CHAPTER 2

MATERIALS AND METHODS

Four species of genus Montipora were collected from the reefs of Sesoko Island (127° 52′ E, 26° 38′ N), East China Sea for this study. These species were Montipora foliosa (folioceous), M. ehrenbergii (encrusting), M. digitata (branching) and M. foveolata (massive with encrusting base). For M. foliosa, there are two color morphs which are obviously distinguished, i.e., brown colony morph and purple-edge colony morph. Taxonomical identification of these species was based on Bernard 1897 and Veron and Wallace (1984). Since there are some difficulties in the identification of scleractinian corals in genus Montipora, these species will be confirmed again. In the present study, short-term laboratory experiments, long-term experiments and field observations were performed.

2.1 Short-term laboratory experiments

Short-term laboratory experiments consisted of two sets of experiments.

2.1.1 Set I experiment

In order to observe the intial mechanisms of interactions in xenogeneic combinations of the four species of genus Montipora, set I of short-term laboratory experiments were conducted in December, 1985 and August, 1986. Ten sets of xenogeneic combinations were prepared, one set consisted of six pairs among four

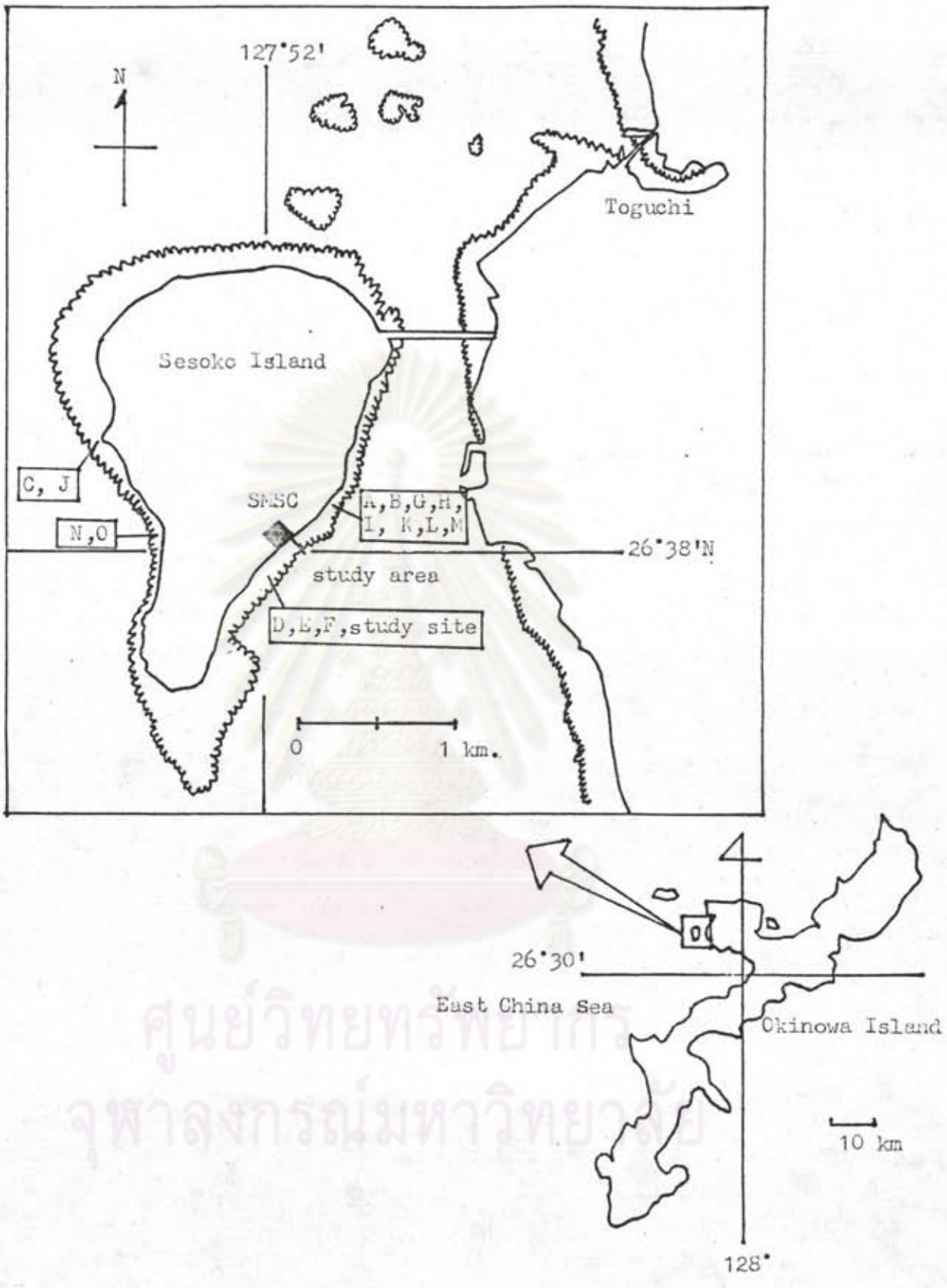

Fig. 1 Map of Okinawa and Sesoko Island showing the locations of Sesoko Marine Science Center, study site, study area and coral aggregate areas where coral colonies used for the experiments were collected from.

SMSC : Sesoko Marine Science Center.

species, i.e., M. foliosa - M. ehrenbergii, M. foliosa - M. digitata, M. foliosa - M. foveolata, M. ehrenbergii - M. digitata, M. ehrenbergii - M. foveolata and M. digitata - M. faveolata. The colonies used in this experiment were collected from the reefs of Sesoko Island and were broken into fragments. All fragments (3.5 cm. in diameter) were carefully fixed by underwater adhesive in small cups (12 cm. in diameter and 6 cm. in depth) and were cultured in aquariums supplied with running sea water. Observations under binocular mi microscopes were conducted at 1, 3, 6, 12, 18, 24 hours after setting and every six hours for one week there after. The pairs were returned to aquariums after short observation period.

2.1.2 Set II experiment

