Vol. 201: 179–187, 2000 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published August 9

Reproductive isolation and genetic differentiation of a scleractinian coral *Mycedium elephantotus*

C.-F. Dai[1,*], T.-Y. Fan[1,2], J.-K. Yu[1]

[1]Institute of Oceanography, National Taiwan University, Taipei, Taiwan, ROC
[2]National Museum of Marine Biology and Aquarium, 2 Houwan Road, Checheng, Pingtung 944, Taiwan, ROC

ABSTRACT: Sexual reproduction and genetic variation of the scleractinian coral *Mycedium elephantotus* at Maoao (MO), Yenliao Bay, northern Taiwan, and at Tiaoshi (TS), Nanwan Bay, southern Taiwan, were studied from 1990 to 1996. *M. elephantotus* is a hermaphroditic broadcast spawner with an annual reproductive cycle. Gametogenesis of the MO population began in January, and spawning occurred in July-August. In the TS populations, there were 2 reproductive groups with different timing of gametogenesis and spawning. Gametogenesis of the first group started in November, and spawning occurred in April or May, while gametogenesis of the second group started in March and spawning occurred in August or September. The temporal reproductive isolation of the 2 groups was possibly the byproduct of adaptation. Allozyme electrophoresis was applied to study the genetic variations among regional coral populations and the 2 reproductive groups. Significant genetic variations were detected among the MO and TS populations and between the 2 reproductive groups at TS, but no fixed differences were found. The MO population demonstrated larger genetic distances with TS populations (Nei's unbiased $D = 0.229$ and 0.165, for the first and second reproductive groups respectively) reflecting the effect of isolation by distance. Significant genetic variation and moderate genetic distance ($D = 0.045$) between the 2 reproductive groups at TS indicate the effect of genetic differentiation due to reproductive isolation in sympatric populations. The lack of morphological differences and the moderate genetic distance between the 2 reproductive groups suggest recent diversification within the taxon. The presence of 2 reproductive groups of *M. elephantotus* in Nanwan Bay may represent the initial stage of sympatric speciation in marine environment or a secondary contact of allopatric populations.

KEY WORDS: Reproductive isolation · Genetic variation · Scleractinian coral

INTRODUCTION

Genetic subdivision among populations is a crucial component of the evolution of phenotypic and ecological diversity within species and ultimately in the splitting of lineages to form new species. The degree to which natural populations become differentiated genetically depends largely on the amount of gene flow between them and the nature of selection on genes within populations. Genetic differentiation may arise due to some physical or physiological boundaries that keep 2 or more groups from genetically mixing (Coyne 1992, Palumbi 1994). It is generally suggested that marine species with high dispersal potential have little genetic differentiation over large geographic scales. However, recent studies have shown that genetic differentiation in marine systems may be driven by isolation through physical barriers (Knowlton et al. 1993), long distance (e.g. Hellberg 1994, Palumbi et al. 1997, Johnson & Black 1998), surface circulation vicariance (Yu et al. 1999), reproductive timing (Knowlton et al. 1997), behavioral patterns (Duffy 1996) and other physiological barriers (Palumbi 1994). For sympatric marine invertebrates, reproductive isolation can be the result of different reproductive characteristics, such as mode or timing of reproduction, or spawning patterns, that prevent gametes of different species from crossing.

*Corresponding author. E-mail: corallab@ccms.ntu.edu.tw

Many marine species spawn eggs and sperm into the water column. Fertilization success of these species is closely related to the timing of spawning. As a result, selection for spawning synchrony may occur in these species, and closely related species or populations can be isolated by temporal separation of spawning. Such reproductive isolation constrains gene flow between populations, with or without reinforcing selection, and may finally lead to genetic divergence and speciation (Coyne 1992). There are many studies documenting the ecological or behavioral changes that give rise to reproductive isolation, but few provide the link between reproductive isolation and genetic diversification (Palumbi 1994).

Several widespread coral species display local and geographic variations in their life histories. Latitudinal and temporal variations in reproductive characteristics have been reported in several coral species (Harrison & Wallace 1990, Richmond & Hunter 1990). These variations could reflect high levels of phenotypic plasticity along environmental gradients or genetic differentiation within a species. For example, the brooding coral *Seriatopora hystrix* exhibits extremely high levels of genetic differentiation among 12 neighboring reefs along 90 km of the Central Great Barrier Reef (GBR) (Ayre & Dufty 1994). Conversely, the widely separated populations of *Pocillopora damicornis* are genetically homogeneous along the length of the GBR (Ayre et al. 1997).

Species boundaries in scleractinian corals are based primarily on morphology, and it is generally assumed that morphological differences between coral species reflect reproductive isolation and equate with genetic differentiation (Lang 1984, Willis 1990). However, recent studies on reproductive isolation and genetic variation of corals have revealed conflicting results. Some studies have shown that morphologically different species are genetically distinct and hence reproductively isolated. For example, the 3 morphological variants of *Montastraea annularis* were considered as sibling species (Knowlton et al. 1992, Knowlton 1993, Weil & Knowlton 1994), the 2 morphs of *Montipora digitata* were identified as reproductively isolated species (Stobart & Benzie 1994). In contrast, no clear genetic distinction was found among morphological species within the coral genus *Platygyra* (Miller & Babcock 1997, Miller & Benzie 1997). The difference in the timing of reproduction has also been applied to separate isoporans into 2 species, *Acropora palifera* and *A. cuneata* (Kojis 1986) and it was subsequently supported by biochemical data (Ayre et al. 1991). However, the 2 types of *Pocillopora damicornis* with different timing of planulation (Richmond & Jokiel 1984) were not genetically distinct (Stoddart 1986). These results suggest that the variation of reproductive timing in relation to genetic and environmental variations is complex.

