11. Distribution of Fusion Incompatibility Types in Natural Populations of the Compound Ascidian, *Botryllus primigenus*¹⁾

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Since the question of fusion in *Botryllus*, a compound ascidian, was first taken up by Bancroft (1903), it has been well known that two colonies show either a complete fusion or a complete rejection when there is contact between them. According to Oka and Watanabe (1957, 1960, 1967) and Oka (1970), in the Japanese species, *B. primigenus*, the colony specificity or fusion incompatibility is controlled by a series of multiple alleles at a single locus. No systematic research on the distribution of fusion incompatibility types in the naturally occurring colonies, however, has yet been published. This is the subject of the present report.

Materials and methods. The materials used were the living colonies of *Botryllus primigenus*, occurring attached to rocks and seaweeds in the coastal belt of the open sea in the vicinity of the Shimoda Marine Biological Station, Japan. Along the coastal zone of the Izu Peninsula, we fixed on three stations, designated N, T and S, being two or three kilometers distant from one another, and each being confined within narrow limits of about 30 m.

Ten colonies from respective stations, 30 colonies in all, were collected. They were designated N1, N2, . . . , T1, T2, . . . , and so forth. The colonies thus collected were reared on glass-plates set immersed in the bay near the Station. The fusibility was tested by bringing the cut surfaces of two colonies in contact. This test was performed in every combination of colonies.

The experiments were carried out at the Shimoda Marine Biological Station. It is our pleasant duty to acknowledge our indebtedness to the staff for providing us facilities for carrying out the experiments.

Results and discussion. The results obtained are presented in Figure 1. Among 30 colonies employed, two colonies (S2 and T2)

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Fig. 1. Results of fusion experiments in *B. primigenus*. The fusible combination of colonies is represented by the square filled in with "1". To each colony, the genotype is assigned on the basis of Oka and Watanabe's hypotheses. Every one of the genes in parentheses can be replaced by a new gene.

Table I. Fusion rates in *B. primigenus*

Combination of stations	Number of combinations of colonies	Number of fusible combinations	Fusion rate (%)
N—N	45	3	6.7
T—T	45	3	6.7
S—S	45	3	6.7
N—T	100	5	5.0
N—S	100	3	3.0
T—S	100	4	4.0
Total	435	21	4.8

were each fusible with four other colonies and 10 colonies were each fusible with none of the other colonies. The remaining 18 colonies each was fusible with 1 to 3 other colonies.

Fusion rates, as represented by the percentage of fusible combinations, have been calculated from Figure 1 and listed in Table I. Roughly speaking, only several per cent of combinations were fusible. The fusion rate in the combinations of colonies within the same station (6.7%) was somewhat higher than that in the combinations of colonies between different stations (3.0 to 5.0%). The year before the experiment stated above, another fusion experiment was carried out with 20 colonies of *B. primigenus* collected from station N. Out of 190 combinations examined, 21 (11.1%) were fusible. We should be very careful, therefore, in generalizing these figures.

From exhaustive investigations on the genetic control of fusibility in *B. primigenus*, Oka and Watanabe (1957, 1960, 1967) have come to the following conclusions: (1) Each colony in nature is heterozygotic in respect to the gene governing fusibility; (2) the gene is represented by a series of alleles like the *S* gene governing self-incompatibility in flowering plants; and (3) colonies containing at least one gene in common are fusible with one another. The multiple alleles were designated F^1 , F^2 , F^3 , and so on by Oka (1970).

On the basis of Oka and Watanabe's hypotheses, we tried to assign a pair of alleles to every one of the colonies used in the present experiments (Fig. 1). The genotype of all the colonies, however, could not be unequivocally determined. That is, the genes in parentheses in Figure 1 can be replaced each by a new gene which is not shared with any other colonies, *i.e.*, by F^n , n being 40 and over. As is clear from Figure 1, no exceptions to the rules proposed by Oka and Watanabe were found, so implying very strongly their validity. The number of genes required came to 39 or more. Of these, at least 23 genes were involved in a single colony and the others in 2 to 4 colonies in common.

As has been summarized in Table II, every station has some genes in common with one of the other two stations. Judging from these facts, there seems no doubt that many more genes are involved in the fusion incompatibility of naturally occurring colonies of *B. primigenus*. It should be noticed here that the number of fusion incompatibility types comes, theoretically at least, to $n(n-1)/2$, where n is the number of genes.

Summary. 1. Fusion experiments were carried out with naturally occurring colonies of *Botryllus primigenus*. Thirty colonies collected from three stations were used.

2. Several per cent of combinations were fusible. No exceptions were found to the rules presented by Oka and Watanabe for the genetic control of fusion incompatibility.

Table II. Gene distribution in *B. primigenus*
The genes involved only in a single colony are omitted from this table. The number of such genes is 6, 8 and 9 in stations N, T and S, respectively

Station Gene	N	T	S	Total
<i>F</i> ¹	2	0	0	2
<i>F</i> ³	2	0	0	2
<i>F</i> ⁵	2	0	0	2
<i>F</i> ¹⁸	0	0	2	2
<i>F</i> ¹⁹	0	0	1 + (1) *	1 + (1)
<i>F</i> ¹²	1	3	0	4
<i>F</i> ¹⁴	1	1	0	2
<i>F</i> ¹⁶	1	1	0	2
<i>F</i> ¹³	1	(2)	0	1 + (2)
<i>F</i> ¹⁵	1	(1)	0	1 + (1)
<i>F</i> ¹⁷	1	(1)	0	1 + (1)
<i>F</i> ⁴	1	0	2	3
<i>F</i> ⁷	1	0	1	2
<i>F</i> ⁸	0	1	2	3
<i>F</i> ⁹	0	1	1	2
<i>F</i> ¹¹	0	1	1	2

* The figures in parentheses correspond to the genes parenthesized in Figure 1.

3. At least 39 genes (multiple alleles) were required to explain the experimental results. It is suggested that many more genes are involved in the natural population of this species.

References

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