station A on 12 July 2002 were mainly composed of type A (40%) and B (50%). On the other hand, most (85%) of the polyps in harbour B were type A. The frequencies of the polyps in harbour C (type A: 55%, type B: 30%) and in harbour D (type A: 50%, type B: 40%) were similar to that of the adult medusae invading the power station. The frequency distribution of ITS-1 sequence type is not significantly different among three polyp habitats (harbours) (χ^2=7.7, df=4, P>0.1). However, the frequency is significantly different between harbour B and power station A (χ^2=9.3, df=2, P<0.01), while not significantly different between harbour C and station A (χ^2=1.3, df=2, P>0.1), harbour D and station A (χ^2=0.55, df=2, P>0.1).

The partial sequences of CO1 showed 14 polymorphic sites in 360 bp (Figure 3). Based on the variation of three sites (12th, 192nd and 303rd), specimens were grouped into three genetic types a, b and c. The frequency of CO1 genetic types is shown in Figure 4. Only type a was found in the adult jellyfish invading the power station on 12 July 2002, and the polyps in harbour C. On the other hand, most (90%) of the polyps in harbour B were type c. In harbour D, both type a and type c were seen. In the adults invading the power station in 2003, type a was dominant as in 2002 although a small number of type b and c was also found. The frequency distribution of CO1 sequence type is significantly different among three polyp habitats (harbours) (χ^2=22.7, df=4, P<0.01). Moreover, the frequency is significantly different between harbour B and power station A in 2002 (χ^2=13.9, df=2, P<0.01), harbour B and A in 2003 (χ^2=16.4, df=2, P<0.01). On the other hand, the frequency is not significantly different between harbour C and station A in 2002 (χ^2=0, df=2, P>0.1), C and A in 2003 (χ^2=3.7, df=2, P>0.1), D and A in 2002 (χ^2=4.3, df=2, P>0.1), D and A in 2003 (χ^2=2.0, P>0.1).

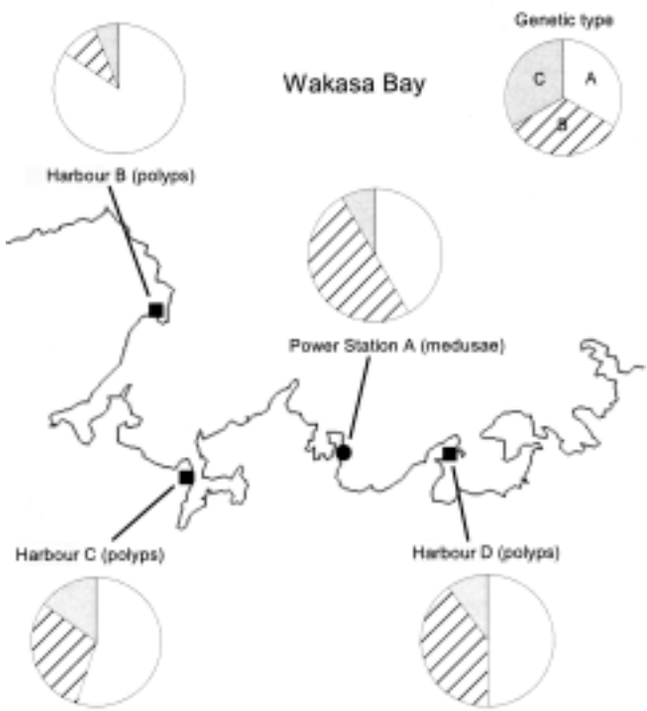

Figure 2. Frequency of ITS-1 sequence types. Sequence data of ITS-1 were obtained in six adult medusae collected at power station A, 23 polyps collected at harbour B, 14 polyps at harbour C and 13 polyps at harbour D. Sequences of polyps collected in a small area (approximately 400 cm^2) were all identical, and the number of clones was 16 at harbour B, 10 at harbour C and 5 at harbour C, respectively. Frequency of each sequence type is based on the number of clones.