Mycedium elephantotus (Pallas, 1766) is a widely distributed scleractinian on many Indo-Pacific reefs, where it may form large assemblages and dominate certain reef areas (Dai 1993, Veron 1993). Little information is available on its reproductive biology other than that it is a hermaphroditic spawner and that it participates the mass spawning event in the Great Barrier Reef (Willis et al. 1985, Babcock et al. 1986) and the reefs in Western Australia (Babcock et al. 1994), southern Taiwan (Dai et al. 1992), and Okinawa (Hayashibara et al. 1993). In this study, we present detailed information on the timing of gametogenesis and spawning of *M. elephantotus* in northern and southern Taiwan. We also document the temporal reproductive isolation of 2 sympatric groups in Nanwan Bay, southern Taiwan. Genetic variations between geographically isolated populations and between the 2 reproductive groups were studied to illustrate the effect of reproductive isolation on genetic divergence.

MATERIALS AND METHODS

Study sites. Populations of *Mycedium elephantotus* were studied at Maoao (MO), Yenliao Bay, northern Taiwan (25° 02′ N, 121° 59′ E), and at Tiaoshi (TS), Nanwan Bay, southern Taiwan (21° 55′ N, 120° 45′ E; Fig. 1).

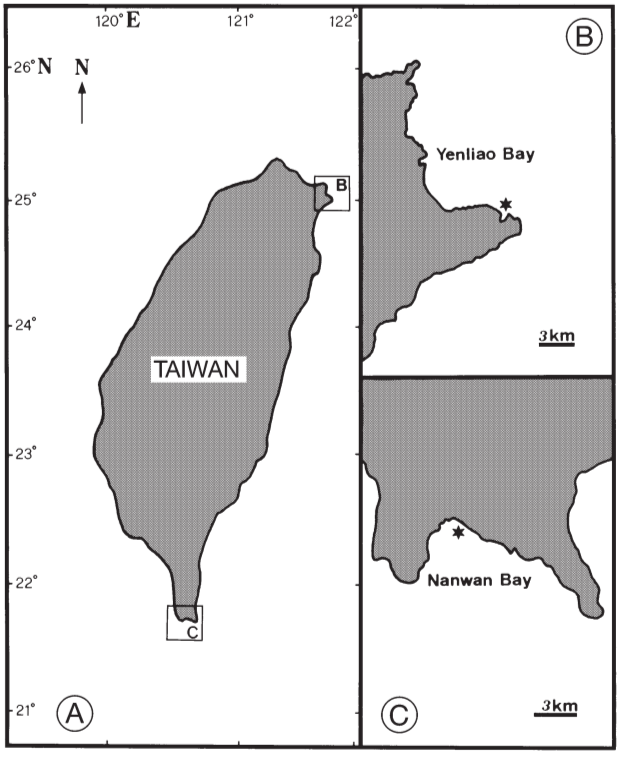

Fig. 1. Map of Taiwan showing the study sites indicated by stars

Coral communities in Yenliao Bay are characterized by a depauperate scleractinian fauna (ca 100 species) and are dominated by species of Faviidae, Agariciidae, and Pectiniidae. *M. elephatotus* is usually abundant on the seaward slopes at 8 to 15 m depth. Monthly mean sea temperatures range from 18.0 to 28.0°C. Nanwan Bay is surrounded by well-developed fringing reefs and inhabited by a rich coral fauna (ca 250 species) (Dai 1991). Foliaceous corals such as *M. elephatotus* and others dominate the reef slope at 15 to 25 m depth (Dai 1993). The marine environment of Nanwan Bay is influenced by the warm Kuroshio current; monthly mean sea temperatures vary from 23.5 to 28.3°C (Dai 1991).

Coral reproduction. Coral samples from 10 to 20 large colonies (>20 cm in diameter) were collected monthly from Yenliao Bay and Nanwan Bay from September 1990 to September 1992. To locate the 2 reproductive groups of *Mycedium elephantotus* in Nanwan Bay, 15 large colonies were tagged and a piece of tissue was sampled monthly from February to September 1992.

Samples were fixed with 10% formalin in seawater for at least 24 h, rinsed in freshwater, decalcified in 8% formic acid, and stored in 70% alcohol. Tissue samples were dehydrated with increasing concentrations of alcohol, cleared with xylene, and embedded in Paraplast. Serial sections 6 to 8 µm thick were prepared and stained with Mayer's hematoxylin and eosin. These slides were examined for gamete development under a compound microscope. The length and width of at least 6 oocytes in nucleolar section were measured for each colony using a micrometer eyepiece. The monthly variation of oocyte size was used to determine the seasonal pattern of oogenesis. The mature egg size was represented by the average of the oocytes collected prior to gamete disappearance.

Allozyme electrophoresis. Tissue samples (fragments of approximately 3×3 cm^2) of *Mycedium elephantotus* were collected randomly from 29 colonies in Yenliao Bay and from 61 tagged colonies in Nanwan Bay in 1995 and 1996. Sexual reproduction of the tagged colonies were then monitored to determine their reproductive groups. After removing epiphytic organisms, chips from a given colony were placed in liquid nitrogen within 2 h of collection, transported to the laboratory, and stored at −70°C.

The coral tissues were scraped off and ground in 2 drops of indicator-extractant (Stoddart 1983) and then homogenized in half of the sample volumes of indicator extractant using a sonicator. Homogenates were centrifuged at $17\,000 \times g$ for 40 min at 4°C, and the supernatants were then stored at −70°C until electrophoresis.

