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Genetic and cytological aspects of histocompatibility in ascidians

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ABSTRACT

The three types of intraspecific histocompatibility in ascidians – fusibility, allograft rejection or retention, and cell contact reaction with mutual lysis – are reviewed and compared under both genetic and histological profiles. The differences between solitary and colonial species are outlined. The genetics of fusibility in different populations of *Botryllus schlosseri* is reconsidered with special reference to its bearing on sexual incompatibility, also in the light of new data. The different modes of allorejection are discussed with regard to the cellular and humoral factors involved. Some histological data on nonfusion reactions in *B. schlosseri* are presented. The difficulties of finding a unifying model for the three types of ascidian alloreactivity are outlined.

KEY WORDS: Ascidians - Histocompatibility - Allorecognition genetics - Allorejection histology.

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ASCIDIAN ALLOREACTIVITY

Intraspecific histocompatibility in ascidians is expressed in various ways: (a) Fusion or nonfusion occurs between conspecifics cosettled in the wild, or juxtaposed in the laboratory. This has been demonstrated in many families of colonial ascidians as «colony specificity» (see Taneda *et al.*, 1985 for a review) and, more recently, also in some solitary species (Schmidt, 1982; Kingsley *et al.*, 1989); (b) Autograft retention and allograft rejection have been studied in the solitary ascidians *Ciona intestinalis* (Reddy *et al.*, 1975) and *Styela plicata* (Raftos *et al.* 1988); (c) Solitary ascidians of different species have also been shown to be capable of allogeneic cell «contact reaction» leading to mutual lysis (Fuke, 1980).

GENETICS OF ALLORECOGNITION

The fusibility gene locus of Botryllus

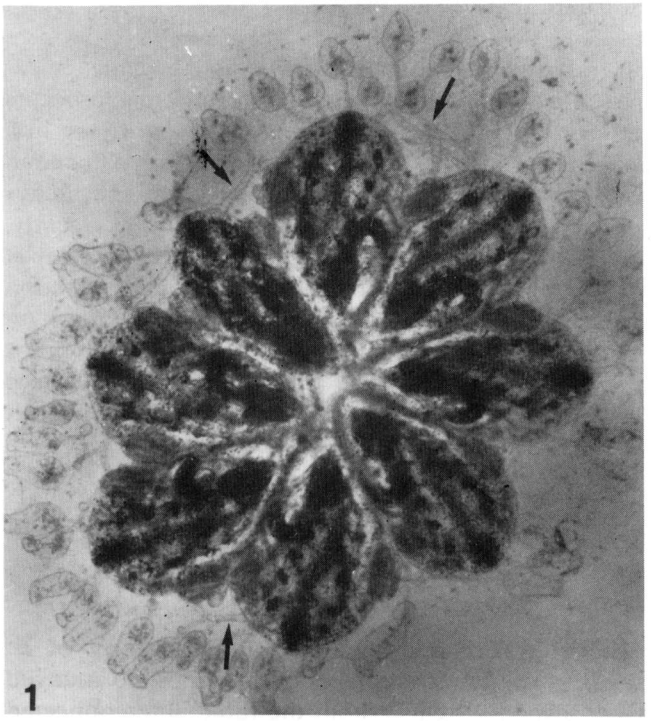
The zooids making up the clonal colony of *B. schlosseri* and of other species of the genus are arranged in star-shaped systems within the tunic, interconnected by blood vessels joining a marginal vessel which expands into a crown of stalked ampullae (Fig. 1). Contact between compatible colonies entails fusion both of opposite tunics and interdigitating ampullae, leading to a common fused vascular system (Fig. 2). Instead, necrotic zones mark the area of contact between incompatible colonies (Fig. 3).

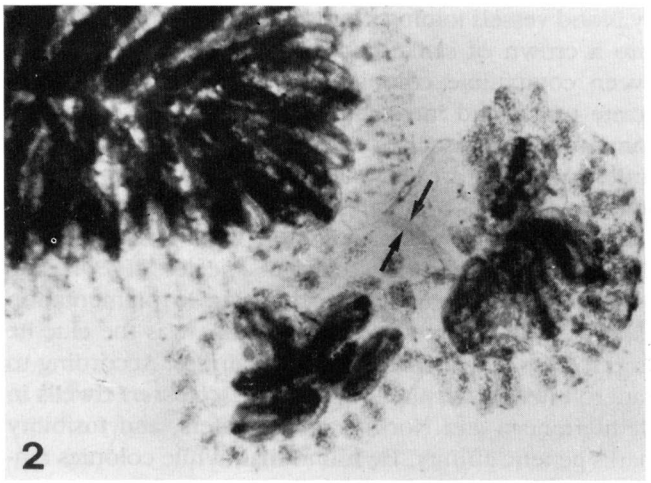
Fusibility in *Botryllus* has long been known: Giard (1872) mentioned many different species of *Botryllus* present on European coasts, differing in pigmentation patterns. The ability of colonies to fuse was the clue he used to resolve classification uncertainties. According to Bancroft (1903) the single species *B. schlosseri* dwells in Mediterranean and North Atlantic waters, and fusibility marks genetic affinity. He found that, while colonies randomly collected in the field rarely fuse, there is more frequent fusion between offspring from the same mother, and constant fusion between pieces of the same colony.