In order to examine the initial mechanism of interaction in xenogeneic combinations under four different conditions, i.e., dark-high temperature (0 lux, 30 ^{o}C), dark-low temperature (0 lux, 20 ^{o}C), light-high temperature (5500 lux, 30 ^{o}C) and light-low temperature (5500 lux, 20 ^{o}C). Set II of short-term laboratory experiments were conducted in December, 1986. Five colonies of each species (M. foliosa, M. foliosa (purple), M. ehrenbergii, M. digitata and M. foveolata) were collected from the reefs of Sesoko Island, broken into fragments and acclimatized before setting the experiments. There were nine xenogeneic combinations : M. foliosa - M. ehrenbergii, M. foliosa (purple) - M. ehrenbergii, M. foliosa - M. digitata, M. foliosa (purple) - M. digitata, M. foliosa - M. foveolata, M. foliosa (purple) - M. foveolata, M. ehrenbergii - M. digitata, M. ehrenbergii - M. foveolata and M. digitata - M. foveolata. Each xenogeneic combination consisted of 20 pairs which were combined

from five colonies of each species; five pairs were used in each experimental conditions. Setting the experiments were carried out in the following way. Fragments (3 - 5 cm. in diameter) were brought into contact in plastic cups which were maintained in water baths with controlled light intensity and temperature for the four experimental conditions. Observations were conducted every 15 minute intervals until the end of the experiments (36 - 48 hours after setting).

In addition, 60 allogeneic pairs including allogeneric pairs between the different color morphs of M. foiosa and 50 isogeneic pairs of the four Montipora species were performed in the same manner with set I experiment.

2.2 Long-term experiments

In order to investigate processes of interactions in long-term, setting of field experiments and laboratory experiments were conducted during December, 1985 and December, 1986. Setting of field experiments were performed in the following way. The corals were collected and broken by a hammer and chisel into several small fragments (3 - 5 cm. in diameter) only unharmed fragments were used for grafting experiments. Pairs of fragments were arranged in contact with each other and fixed on concrete blocks by underwater adhesive (5 pairs/block). The cement blocks were fixed on hard bottom by cement. The study site was located near the edge of fringing reef, 95 m. offshore (depth, 2.3 - 3.5 m, MSL) of the coast of Sesoko Island, the same place where Nakaya (1984) conducted his experiments (see Fig. 1). Long-term

laboratory experiments were conducted in the same way with field experiments but pairs of fragments were fixed on plastic plates by underwater adhesive and maintained in a deep holding tank supplied with running sea water by hanging plastic plates at 1 m. in depth. Types of interactions for long-term experiments were inspected every two-week interval until the end of the experiments (14 or 18 weeks after setting). Long-term experiments consisted of xenogeneic grafting experiments, allogeneic grafting experiments and isogeneic grafting experiments.