Horizontal electrophoresis with 12% starch gel was performed at 4°C under different buffer systems and electrical conditions. The following 8 enzyme systems were used: malate dehydrogenase (MDH; E.C. #1.1.1.37); esterase (EST; E.C. #3.1.1.-); phosphoglucomutase (PGM; E.C. #5.4.2.2); dipeptidase using glycyl-leucine as a substrate (PEPA; E.C. #3.4.-.-); mannose-6-phosphate isomerase (MPI; E.C. #5.3.1.8); malic enzyme ($NADP^+$) (MEP; E.C. #1.1.1.40); and tri-peptide aminopeptidase using leucyl-glycyl-glycine as a substrate (PEPB; E.C. #3.4.-.-). MDH, EST and PGM were run using a Tris EDTA citrate pH 7.0 (TEC 7.0) buffer (modified buffer of TEC 7.9 in Benzie et al. 1995); PEPA, MPI, and MEP were run using a Tris citrate pH 8.0 buffer; and PEPB was run using a Tris citrate borate LiOH buffer (Redfield & Salini 1980). Alleles were assigned a value based on the ratio of their electrophoretic mobility relative to that of the most common allele.

Statistical analysis. Gene frequencies, basic measures of genetic variability, analyses of heterogeneity among populations, genetic distances and cluster analyses were done using programs in BIOSYS-1 (release 1.7, Swofford & Selander 1989). The hypotheses that each population is in Hardy-Weinberg equilibrium was tested with chi-square tests. When more than 2 alleles are observed at a locus, BIOSYS-1 pools genotypes into 3 classes and repeats the tests. This is accomplished by considering all alleles except the most common one as a single allele.

Two measures were used to assess the possible effects of asexually derived recruits on the genotypic diversity of collections. First, each colony was assigned a multilocus ('clonal') genotype. The number of multilocus genotypes detected (N_G), is thus an estimate of the minimum number of clones that are present within a population; and the ratio N_G:N_I (number of collected individual colonies) provides the simple index of the effects of asexual reproduction on genotypic diversity. Second, the ratio of observed multilocus genotypic diversity (G_O) to that expected under conditions of sexual reproduction (G_E) was calculated following Stoddart & Taylor (1988). Departure of G_O:G_E from unity provides an index of the combined effects of departures from single-locus Hardy-Weinberg equilibrium and multilocus linkage disequlibrium. A genetically variable population with high levels of asexual recruitment should display a low ratio of observed to expected genotypic diversity.

F-statistics use departures from levels of heterozygosity expected under complete panmixia to partition total inbreeding (F_{IT}) into components due to inbreeding within subpopulations (F_{IS}) and subdivision among populations (F_{ST}). The significance of F_{ST}, a measure of genetic differentiation among populations, and of F_{IS}, a measure of genetic variation within populations, was calculated using equations given in Waples (1987).

RESULTS

Gametogenesis

Mycedium elephantotus is a simultaneous hermaphrodite. The oocytes and spermaries are within the mesenteries between the retractor muscles and the mesenterial filaments. Five developmental stages of gametes were classified according to the histological characteristics and relative sizes of oocytes and spermaries. The stages were: (1) oogonia, (2) developing oocytes, (3) developing oocytes and early spermaries, (4) developing oocytes and spermaries, and (5) mature oocytes and spermaries. In Stage 1, few oogonia were found in the mesoglea of the mesenteries and the oogonia were <50 µm in diameter. The oogonium had a nucleus with a prominent nucleolus and a thin layer of cytoplasm. In Stage 2, the oogonia underwent vitellogenesis and were 50 to 150 µm in diameter. In Stage 3, the oocytes were 150 to 200 µm and the spermaries appeared. The spermaries are composed of several discrete sacs which contain a few spermatogonia. In Stage 4, the oocytes were 200 to 250 µm in diameter. The spermaries increased in diameter and the spermatocytes became more numerous and smaller. In Stage 5, the mature oocytes reached a diameter of 260 to 350 µm. The sperm were arrayed in a bouquet with the heads located peripherally and the tails projecting toward the lumen. Mature eggs of *M. elephantotus* before spawning were red.

Reproductive seasonality

Monthly changes of mean oocyte diameters and frequencies of colonies containing different stages of gametes in *Mycedium elephantotus* indicated a clear annual gametogenic cycle (Figs. 2 & 3). In Yenliao Bay, none of the colonies from October to December 1990 nor from September to December 1991 contained gametes (Fig. 3A). In 1991 and 1992, oocytes first appeared in January and increased in size until August. Spermatogenesis started in June (Fig. 3A). All colonies contained mature gametes in July 1991 and 1992. The sharp decline of colonies containing gametes in July and August 1991 and 1992 was possibly a result of spawning.

In Nanwan Bay, 2 reproductive groups which were separated in the timing of gametogenesis and spawning could be distinguished (Figs. 2B & 3B). Colonies within each reproductive group demonstrated a synchronous annual reproductive cycle, while colonies between reproductive groups were different. Oogenesis of the first reproductive group started in November 1990 and October 1991 (Fig. 3B) and oocytes increased in size until May or June. Spermatogenesis began in February 1991 and 1992 (Fig. 3B). The sharp decline of colonies containing mature gametes between May and July 1991 and between June and July 1992 was possibly a result of spawning. In the second reproductive group, oocytes appeared in March 1991 and January 1992 (Fig. 3B) and increased in size until November. Spermatogenesis started in July 1991 and 1992 (Fig. 3B). Spawning of the second group probably occurred from October to December. These results indicated that timing of gametogenesis and spawning of the second reproductive group was approximately 3 to 4 mo later than those of the first group. Individual colonies of *Mycedium elephantotus* had an annual cycle of gametogenesis; this was confirmed by surveying the reproduction of tagged colonies (n = 15). The ratio of colonies belonging to the first and the second reproductive groups was about 7:3.

