Reference: *Biol. Bull.* **169:** 631–637. (December, 1985)

PATTERN OF CELLULAR ALLOREACTIVITY OF THE SOLITARY ASCIDIAN, *HALOCYNTHIA RORETZI,* IN RELATION TO GENETIC CONTROL

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ABSTRACT

The pattern of cellular alloreactivity in the solitary ascidian, *Halocynthia roretzi* (Drashe), was examined using 435 combinations of 30 individuals. A high level of polymorphism was revealed and no two individuals had identical histocompatibility even though they were sampled in a relatively small area of Mutsu Bay.

In *H. roretzi,* the allogeneic reaction seems to be triggered by an absence of common self markers, as in the colony fusibility of *B. primigenus,* rather than by a specific reaction to non-self markers as in higher vertebrates. We analyzed the allelic compositions of 30 animals assuming that a non-reactive pair share an allele. Since at most four "alleles" are carried by each individual, the solitary ascidian most probably has two genes that control alloreactivity.

INTRODUCTION

Recent studies indicate that the ability to distinguish self and non-self components is one of the intrinsic characteristics of living organisms (Hildeman, 1974; Cooper, 1976; Coombe *et al.,* 1984). This ability is widespread throughout the biosphere from lower organisms, such as the slime mold (Carlile, 1972) and sponge (Hildeman, 1979; Curtis *et al.,* 1982), to higher vertebrates such as the mammals (Klein, 1975; Götze, 1977). Whether there is a homologous relationship among different animals, especially between invertebrates and higher vertebrates, remains to be determined.

Oka and his colleagues have shown that the hermaphroditic colonial ascidian *Botryllus primigenus* Oka (Stylidae) has a single multiallelic histocompatibility (H) gene (Oka, 1970; Tanaka and Watanabe, 1973). Ascidians represent a class of Tunicata, the most primitive living branch of the phylum Chordata (Berrill, 1955). It is therefore possible that the major histocompatibility complex (MHC) of the vertebrates has evolved from a gene not very different from the H gene of *B. primigenus* (Scofield *et al.,* 1982). This gene controls two types of cell recognition systems: intercolonial compatibility at the somatic cell level and fertilization at the gamete cell level. When cut surfaces of two colonies are brought into contact, they either fuse or reject each other. Fusion ensues when the two colonies share an allele of the H gene. If the colonies do not share an allele, mutual rejection ("nonfusion reaction") occurs. Thus, colony fusibility is determined by the presence or absence of self (*i.e.,* shared) rather than non-self (*i.e.,* not shared) H gene controlled markers, a crucial point discussed previously by several authors (Burnet, 1971, 1976; Mäkelä *et al.,* 1976; Laffery and Woolnough, 1977). Fertilization takes place if the single allele carried by spermatozoa is not shared by the egg donor, presumably because self recognition between haploid spermatozoa and the diploid cell-derived chorion of the egg prevents fertilization (Oka,

Received 28 May 1985; accepted 18 September 1985.

1970). All individuals of natural *B. primigenus* populations are, therefore, heterozygous for the H gene, suggesting that the gene has evolved as a genetic mechanism for obligatory outbreeding, like the *S* genes of flowering plants (Lewis, 1976; Clarke and Knox, 1978). The remarkably high frequency of *H-2* heterozygosity in wild mice (Duncan *et al.*, 1979a) may indicate that a similar function is retained by the murine MHC and/or other related genes on the same chromosome (Hammerberg and Klein, 1975).

An *in vitro* cellular counterpart of the intercolonial nonfusion reaction has recently been described for the solitary (as opposed to colonial) ascidians (Fuke, 1980). The contact reaction, as the *in vitro* reaction was termed, requires direct contact between xenogeneic or allogeneic blood cells, is reciprocal in that two cells in contact lyse each other, and is a rapid reaction that is complete within a few minutes. The allogeneic reactions were observed between blood cells from some but not all donor combinations. In the present communication, we report a genetic analysis of alloreactivity in *Halocynthia roretzi* (Drashe), a hermaphroditic solitary ascidian of the family Pyuridae.

Materials and Methods

Three variant types of *H. roretzi* (Type A, B, C) inhabit the coast of northern Japan (Numakunai and Hoshino, 1973, 1974). Type A individuals, which could be readily distinguished from other types by morphological criteria, were used for this study. They were randomly collected at 10 to 20 meters by SCUBA diving along approximately 120 meters of the shoreline of Futagojima Island in Mutsu Bay, Aomori.

The "contact reactions" were tested as follows: blood cells were collected from the mantle-test interspace by withdrawing body fluid. Initially, the blood cells from one animal were cultured in a small glass chamber and vitally stained. The suspension of the cells from a second animal was introduced into the chamber. Contact reactions began immediately and were observed under phase contrast microscopy. Typically, after two cells from different individuals came into contact, one cell moved around the other as if it had been scouting for the other. In a short time, the former stopped and pressed itself to the latter. Within seconds, one cell was lysed, followed immediately by lysis of the other. To determine whether a reaction occurred the cells were observed one hour after mixing. A full account of experimental procedures and details of the contact reaction were given in a previous paper (Fuke, 1980).