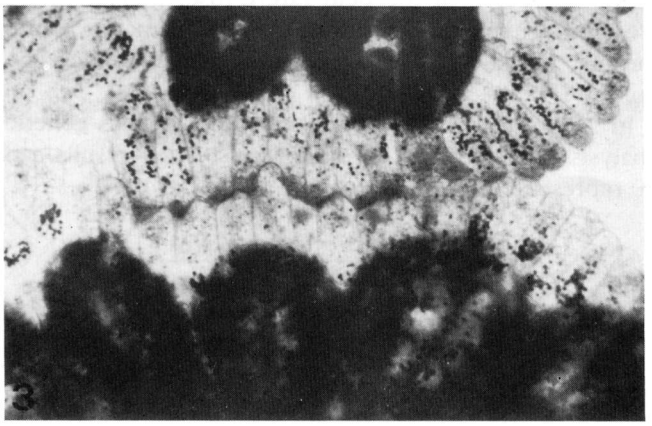
In 1957 Oka & Watanabe embarked on the genetic analysis of fusibility in *B. primigenus*. Their results can be represented as follows:

P			AB		×		CD								
F_1			AC		AD		BC		BD						
	AC		×		BD			AD		×		BC			
F_2	AB	AD	BC	CD				AB	AC	BD	CD				
	♂ BC		×		BD			♂ BD		×		BC			
F_2	BC				CD			BD				CD			

Besides confirming the data of Bancroft in *B. schlosseri*, they showed that progeny from nonfusible







Figs. 1-3 - *Botryllus schlosseri* colonies from ventral side. ***Fig 1***. Zooids embedded within tunic are connected by a vascular network with marginal vessel (arrows) expanding into a crown of vascular ampullae; ×12. ***Fig. 2***. Vessel anastomosis (arrows) marks fusion of two histocompatible colonies; ×8. ***Fig. 3***. Histoincompatible colonies in process of rejection; ×21.

colonies, AB × CD with reference to a presumed locus of fusibility, actually segregated into four classes - AC, AD, BC, BD - with 75% fusibility, each class being fusible with two others and all the offspring with both parents. This was also true of progeny from F_1 incompatible colonies (Oka & Watanabe, 1960). These authors came to the conclusion that fusibility is controlled by one gene locus with condominantly expressed alleles: fusion takes place whenever even a single allele is shared. No selfing occurs in this species. At first a completely mutual relation between somatic compatibility and sexual incompatibility was considered: sharing of even only one allele would have involved cross-sterility as well. It was later shown that this sterility is partial: only sperm sharing their allele with the ovular envelopes are sterile, like the gametophytic type of sterility in some flower plants (Oka, 1970). The double way cross between F_1 BC and BD colonies yields F_2 offspring only segregating into two classes – BC, CD and BD, CD respectively – distinguished by the fact that CD colonies fuse with all the F_1 progeny. In conclusion, *B. primigenus* colonies are all heterozygous at the locus of fusibility.

The situation is different in *B. schlosseri*. The protogyny of this species (Milkman, 1967), with all zooids within each generation maintained at the same sexual stage by common circulation, may be circumvented by intercrossing pieces of the same colony at different sexual stages; this makes possible both selfing and, of course, cross-fertilization between colonies of the same fusibility genotype (Sabbadin, 1959, 1971, 1982). Therefore, in this species, the genetic analysis of fusibility does not suffer from the restrictions met within *B. primigenus*, as shown in Table I, which summarizes our previous (Sabbadin, 1962, 1982, 1989) and some new data. One gene locus with codominant alleles also controls fusibility in this species. Offspring from parental as well as filial incompatible colonies segregate into four classes of equal frequency; offspring from colonies of the same class segregate into three classes in a 1:2:1 ratio, two of them homozygous, with 87.5% fusibility; two classes, with 100% fusibility, segregate from homozygous males crossed to heterozygous females with a shared allele and, of course, a single class from homozygous colonies of identical genotype.

