Reticulate evolution of corals

Masayuki Hatta* and Hironobu Fukami**

*Department of Biology, Ochanomizu University
2-1-1 Otsuka, Bunkyo-ku, Tokyo 112-8610, Japan
mhatta@cc.ocha.ac.jp
** Smithsonian Tropical Research Institute
Unit 0948, APO AA34002-0948, USA

Abstract: Many species of reef-building corals spawn gametes synchronously in the reef, and hybridization has been supposed in such mass-spawning events. We conducted crossing experiments and molecular phylogeny with a number of mass-spawning species in the major coral genus *Acropora*. Interspecific fertilization at high rates was observed in several combinations of particular species despite very different morphologies. The hybrid larvae developed normally, and contained two allelic sequences of a marker gene inherited from the parents in the Mendelian manner. Molecular phylogenetic analyses provided strong evidence that a DNA polymorphism is shared by the hybridizing species. These reproductive and genetic characteristics are consistent with a species complex under the separation or fusion processes predicted in reticulate evolution. Synchronous mass spawning may facilitate the unique evolutionary history in corals.

1. Introduction

Many reef-building corals reproduce sexually in a unique manner "mass spawning". In the Indo-Pacific region, mass spawning occurs typically once a year on a night near the full moon in early summer (Babcock et al. 1986; Hayashibara et al. 1993). Huge quantities of eggs and sperm are released simultaneously within an hour by vast numbers of coral colonies belonging many species and genera. Mass spawning of corals is unique because multiple species are involved in the synchronous spawning. Although the mechanisms are still unknown, synchronous spawning certainly promotes fertilization within each species of the sedentary corals.

Mass spawning, however, possibly raises a problem for species identity. Since colonies of many species, growing side by side, spawn simultaneously, their eggs and sperm are mixed together. Cross-fertilization may occur between closely related species, unless they have effective mechanisms to avoid interspecific fertilization. Based on these reproductive aspects, a "reticulate evolution" hypothesis has been proposed for reef-building corals (Veron 1994). This hypothesis supposes that current coral species have evolved by repeated rounds of species separation and fusion to form a reticulate pattern of many interconnecting lineage, instead of the continual separation of new species along discrete lineage (Figure 1). If such a group of species exists that is currently undergoing separation, the involved species may still be connected reproductively and genetically, although they have developed species-specific features. In reverse, if multiple species are undergoing fusion by hybridization, the involved species may cross-fertilize to some extent and share a common gene pool as a result of gene introgression. This problem will be particularly important for the genus *Acropora*, which dominates Indo-Pacific reefs and contains a large number of species with various morphologies. We tested the possibilities reticulate evolution by conducting crossing experiments and DNA analyses systematically for a number of sympatric mass-spawning species in *Acropora*.