All 435 different combinations of the 30 animals were tested at least twice. All experiments were carried out at aquarium facilities of the Biological Station, Asamushi, Aomori.

Results

The results of the mixed culture tests involving 435 combinations, are shown in Table I. No individuals reacted with all of the other 29 animals. The minimum number of non-reactive combinations found in each of 30 individuals was one (No. 20 and 27), while the maximum number was 9 (No. 18). Thus, the frequency of nonreactive allogeneic combinations for each animal ranged from 3.4% to 31% and averaged 15.4%, which was similar to the frequency observed in other types of *H. roretzi* (Fuke and Numakunai, 1982).

Absence of alloreactivity between two individual mice indicates lack of disparity in their histocompatibility antigens. In *H. roretzi,* however, absence of alloreactivity between two animals does not necessarily mean that they are identical in their histocompatibility. For example, animals No. 1 and No. 3 did not react with each other and shared parallel reactivity towards No. 4 and No. 29, yet differed in their reactivity to No. 7, No. 10, etc.

TABLE I

Reciprocal alloreactivity of 30 individuals *

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	–		–	–			–			–									–									–	
2		–													–										–	–				
3	–		–	–				–	–								–											–	
4	–		–	–				–				–						–	–									–	–
5					–						–			–							–								–
6						–							–			–								–			–		
7	–						–			–							–												
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28						–										–				–				–				–	
29	–		–	–															–						–			–	
30				–	–			–			–	–						–											–

* A minus (−) sign indicates absence of reactivity; unmarked combinations were reactive.

Among the 30 animals, there were some pairs or groups which had similar, but not identical, alloreactivity. For example, individuals No. 12 and No. 30 did not react with each other and showed the same reactivity to No. 4, No. 8, and No. 18 but differed in reactivity to No. 5 and No. 11.

DISCUSSION

In a previous study (Fuke, 1980), two animals, behaving as if they were "syngeneic," were found in a smaller panel employing 16 individuals. The present data, however, obtained from a larger number of animals show that none of the mutually nonreactive animals have the same pattern of reactivity against the rest of the animals and suggest that there are no syngeneic pairs in nature.

The results can be explained if we assume that the reaction took place only when two individuals did not share an allele of an H gene or genes. This simple genetic model is identical with the one that accounts for colony fusibility of *B. primigenus*. It is possible that more complex genetic models also explain the data, but the Occam's choice would be the one presented above. According to this model, a nonreactive pair must share at least one allele. We now assume that such a pair shares only one allele. An arbitrary letter was assigned to each of the alleles identified by histocompatible groups of two or more mutually nonreactive animals. This operation is illustrated in Figure 1 for the first seven alleles, *a* through *g*. The allelic compositions of the 30 animals thus obtained are listed in Table II. Since as many as four "alleles" are carried by some individuals, the solitary ascidian must have two or more H genes that control alloreactivity. As implied by the genetic model on which this analysis is based, these H genes are functionally equivalent, at least as far as their control of alloreactivity is concerned.