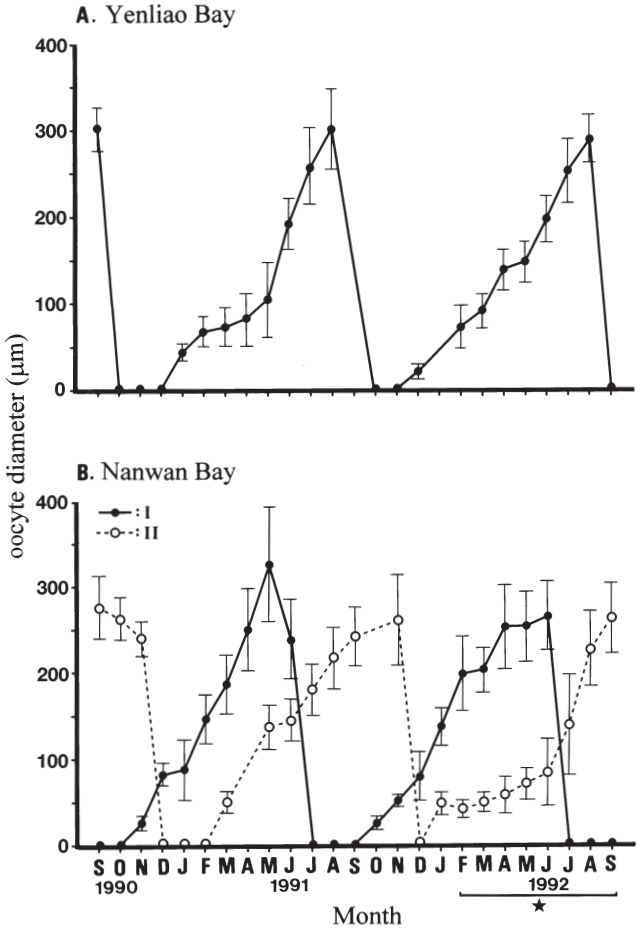

Fig. 2. *Mycedium elephantotus*. Seasonal cycle of mean oocyte diameters of populations in (A) Yenliao Bay, northern Taiwan and (B) Nanwan Bay, southern Taiwan. I: first reproductive group; II: second reproductive group. ★: samples collected from tagged colonies

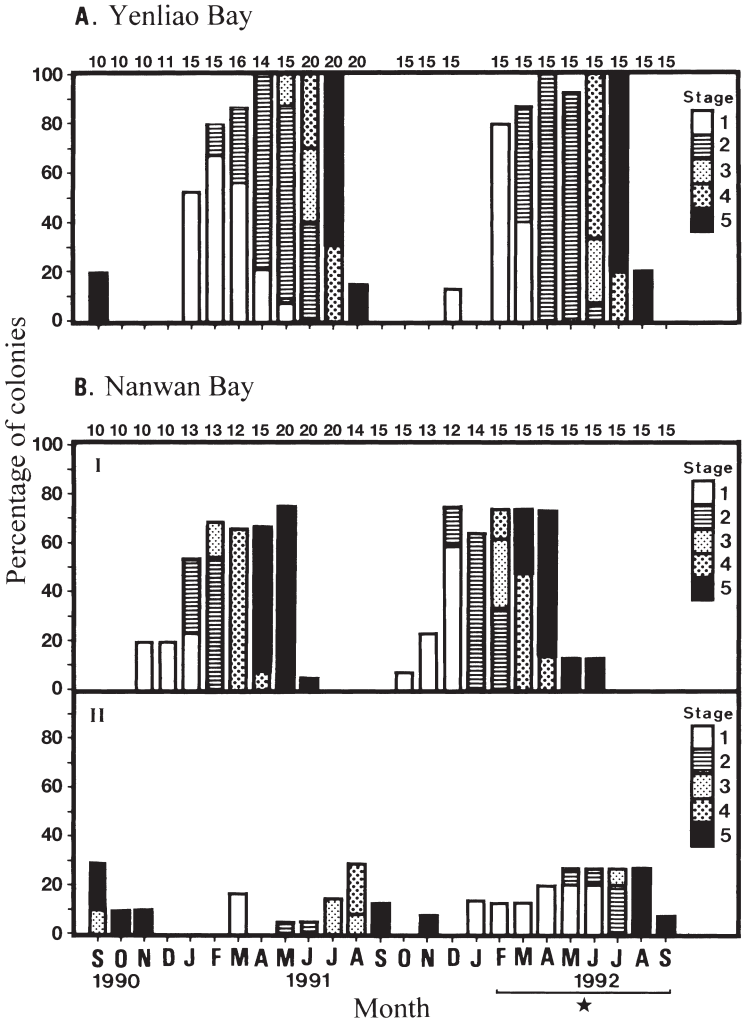

Fig. 3. *Mycedium elephantotus*. Monthly changes in percentage of colonies containing gametes of population in (A) Yenliao Bay, northern Taiwan and (B) Nanwan Bay, southern Taiwan. Numbers above bars indicate the number of colonies sampled. I: first reproductive group; II: second reproductive group. ★: samples collected from tagged colonies

Genetic variation

Seven polymorphic and 1 monomorphic loci were encoded. The mean numbers of alleles per locus (N_a) were 2.9, 3.1 and 3.3 in the northern Taiwan population and the 2 reproductive populations in southern Taiwan, respectively (Table 1). Percentages of polymorphic loci (P_{95}) ranged from 62.5 to 75% indicating that the genetic variability is high. The observed heterozygosities, ranging from 0.219 to 0.290, were generally lower than expected under Hardy-Weinberg equilibrium (Table 1).