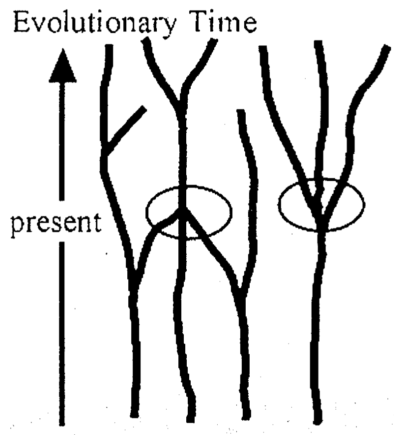

Figure 1. Concept diagram of reticulate evolution

2. Results

2.1 Reproductive Property

First, we carried out crossing experiments using dominant mass-spawning species of *Acropora* in Okinawa, Japan. Colonies were transferred to the laboratory just before spawning and allowed to release gametes independently in separate buckets. Acroporids are hermatypic and release bundles of eggs and sperm. Buoyant gamete bundles were collected, and separated to eggs and sperm suspension. Eggs and sperm taken from individuals were mixed in various combinations within and between species, and the fertilization rate was scored for each individual cross. Table 1 shows examples of individual crosses done on June 6th, 1996. The fertilization rate within species was high in most cases, ≥95% in 7 cases and ≥70% in 2 cases in this table. Self-fertilization was near 0% in every case. Between the cryptic species, *A. formosa*-A and –B, cross-fertilization was not observed although they are very similar in morphology. On the other hand, apparent cross-fertilization occurred between *A. nasuta* and *A. formosa*-A in spite of their different morphologies; *A. nasuta*-A is azalea-bush type and *A. formosa*-A is dendritic (Figure 2, see Hatta et al. 1999 for the cryptic species). The values of individual cross-fertilization varied from 3% to 100% by combinations. This kind of crossing experiments were done every year from 1993 to 1998, and interspecific fertilization was observed among 17 combinations of 11 species out of 19 species (data not shown). Part of the results is represented in Figure 3 (details in Hatta et al. (1999).

Table 1. Examples of individual crosses

sperm	*A. nasuta*-A			*A. formosa*-A		*A. formosa*-B	
egg	C3	D3	E3	F3	G3	H3	I3
C3	0	95	84	75	61	0	0
D3	98	0	99	19	7	0	0
E3	99	99	0	16	3	0	0
F3	72	15	100	0	nd	0	0
G3	97	100	96	100	2	4	2
H3	0	2	1	4	1	0	97
I3	2	0	0	0	1	72	0

Note: Values represent % fertilization. nd stands for not done. Individuals are shown with colony numbers.

Figure 2. Two species in the field

2.2 DNA Diagnosis

All embryos produced by crossing experiments, including hybrids, developed normally to planula larvae. We confirmed that the obtained hybrid larvae are really hybrids by DNA analyses. A nuclear single copy gene, mini-collagen (Wang et al. 1995), was used as a marker. Two allelic sequences of the mini-collagen gene were obtained from each colony and each larva by PCR and sequenced after cloning. and metamorphosed to primary polyps. When the alleles of one larva were compared with those of its parents, one of the larval alleles was identical to one allele of a parent and another larval allele was identical to one of the other parent. An example case is shown in Table 2. Thus, it was clearly shown that the hybrid larvae are produced by equal contribution of two parental genomes. This is the first genetic evidence of hybrid formation in corals.

Table 2. Examples of Mendelian inheritance in hybrids

Individual animal	part of allelic sequences	allele no.
Egg donor		
A. nasuta-A	ATTATTTCCTTTCTTT	naA-B2-1
colony B2	ATTATCTCTATGCGCT	naA-B2-2
Sperm donor		
A. formosa-A	ATCATTTCCTGTCTTC	foA-S2-1
colony S2	ATTACTTCCTTTCTTT	foA-S2-2
Hybrid larva 1	ATTATTTCCTTTCTTT	naA-B2-1
	ATTACTTCCTTTCTTT	foA-S2-2

2.3 Genetic relationships

It is interesting and important to see morphologies and fertility of the hybrids, however it is very difficult to grow acroporids from primary polyps to mature colonies. So, we tried to detect gene introgression by DNA phylogenetic analyses. DNA sequences of an intron of the mini-collagen gene about 250bp long were subjected to phylogenetic analyses based on the Neighbor-Joining method. Figure 3 shows a schematic phylogenetic tree. Eight mass-spawning species were divided to 3 groups in which alleles of different species were intermingled (details in Hatta et al. 1999). Figure 3 also reveals a summary of reproductive relationships. Interspecific fertilization was observed only within the group, on the other hand, each group contains species of very different morphologies such as *A. nasuta*–A and *A. formosa*-A (Figure 2).