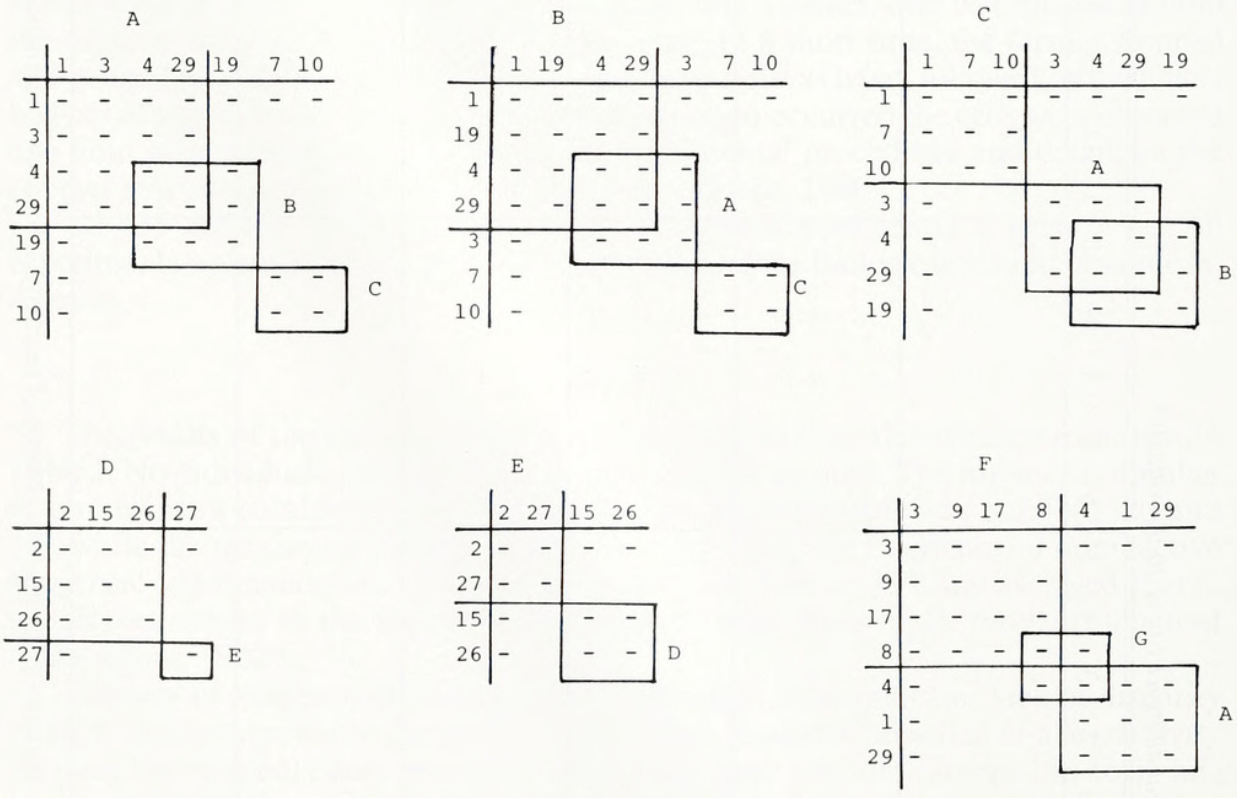

FIGURE 1. Illustration of histocompatibility group analysis. The group *A* through *G* identify alleles *a* through *g*, respectively.

TABLE II

Allelic compositions of the H genes in individuals

Animal No.	Allelic composition	No. of alleles
1	a b c	3
2	d e	2
3	a f g	3
4	a b g h	4
5	i j k	3
6	l m	2
7	c n	2
8	f g h o	4
9	f o p	3
10	c	1
11	i j q	3
12	h	1
13	l p q	3
14	k r	2
15	d o	2
16	m s	2
17	f n o	3
18	h o t u	4
19	b s	2
20	v	1
21	i k	2
22	t w	2
23	p w	2
24	l m r u	4
25	q x	2
26	d	1
27	e	1
28	m v	2
29	a b x	3
30	h j	2

Assuming that the solitary ascidian has only two H loci and that, unlike the *B. primigenus* H gene, one or both of the loci can be homozygous, we find the total minimum number of alleles represented in the panel to be 29 (those organisms which were assigned only one or two alleles were assumed to be homozygous for both loci; those with three alleles were considered to have one homozygous and one heterozygous locus). On the other hand, if none of the organisms tested were homozygous at either of the loci, 73 would be the maximum number of alleles represented by the panel. The average frequency of alleles in these cases would be 6.9 and 2.7, respectively (in the latter case, for example, the frequency of the *h* or *o* allele is 5/60, that of *a, b, f,* or *m,* is 4/60, etc., and 49 unnamed alleles appear only once with a frequency of 1/60). Since the organisms of this panel were sampled in a relatively small area of a bay, even the lower value could be an overestimate. For comparison, a similar analysis was based on published data regarding the *B. primigenus* H gene. Since all naturally occurring colonies are heterozygous for the H gene, as pointed out earlier, Tanaka

and Watanabe's data (1973) indicate that 80 to 83 alleles were represented by their panel of 45 randomly collected colonies, yielding an average alleleic frequency of 1.2%. As 11 out of 990 different pairs from 45 colonies were nonreactive (*i.e.*, sharing an allele), the total number of alleles of the H gene should be several hundred according to Bateman's methods (1947) of estimation. Judging from the average allelic frequencies, the extent of H gene polymorphism per locus seems somewhat higher in the colonial than in the solitary species. However, the existence of two H loci in the latter organism should confer as much or even more overall genetic diversity as the single locus does for the colonial species. Interestingly, the polymorphism of the mouse MHC (the H-2) class I loci is of similar magnitude (Duncan *et al.*, 1979b).

B. primigenus and *H. roretzi* belong to two related families of the same suborder (Millar, 1966). Furthermore, according to Millar (1966), the family Pyuridae to which *H. roretzi* belongs may have been derived from a primitive stock of Styelidae, which includes the genus *Botryllus*. If Millar's view is correct, it is possible that the redundant H genes of the solitary ascidian arose by a recent duplication and that the H genes of these organisms represent two successive stages in a monophyletic line of MHC evolution.

In a previous experiment, fertilization was observed between gametes from alloreactive as well as nonreactive donors (Fuke, 1980). The results are consistent with the haploid-diploid compatibility postulated for *B. primigenus* fertilization, but do not exclude a number of other possibilities that include, for instance, total lack of H gene control over fertilization. Therefore, it remains to be seen whether or how these H genes control the reproduction of *H. roretzi*. Experiments with "mosaic eggs" have recently revealed that the chorion of the eggs has specific ability to recognize self components of sperm (Fuke, 1983). Immunochemical studies of the coelomocyte's membranes and the chorion of unfertilized eggs are in progress to determine whether there are common factors responsible for recognition of self *versus* non-self.

ACKNOWLEDGMENTS

We are indebted to Dr. Takaharu Numakunai for his help and advice during this study. We wish to thank the late Dr. Setsuro Hirai and the staff of Asamushi Marine Biological Laboratory for their hospitality during our residence.

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