The ratios of the number of observed genotypes (N_G) to the number of individuals sampled (N_I) were close to 1 (0.963 to 1.000) indicating that almost as many genotypes as individuals were found in all the *Mycedium elephantotus* populations (Table 2). The ratios of G_O:G_E among collection sites were high (0.9 to 1.0) suggesting high degrees of sexual reproduction.

Allele frequencies of *Mycedium elephantotus* populations were markedly different among the 3 populations (Table 3). The MDH-1-85, MDH-2-90, PEPA-110, -95 and -83 appeared only in southern Taiwan populations, while EST-100 appeared only in northern Taiwan population. Significant differences on allele frequencies were also found between the 2 reproductive groups in southern Taiwan, especially for those of PEPA ($\chi^2 = 32.1$, df = 1, $p < 0.001$) and MPI ($\chi^2 = 26.3$, df = 1, $p < 0.001$). The MPI-105 and PGM-115 did not appear in the first reproductive group, while PEPA-112 did not appear in the second reproductive group. The frequencies of PEPA-83 and MPI-100 were higher in the first reproductive group, while those of PEPA-100, and MPI-82, -105 were higher in the second reproductive group in southern Taiwan (Table 3).

Significant values of F_{ST} were detected among the 3 populations in 5 out of 7 loci, the exceptions being MDH-2 and MEP (Table 4). Significant values of F_{ST} were also found between the 2 reproductive groups for PEPA ($F_{ST} = 0.094$, $p < 0.001$) and MPI ($F_{ST} = 0.090$, $p < 0.001$). Genetic differentiation among the 3 populations was highly significant ($F_{ST} = 0.155$, $p < 0.001$). Paired comparisons between populations showed that significant genetic differentiation was found between

Table 1. *Mycedium elephantotus*. Genetic variability for coral populations in Yenliao Bay, northern Taiwan (MO) and the 2 reproductive groups in Nanwan Bay, southern Taiwan (TS-1 and TS-2), with standard errors in parentheses

Population	Mean no. of alleles/locus	% polymorphic loci (P_{95})	Observed heterozygosity	Expected heterozygosity
MO	2.9	62.5	0.219	0.334
	(0.6)		(0.078)	(0.106)
TS-1	3.1	75.0	0.233	0.312
	(0.7)		(0.072)	(0.096)
TS-2	3.3	75.0	0.290	0.377
	(0.7)		(0.089)	(0.118)

Table 2. Effects of asexual reproduction on genotypes within samples of individual coral colonies (N_I) collected from 3 populations. Level of multilocus genotypic variation within each population was expressed as no. of multilocus genotypes (N_G). Relative contribution of asexual reproduction was estimated both by ratio of N_G/N_I and ratio of observed genotypic diversity (G_O) to that expected (G_E). Population abbreviations as in Table 1

Population	N_I	N_G	N_G/N_I	G_O	G_E	G_O/G_E
MO	29	29	1.000	29.00	29.00	1.000
TS-1	34	33	0.971	31.24	34.00	0.919
TS-2	27	26	0.963	24.30	27.00	0.900

Table 3 *Mycedium elephantotus.* Allele frequencies in 3 populations (abbreviations as in Table 1); (n) no. of samples

Locus	Population		
	MO	TS-1	TS-2
MDH-1			
(n)	29	34	27
100	1.000	0.912	0.907
85	0.000	0.088	0.093
MDH-2			
(n)	29	34	27
108	0.034	0.059	0.056
100	0.966	0.926	0.926
90	0.000	0.015	0.019
EST			
(n)	29	34	27
100	0.845	0.000	0.000
97	0.155	1.000	1.000
PEP-A			
(n)	29	34	27
112	0.034	0.015	0.000
110	0.000	0.029	0.019
105	0.310	0.221	0.389
100	0.586	0.088	0.296
95	0.000	0.074	0.148
91	0.069	0.044	0.074
83	0.000	0.529	0.074
MPI			
(n)	26	28	26
105	0.038	0.000	0.192
100	0.346	0.714	0.308
94	0.327	0.107	0.269
88	0.115	0.089	0.038
82	0.173	0.089	0.192
PGM			
(n)	28	33	27
115	0.000	0.000	0.037
112	0.143	0.121	0.167
105	0.089	0.364	0.222
100	0.482	0.439	0.444
93	0.214	0.076	0.130
85	0.071	0.000	0.000
MEP			
(n)	29	32	27
103	0.121	0.156	0.167
100	0.776	0.734	0.648
96	0.103	0.109	0.185
PEPB			
(n)	29	34	27
100	1.000	1.000	1.000

coral populations from northern Taiwan and southern Taiwan (F_{ST} = 0.181, p < 0.001, for MO vs TS-1; F_{ST} = 0.127, p < 0.001, for MO vs TS-2), and between the 2 reproductive groups in southern Taiwan (F_{ST} = 0.049, p < 0.001). The Nei's unbiased genetic distance between the first and second reproductive groups (D = 0.045) also indicated a marked difference (Table 5). However, higher genetic distances were found between the northern Taiwan and southern Taiwan populations (D = 0.229 between MO and TS-1; D = 0.165 between MO and TS-2).