DISCUSSION

In the present study, some genetic variations were detected in the jellyfish, *Aurelia* in Wakasa Bay and locally specific frequencies of each haplotype were observed. These results show that DNA sequencing analyses of ITS-1 and CO1 are useful for the study on intra-species genetic variation in adult medusae and wild polyps. On the other hand, all sequences of the polyps examined in a small area (approximately 400

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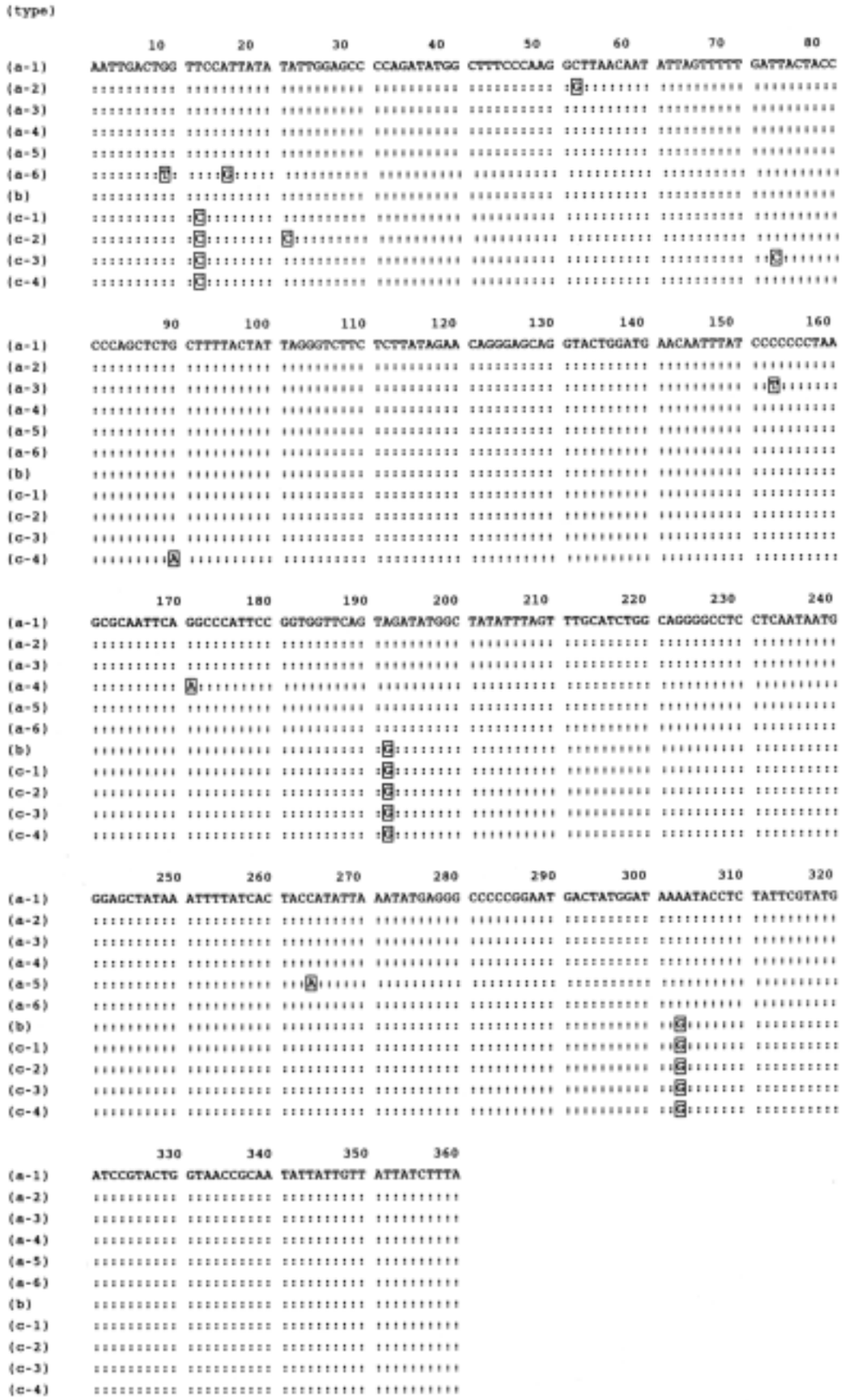