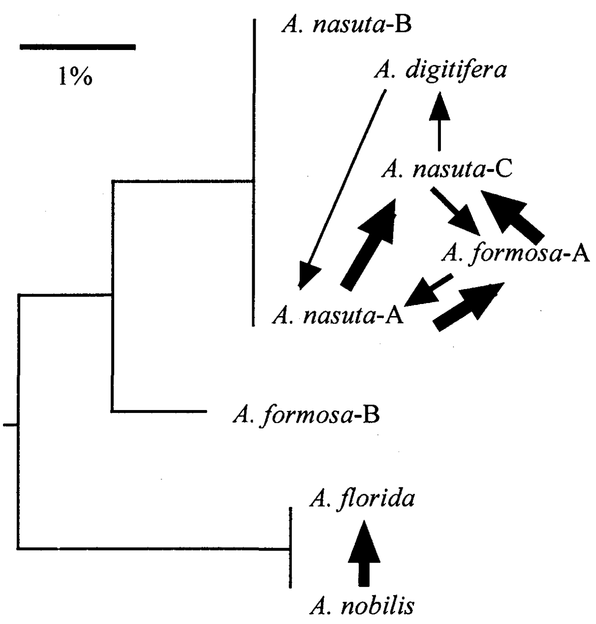

Figure 3. Schematic diagram of genetic and reproductive relationships Arrows indicate the direction of cross-fertilization from sperm to egg, and the thickness of the arrow indicates the frequency: $\geq$50% average=thick, 20-50%=moderate, 3-20%=thin.

3. Discussion

As indicated by circles in Figure 1, two scenarios can be invoked to explain the reproductive and genetic characteristics revealed in this study.

(1) The process of speciation. The species in the group are currently undergoing reproductive isolation from a single common ancestral species that was highly polymorphic in morphology. Each species has already fixed species-specific morphologies. According to this scenario, morphologically similar species are explained that they speciated from a common ancestor and still remains the DNA polymorphism. However, it is difficult to conceive that multiple species arose from a single ancestor at the same time in sympatry, and that very different morphologies have been fixed in each species without fixing species-specific DNA polymorphisms.

(2) The process of species fusion. An alternative scenario is based on the view of gene introgression via hybrids produced in mass-spawning events. In an evolutionary time scale, established species have recently become able to cross-fertilize by mutations in egg-sperm recognition. If hybrids are fertile and can backcross with their parental species, the genes are transmitted from one species to another. Such interspecific gene flow may become wider with time after repeated hybridization and backcrossing. The hybridizing species now share a gene pool as a result of extensive gene introgression.

Irrespective of the scenarios, the interconnection of multiple species described in our study reveals the characteristics that are consistent with a section of a reticulate evolutionary history at a present time point. A significant number of hybrids will be produced in nature every year by synchronous mass spawning, because colonies of hybridizing species often grow side by side as shown in Figure 1. Since hybrids do not necessarily express intermediate phenotypes, some hybrid acroporids might form unexpected morphologies and currently be recognized as independent species. Hybridization might mediate diversification of morphology by mixing genetic information in *Acropora*. Mass spawning may have facilitated the unique evolutionary history of corals, and the corals may be evolving just now.

[References]

Babcock, R.C., Bull, G.D., Harrison, P.L., Heyward, A.J., Oliver, J.K., Wallace, C.C., Willis, B.L. 1986. "Synchronous spawning of 105 scleractinian coral species on the Great Barrier Reef" Mar. Biol. 90:379-394.

Hatta, M., Fukami, H., Wang, W., Omori, M., Shimoike, K., Hayashibara, T., Ina, Y., Sugiyama, T. 1999. "Reproductive and genetic evidence for a reticulate evolutionary history of mass-spawning corals" Mol. Biol. Evol. 16:1607-1613.

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

Veron, J.E.N. 1995. "Corals in space and time" Comstock/Cornell, Ithaca and London.

Wang, W., Omori, M., Hayashibara, T., Shimoike, K., Hatta, M., Sugiyama, T., Fujisawa, T. 1995. "Isolation and characterization of a mini-collagen gene encoding a nematocyst capsule protein from a reef-building coral, *Acropora donei*" Gene 152:195-200.