DISCUSSION

Mycedium elephantotus is a simultaneous hermaphrodite with an annual reproductive cycle. Reproductive characteristics such as gonad structure and the length of gametogenesis were similar, but the timing of gametogenesis and spawning were different, among the 3 populations in northern and southern Taiwan.

In southern Taiwan, the first reproductive group of *Mycedium elephantotus* spawned at the same time as most scleractinian coral species in that region, in late April or early May (Dai et al. 1992). The second reproductive group of *M. elephantotus*, together with other species including *Echinopora lamellosa*, *Merulina ampliata* and a second reproductive group of *Echinophyllia aspera*, spawned in August and September (Fan & Dai 1995, 1998, Fan 1996). The separation of reproductive season for corals in southern Taiwan suggests that the environmental conditions favorable for the survival of coral larvae are diverse (Dai et al. 1992, Fan & Dai 1995). It is possible that the warmer and smaller range (5.0°C) of sea temperatures in southern Taiwan provide a longer period during which sea temperature is appropriate for successful reproduction. In this case, coral species respond to one or more forcing functions in the environment which constrain the time of optimal spawning (Oliver et al. 1988).

Table 4. *Mycedium elephantotus*. Estimates of deviations from Hardy-Weinberg proportions, and *F*-statistics (Nei 1977) of 3 populations. Rare alleles are pooled in the chi-square test and the degree of freedom in each test is 1. *F*-statistics indicate deviations from Hardy-Weinberg proportions within the total population (F_{IT}), within populations (F_{IS}) and among populations (F_{ST}). Population abbreviations as in Table 1 (significant level: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$)

Population	Locus						
	MDH-1	MDH-2	EST	PEPA	MPI	PGM	MEP
MO	–	−0.036	0.868***	0.067	0.419	0.325	0.352
TS-1	−0.097	−0.066	–	0.281*	0.614***	0.166	0.190
TS-2	−0.102	−0.064	–	0.135	0.289	0.214	0.499*
F_{IT}	−0.064	−0.054	0.972	0.282	0.458	0.256	0.365
F_{IS}	−0.099	−0.059	0.868***	0.165	0.415***	0.236***	0.358***
F_{ST}	0.032*	0.005	0.784***	0.140***	0.074**	0.026*	0.010

Table 5. Nei's unbiased genetic distance (below diagonal) and unbiased genetic similarity (above diagonal) (Nei 1978) between populations. Population abbreviations as in Table 1

Population	MO	TS-1	TS-2
MO	–	0.796	0.848
TS-1	0.229	–	0.956
TS-2	0.165	0.045	–

The 2 reproductive groups of *Mycedium elephantotus* in Nanwan Bay, southern Taiwan could be separated by differences in the timing of gametogenesis and spawning. A similar phenomenon has also been described in sympatric colonies of *Echinophyllia aspera* in Nanwan Bay (Fan 1996). The existence of 2 reproductive groups was clearly demonstrated by separation of reproductive timing among sympatric colonies, while the reproductive activity of coral colonies within each group was synchronous. This is different from those broadcast-spawning species which have multiple gametogenic cycles within a colony in a year, such as *Acropora formosa* and *A. hyacinthus* in Papua New Guinea (Oliver et al. 1988) and *Montipora digitata* at Magnetic Island, Great Barrier Reef (Stobart & Benzie 1994). It is also not a case of split-spawning (*sensu* Willis et al. 1985) because reproductive activities of the 2 groups are separated by 3 to 4 mo.

As eggs and sperm are viable for 4.5 to 8 h after spawning (Heyward & Babcock 1986; Willis et al. 1997), fertilization is impossible between coral populations spawned on different mo. The 2 sympatric reproductive groups of *Mycedium elephantotus* in southern Taiwan with a temporal separation of 3 to 4 mo are reproductively isolated and genetic exchange between them is unlikely to occur in the present condition. Yu et al. (1999) demonstrated that the allopatric populations of the first reproductive group of *M. elephantotus* in southern Taiwan, which were separated by a distance of more than 10 km, were genetically homogeneous. Nei's unbiased genetic distance between the 2 reproductive groups ($D = 0.045$) is much higher than those among allopatric populations of the first reproductive group ($D \leq 0.015$; Yu et al. 1999). Furthermore, the UPGMA dendrogram based on genetic distance showed a clear subdivision between the 2 groups (Fig. 4). All of these data suggest that the 2 reproductive groups are separated genetically.

The existence of 2 reproductive groups of *Mycedium elephantotus* may result from either recent divergence in sympatric populations or secondary contact of populations with different origins. Richmond & Jokiel (1984) suggested that asynchrony among sympatric populations of an identified species may be the result of the immigration of planulae from one region into another. However, the lack of qualitative and quantitative morphological differences between the 2 groups is also consistent with recent diversification within taxon (McPeek & Wellborn 1998).

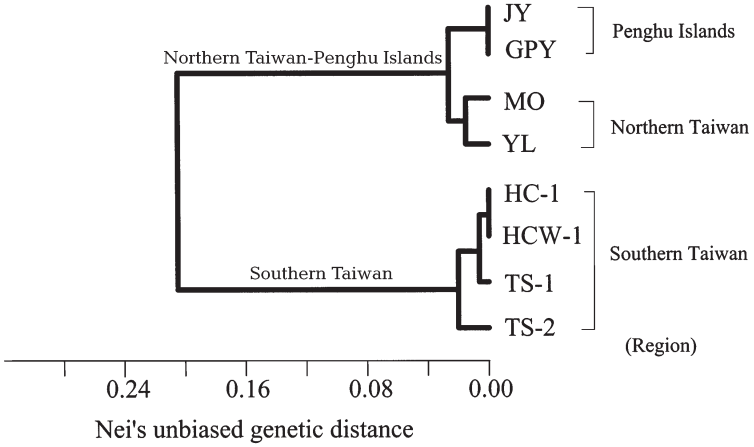

Fig. 4. UPGMA dendrogram showing relationships among populations based on Nei's unbiased genetic distances (*D*) in Table 5 and the data in Yu et al. (1999)