Figure 3. Sequence variation of CO1. The partial sequences of CO1 in polyps and adult medusae showed 14 polymorphic sites in 360 bp. Based on the variation of three sites (12th, 192nd and 303rd), three genetic types, a, b and c were grouped.

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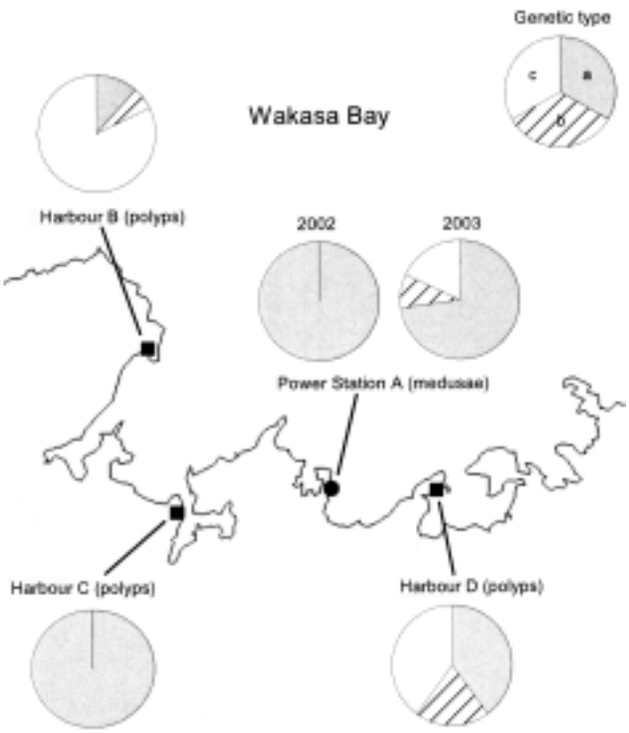

Figure 4. Frequency of CO1 genetic types. Sequence data of CO1 were obtained from five and 22 adult medusae collected at power station A in 2002 and 2003, respectively, 20 polyps collected at harbour B, 15 polyps at harbour C and 12 polyps at harbour D. Sequences of polyps collected in a small area (approximately 400 cm^2) were all identical, and the number of sampled clones was 14 at harbour B, 11 at harbour C and 5 at harbour C, respectively. Frequency of each sequence type is based on the number of clones.

cm^2) were identical (data not shown), suggesting their asexual reproduction by budding. These indicate that wide collecting points of polyps in each area are necessary for the examination of genetic variation of polyp colony. In the present study, we collected polyp samples at several points covering a wide area in each harbour, but collecting at more points will lead to a more accurate analysis.

The haplotype frequency of the adults collected at power station A was similar to that of the polyps at harbour C and D in embayed area, but not at another harbour B at the western entrance of the bay. Statistical analysis showed that the frequency in ITS and CO1 is significantly different between harbour B and power station A. These results indicate the possibility that the original polyp habitat is a source of the adult medusae invading the power station A and is located in an embayed area such as harbour C. However, more sequence data will be necessary to reveal the detailed relationship between the adult medusae invading power stations and the polyp colonies. At the same time, systematic investigations of jellyfish behaviour and ecology and ocean currents are necessary. Nevertheless, the present study indicates that DNA sequencing analyses of molecular markers such as CO1 are valid to examine the original polyp habitat of fouling medusae.

Our sequence data also indicate that species of the jellyfish in Wakasa Bay is thought to be *Aurelia aurita* (Schroth et al., 2002) or *Aurelia* sp. 1 (Dawson & Jacobs, 2001).

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