Scofield *et al.*, (1982a, b), working with *B. schlosseri* populations from Woods Hole and from Monterey Bay, chose a different genetic approach. They determined the percentage of fusibility of pairs of oozooids from colonies fertilized in the field or from controlled crosses in the laboratory. Since fusibility never exceeded 75%, they confirmed that it requires the condivision of one allele. They were surprised at the absence of homozygotes in some dozens of colonies tested, and thought that this absence might perhaps be explained by the same mechanism of shared allele exclusion acting in *B. primigenus*, although scanty offspring from isolated colonies were obtained. One fact supporting their

TABLE I - *Genetic analysis of fusibility between colonies of* Botryllus schlosseri.

Parental genotypes	No. of crosses	Offspring genotypes			
		AC	AD	BC	BD
AB × CD	11	64	57	68	63
		AB	AD	BC	BD
AC × BD	9	62	60	68	50
		AA	AB	BB	
AB × AB	4	30	79	32	
		AB	AD		
AA × BD	6	88	81		
		AA			
AA × AA	10	186			

In our laboratory, alleles are given a progressive number; in Table I numbers are replaced by letters A, B, C, D. (The fusibility genotype of any given colony was determined step by step by juxtaposing its systems of zooids with those from tester colonies of known genotype).

hypothesis was the finding of 100% fusibility among the pairs of oozooids from some crosses between F_1 and some crosses between Monterey colonies collected on the same algal blade.

The conclusion of Scofield *et al.* (1982b) – that in *B. schlosseri* there is a gametic barrier of the type found in *B. primigenus* – does not fit our results with Mediterranean *B. schlosseri*, shown in Table II. This may be due to differences between populations. Slight morphological and consistent allorejection differences have been found even between the two populations considered by the above authors (Boyd *et al.*, 1990). Actually, as shown in Table II, a penalization of the sperm sharing their alleles with ovular envelopes was observed in some of our crosses between AB and BD colonies and between BD males and BB females, contrary to what happens in reciprocal crosses between BB males and BD females (Sabbadin, 1989, and new data). In any case, only shortages but not the expected absence of classes of offspring were observed. It is worth noting that the two alleles involved in this segregation distortion came from two colonies from the Tyrrhenian Sea whose offspring were crossed to colonies from the Lagoon of Venice.

Relationships between the distorting factor(s) and the fusibility locus must be determined. There may be a gene complex (a «MHC-like system», according to Scofield *et al.*, 1982a) that controls other phenomena besides fusibility: by means of a genetic marker represented by a rare allele of PGI locus, Grosberg & Quinn (1986) showed the aggregative settlement of marked larvae on the basis of alleles shared at the fusibility locus, while Rinkevich & Weissman (1987) demonstrated the involvement of the same locus in the resorption phenomenon which affects one of the members in fused pairs, and its possible involvement in the «retreat growth» of one of the members of incompatible pairs (1988). Using the tunic deprived of zooids as a culture medium for grafting young buds, we have shown (Sabbadin, 1982) that grafted buds can be vascularized and grow to maturity only if donor and recipient are fusible.

The fusibility locus is highly polymorphic: Oka & Watanabe (1957) showed that only two out of 50 colonies of *B. primigenus*, collected in an area of 1 m^2, behaved exactly in the same way when confronted with all the others. In the same species, out of 968 pairwise combinations of 45 colonies from two localities about 3000 m apart, there were only 11 fusions (Tanaka & Watanabe, 1973). As for *B. schlosseri*, in the Eel Pond at Woods Hole, Karakashian & Milkman (1967) and Grosberg & Quinn (1986) observed fusion frequencies of 5% and 4.2%, respectively, with roughly 80-100 alleles involved, corresponding to a few thousand different genotypes. This accounts for the rarity of homozygous colonies within a population. For instance, so far we

TABLE II - *Genetic analysis of fusibility between colonies of* Botryllus schlosseri.

Parental genotypes	No. of crosses	Offspring genotypes			
		AB	AD	BB	BD
AB × BD	9	82	78	73	84
	1	24	24	17	7*
	1	55	49	20	24**
	1	32	40	15	24**
				BB	BD
♂ BB × BD	11			142	151
				BB	BD
♂ BD × BB	5			48	42
	1			8	21*
	1			4	12*
	1			8	21*
	1			6	24**
	1			2	9

* $P<0.05$; ** $P<0.01$

have found only one homozygote (100% fusibility among the 57 offspring tested, segregated into two classes) out of hundreds of colonies collected in the Lagoon of Venice and crossed in the laboratory with incompatible heterozygous partners.