The subdivision of 2 reproductive groups of *Mycedium elephantotus* in southern Taiwan may represent adaptive divergence of sympatric populations. It is generally considered that space for settlement is one of the most important limiting resources on coral reefs and there is intensive competition among coral populations (Dai 1990, Lang & Chornesky 1990). Spawning of the 2 reproductive groups in different seasons may disperse the reproductive investment (Shlesinger & Loya 1985). Seasonal disturbances in southern Taiwan, including typhoons and heavy rainfall, happen mainly in summer (Dai 1991). Spawning of the first reproductive group occur before the seasonal disturbance and the second group after the disturbance may increase the substrate availability to larvae, avoid the high mortality caused by disturbances and then increase reproductive success (Fan & Dai 1995). Therefore, different reproductive timing of the 2 groups of *M. elephantotus* in southern Taiwan may be considered as a byproduct of natural selection (Coyne 1992) and such subdivided populations may symbolize 2 adaptive peaks envisioned in Wright's (1931) shifting-balance model. The mode of origin of the 2 reproductive groups could be sympatric or allopatric but the present data cannot distinguish these hypotheses.

Acknowledgements. We thank Dr S.-C. Lee and Mr H.-Y. Wang for their help in genetic studies. We also thank Mr D.-S. Chen, C.-S. Wu, S.-D. Huang, Y.-C. Tsay for their assistance in the field. We are grateful to 2 anonymous reviewers for their helpful comments on the manuscript. This study was supported by a grant from the National Science Council, ROC (NSC85-2611B-002A-002).

LITERATURE CITED

Ayre DJ, Dufty S (1994) Evidence for restricted gene flow in the viviparous coral *Seriatopora hystrix* on Australia's Great Barrier Reef. Evolution 48:1183–1201

Ayre DJ, Veron JEN, Dufty SL (1991) The corals *Acropora palifera* and *Acropora cuneata* are genetically and ecologically distinct. Coral Reefs 10:13–18

Ayre DJ, Hughes TP, Standish RJ (1997) Genetic differentiation, reproductive mode, and gene flow in the brooding coral *Pocillopora damicornis* along the Great Barrier Reef, Australia. Mar Ecol Prog Ser 159:175–187

Babcock RC, Bull GD, Harrison PL, Heyward AJ, Oliver JK, Wallace CC, Willis BL (1986) Synchronous spawning of 105 scleractinian coral species on the Great Barrier Reef. Mar Biol 90:379–394

Babcock RC, Willis BL, Simpson CJ (1994) Mass spawning of corals on a high latitude coral reef. Coral Reefs 13:161–169

Benzie JAH, Haskell A, Lehman H (1995) Variation in the genetic composition of coral (*Pocillopora damicornis* and *Acropora palifera*) populations from different reef habitats. Mar Biol 121:731–739

Coyne JA (1992) Genetics and speciation. Nature 355: 511–515

Dai CF (1990) Interspecific competitions in Taiwanese corals with special reference to interactions between alcyonacean and scleractinian corals. Mar Ecol Prog Ser 60: 291–297

Dai CF (1991) Reef environment and coral fauna of southern Taiwan. Atoll Res Bull 354:1–28

Dai CF (1993) Patterns of coral distribution and benthic space partitioning on the fringing reefs of southern Taiwan. PSZN I: Mar Ecol 14:185–204

Dai CF, Soong K, Fan TY (1992) Sexual reproduction of corals in northern and southern Taiwan. Proc 7th Int Coral Reef Symp Guam 1:448–455

Duffy JE (1996) Resource-associated population subdivision in a symbiotic coral-reef shrimp. Evolution 50:360–373

Fan TY (1996) Life histories and population dynamics of foliaceous corals in northern and southern Taiwan. PhD thesis, National Taiwan University, Taipei

Fan TY, Dai CF (1995) Reproductive ecology of the scleractinian coral *Echinopora lamellosa* in northern and southern Taiwan. Mar Biol 123:565–572

Fan TY, Dai CF (1998) Sexual reproduction of the scleractinian coral *Merulina ampliata* in southern Taiwan. Bull Mar Sci 62:897–904

Harrison PL, Wallace CC (1990) Reproduction, dispersal and recruitment of scleractinian corals. In: Dubinsky Z (ed) Ecosystems of the world 25: coral reefs. Elsevier, New York, p 133–207

Hayashibara T, Shimoike K, Kimura T, Hosaka S, Heyward A, Harrison P, Kudo K, Omori M (1993) Patterns of coral spawning at Akajima Island, Okinawa, Japan. Mar Ecol Prog Ser 101:253–262

Hellberg ME (1994) Relationship between inferred levels of gene flow and geographic distance in a philopatric coral, *Balanophyllia elegans*. Evolution 48:1829–1854

Heyward AJ, Babcock RC (1986) Self- and cross-fertilization in scleractinian corals. Mar Biol 90:191–195

Johnson MS, Black R (1998) Effects of isolation by distance and geographical discontinuity on genetic subdivision of *Littoraria cingulata*. Mar Biol 132:295–303

Knowlton N (1993) Sibling species in the sea. Annu Rev Ecol Syst 24:189–216

Knowlton N, Weil E, Weigt LA, Guzmán HM (1992) Sibling species in *Montastraea annularis*, coral bleaching, and the coral climate record. Science 255:330–333