2.2.1 Xenogeneic grafting experiments

The colonies of four species of Montipora, i.e., M. foliosa, M. foliosa (purple), M. ehrenbergii, M. digitata and M. foveolata, were collected from the reefs of Sesoko Island. For combinations among these species, 10 colonies of each species were collected, divided into 50 fragments and provided 50 random pairs of coral fragments for each combination of two species.

2.2.2 Allogeneic grafting experiments

Three species of Montipora; M. foliosa, M. ehrenbergii and M. digitata, were used for allografts. Allografts consisted of intra-reef allografts, inter-reef allografts, across-island allorafts, inter-morph allografts and the effects of fragment size and region of colony in contact on allograft interactions.

2.2.2.1 Intra-reef allografts

Five colonies of each species were collected from the coral aggregate areas and made combinations among them with

5 replicates (50 pairs for each species). The coral colonies were collected from the coral aggregate area A, G, L and M for M. foliosa, B,H and I for M. ehrenbergii, C. J. and K for M. digitata (Fig. 1, p. 6). Intra-reef allografts of the colonies collected from coral aggregate area A, G, B and C (Fig. 1, p. 6) were conducted in field experiments and the remainder in laboratory experiments.

2.2.2.2 Inter-reef allografts

Grafting among colonies collected from different coral aggregate areas were performed. Five colonies of each species collected from one coral aggregate area were combined with five colonies collected from another so as to form a total of 25 pairs. The combinations of colonies collected from the coral aggregate area A - D, A - E and M - D for M. foliosa, B - F and I - F for M. ehrenbergii, C - N and J - O for M. digitata (Fig. 1, p. 6). Inter-reef allografts of the coral colonies collected from A - D, A - E, B - F and C - N (Fig. 1, p. 6) were conducted in field experiments and the remainder in laboratory experiments.

2.2.2.3 Across-island allografts

Grafting among colonies collected from the opposite site of the island were conducted in the field experiments. Five colonies of M. foliosa collected from the coral aggregate area A were combined with five colonies collected from the western coast of Sesoko Island so as to form a total of 25 pairs. The colonies of M. ehrenbergii collected at area B and those of M. digitata collected at area K were treated similarly as above.

2.2.2.4 Inter-morph allografts

There are two color morphs of M. foliosa which are clearly distinguished, brown colony and purple edged colony. Five colonies of each color morphs were collected to make the combinations among them in total of 25 pairs for field experiments. Inter-morphs allografts were also set in laboratory experiments (25 pairs).

2.2.2.5 The effects of fragment size and region of colony in contact on allograft interaction

One set of M. ehrenbergii, two sets of M. digitata and three sets of M. foliosa were prepared in laboratory experiments in order to investigate the effects of the region of colony in contact. For this propose, fragments were taken from the top or side of the colonies for M. ehrenbergii and M. digitata, upper or lower side of colonies for M. foliosa. The colonies used for grafting in this experiment were collected from different coral aggregate areas. Three sets of M. foliosa and M. ehrenbergii were performed to check the effect of fragment size on allograft reactions. Differences in size between large fragments and small ones were approximately five-fold.

2.2.3 Isogeneic grafting experiments

Isografts were conducted in both field experiments and laboratory experiments to make sure that all fragments from the same colony would fuse with each other. Moreover, isografts studying the effects of contact region of colonies and those of fragment size on interactions were performed in the four species of Montipora.

2.3 Field observations

In order to examine the naturally occurring interactions within and between the species of Montipora, a survey of all colonies of M. foliosa (two color morphs), M. ehrenbergii, M. digitata and M. foveolata were conducted in a rectangular area of 10 m x 100 m of the fringing reef in front of Sesoko Marine Science Center, Okinawa in August - September, 1986. The longer axis of the survey area lies perpendicular to the shore line and extends seaward from the shore to reef edge. Number of colonies, coverage area, number of living fragments and types of interactions of both intraspecific and interspecific reactions of the four Montipora species were recorded. An attempt to increase the number of interactions to be observed, random diving to observe types of interactions across the reefs of Sesoko Island had been performed for many dives in November - December, 1986.