Transplantation and cell «contact reaction» in solitary ascidians

Contrary to what happens with *Botryllus*, both fusion and graft retention in the solitary ascidian *Styela plicata* demand identity of both controlling haplotypes (Raftos *et al.*, 1988; Kingsley *et al.*, 1989). As for the «contact reaction» of allogeneic cells leading to mutual lysis, demonstrated by Fuke (1980) in different species, Fuke & Numakunai (1982) showed that, within the three – A,B,C – variants of *Halocynthia roretzi*, the contact reaction does not occur in 13-15% pairwise combinations and no allele need be shared, as is the case of the nonfusion reaction in *Botryllus*. A subsequent thorough analysis of alloreactivity in 30 specimens of the A variant (Fuke & Nakamura, 1985) suggested genetic control by at least two gene loci; one shared allele is sufficient to prevent the reaction.

CYTOLOGY OF ALLOREACTIONS

Rejection between contacting colonies in the family Botryllidae

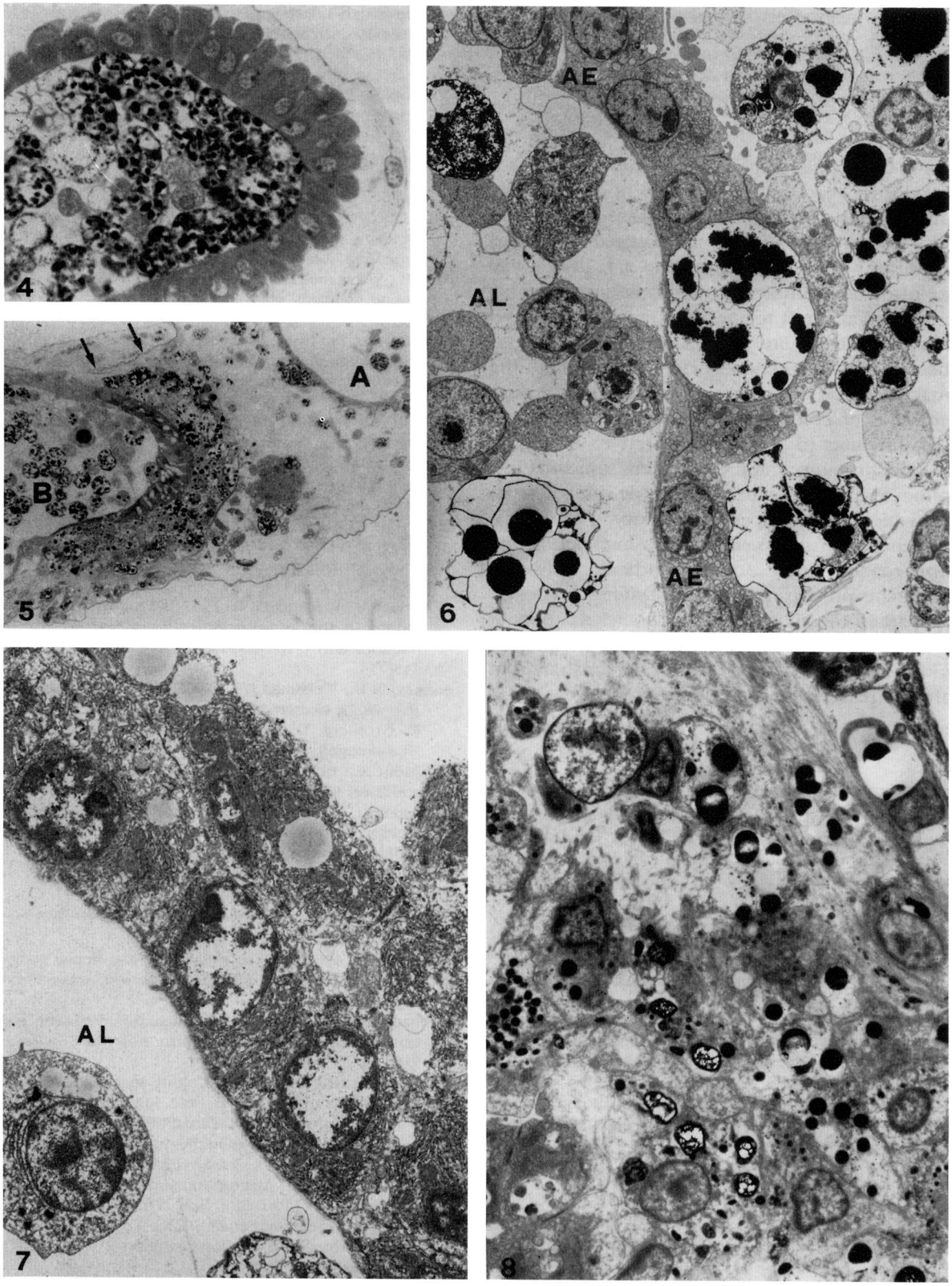
Rejection between colonies contacting at growing edges takes different forms in the species investigated (Taneda *et al.*, 1985; Hirose *et al.*, 1988): (a) «*Botryllus scalaris model*» (Saito & Watanabe, 1982): Rejection takes place within the ampullar lumen soon after tunics and opposite ampullae have fused, possibly as a cytotoxic reaction between allogeneic hemocytes of the «contact reaction» type described by Fuke (1980) in solitary ascidians; (b) «*B. primigenus model*» (Taneda & Watanabe, 1982a; Taneda *et al.*, 1985): Following tunic fusion and ampullar contact, a diffusible factor from the opposite colony reaches the ampullar lumen and acts on the allogeneic hemocytes; (c) «*Botrylloides violaceus and B. simodensis model*» («sub-cuticular reaction», Hirose *et al.*, 1988): Fusion of the opposite tunics, involving the disappearance of surface cuticular layers, only occurs in small areas along the boundary zone of the two colonies, where the necrotic reaction is concentrated, without reaching the ampullar lumen. Together with humoral factor(s) (Saito & Watanabe, 1984), tunic cells may be involved in this type of allorecognition. According to Hirose *et al.*, (1988), in the course of evolution allorecognition shifted from within the vessels to the periphery, that is, from the (a) to (c) model. The «*B. schlosseri model*» (d) does not seem to fit this scheme. According to Boyd *et al.*, (1990), fusion of tunics does not occur between incompatible colonies of the Monterey population, whereas it does, although in limited areas, in the Woods Hole and Venice populations (Milanesi *et al.*, 1978). Working with Woods Hole *Botryllus*, Scofield & Nagashima (1983) described different categories of rejection, making a continuum and reflecting a hierarchy between the fusibility alleles involved. Transient fusion between opposite ampullae, aborted at different stages and involving contact between allogeneic hemocytes as in *B. scalaris*, would correspond to the different nonfusion types.