Knowlton N, Weight LA, Solorzano LA, Mills DK, Bermingham E (1993) Divergence in proteins, mitochondrial DNA, and reproductive compatibility across the isthmus of Panama. Science 260:1629–1632

Knowlton N, Maté JL, Guzmán HM, Rowan R, Jara J (1997) Direct evidence for reproductive isolation among the three species of the *Montastraea annularis* complex in Central America (Panama and Honduras). Mar Biol 127:705–711

Kojis BL (1986) Sexual reproduction in *Acropora* (*Isopora*) species (Coelenterata: Scleractinia). I. *A. cuneata* and *A. palifera* on Heron Island, Great Barrier Reef. Mar Biol 91: 291–309

Lang JC (1984) Whatever works: the variable importance of skeletal and of non-skeletal characters in scleractinian taxonomy. Palaeontogr Am 54:18–43

Lang JC, Chornesky EA (1990) Competition between scleractinian corals — a review of mechanisms and effects. In: Dubinsky Z (ed) Ecosystems of the world 25: coral reefs. Elsevier, New York, p 209–252

McPeek MA, Wellborn GA (1998) Genetic variation and reproductive isolation among phenotypically divergent amphipod populations. Limnol Oceanogr 43:1162–1169

Miller KJ, Babcock R (1997) Conflicting morphological and reproductive species boundaries in the coral genus *Platygyra*. Biol Bull 192:98–110

Miller KJ, Benzie JAH (1997) No clear genetic distinction between morphological species within the coral genus *Platygyra*. Bull Mar Sci 61:907–917

Nei M (1977) F-statistics and analysis of gene diversity in subpopulations. Ann Hum Genet 41:225–233

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590

Oliver JK, Babcock RC, Harrison PL, Willis BL (1988) Geographic extent of mass coral spawning: clues to ultimate causal factors. Proc 6th Int Coral Reef Symp Townsville 2: 803–810

Palumbi SR (1994) Genetic divergence, reproductive isolation, and marine speciation. Annu Rev Ecol Syst 25: 547–572

Palumbi SR, Grabowsky G, Duda T, Geyer L, Tachino N (1997) Speciation and population genetic structure in tropical Pacific sea urchins. Evolution 51:1506–1517

Redfield JA, Salini JP (1980) Techniques of starch-gel electrophoresis of penaeid prawn enzymes (*Penaeus* spp. and *Metapenaeus* spp.). CSIRO Aust Div Fish Oceanogr Rep 116:1–20

Richmond RH, Hunter CL (1990) Reproduction and recruitment of corals: comparisons among the Caribbean, the tropical Pacific, and the Red Sea. Mar Ecol Prog Ser 60: 185–203

Richmond RH, Jokiel PL (1984) Lunar periodicity in larva release in the reef coral *Pocillopora damicornis* at Enewetak and Hawaii. Bull Mar Sci 34:280–287

Shlesinger Y, Loya Y (1985). Coral community reproductive patterns: Red Sea versus the Great Barrier Reef. Science 228:1333–1335

Stobart B, Benzie JAH (1994) Allozyme electrophoresis demonstrates that the scleractinian coral *Montipora digitata* is 2 species. Mar Biol 118:183–190

Stoddart JA (1983) Asexual production of planulae in the coral *Pocillopora damicornis*. Mar Biol 76:279–284

Stoddart JA (1986) Biochemical genetics of *Pocillopora damicornis* in Kaneohe Bay, Oahu, Hawaii. In: Jokiel PL, Richmond RH, Rogers RA (eds) Coral reef population biology. Hawaii Inst. Mar Biol Tech Rep No. 37, p 133–149

Stoddart JA, Taylor JF (1988) Genotypic diversity: estimation and prediction in samples. Genetics 118:705–711

Swofford OL, Selender RB (1989) BIOSYS-1, a computer program for the analysis of allelic variation in the population genetics and biochemical systematics. Illinois Natural History Survey, Champaign, IL

Veron JEN (1993) A biogeographic database of hermatypic corals; species of the central Indo-Pacific, genera of the world. Aust Inst Mar Sci Monogr Ser 9

Waples RS (1987) A multispecies approach to the analysis of gene flow in marine shore fishes. Evolution 41:385–400

Weil E, Knowlton N (1994) A multi-character analysis of the Caribbean coral *Montastraea annularis* (Ellis and Solander, 1786) and its sibling species, *M. faveolata* (Ellis and Solander, 1786) and *M. franksi* (Gregory, 1895). Bull Mar Sci 55:151–175

Willis BL (1990) Species concepts in extant scleractinian corals: considerations based on reproductive biology and genotypic population structures. Syst Bot 15:136–149

Willis BL, Babcock RC, Harrison PL, Oliver JK, Wallace CC (1985) Patterns in the mass spawning of corals on the Great Barrier Reef from 1981 to 1984. Proc 5th Int Coral Reef Congr, Tahiti 4:343–348

Willis BL, Babcock RC, Harrison PL, Wallae CC (1997) Experimental hybridization and breeding incompatibilities within the mating systems of mass spawning reef corals. Coral Reefs 16:S53–S65

Wright S (1931) Evolution in Mendelian populations. Genetics 16:97–159

Yu JK, Wang HY, Lee SC, Dai CF (1999) Genetic structure of a scleractinian coral *Mycedium elephantotus* (Pallas 1766) in Taiwan. Mar Biol 133:21–28

Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

Submitted: August 19, 1999; Accepted: January 27, 2000
Proofs received from author(s): July 10, 2000