In all the species, rejection implies infiltration of blood cells, especially morula cells, from the ampullae into the tunic where they disintegrate, thus forming the bulk of the necrotic material that also involves the tunic cells of the area. Cell infiltration is mediated by increased permeability of the ampullar epithelium, as revealed by the leakage of India ink from the tips of ampullae (Taneda & Watanabe, 1982a); it may be induced by a chemotactic factor (Hirose *et al.*, 1990) which congregates the cells at the ampulla tips before being released into the tunic, in both *B. primigenus* and *B. schlosseri* (Fig. 4).

B. schlosseri morula cell release, with resulting fenestration of the ampullar epithelium, is seen in Figures 5 and 6. The epithelium is damaged in this process (Fig. 7). Degeneration of the released cell mass is shown in Figure 8. The role in the rejection process of morula cells and possibly of other types of cells infiltrated into the tunic is probably passive, since X-irradiation, which kills the least differentiated lymphocyte-like cells, strongly affects rejection (Taneda & Watanabe, 1982b).

Graft rejection

Tunic allograft rejections in *Ciona intestinalis* and *Styela plicata* have been studied by Reddy *et al.* (1975) and Raftos *et al.* (1987a, b), respectively. In *Ciona*, rejection is expressed 6-8 weeks after transplantation in the graft demarcation from host tissues and the accumulation of lymphocyte-like cells, granulocytes and phagocytes along the graft borders. At the end of the 8th week, the frequency of lymphocytes is significantly higher than in autografts, whereas the percentage of morula cells is lower than that found in the normal integument, in both auto- and allografts. As regards *Styela*, by means of first-set, second-set and third-party allografts, specific memory has been demonstrated (Raftos *et al.*, 1987a). At first, both auto-and allograft beds are invaded by amoebocytes, morula, signet-ring and lymphocyte-like cells, all reaching significantly higher frequencies than in the normal tunic. Later, the first three types of cells gradually decrease to normal levels, whereas the lymphocyte-like cells remain at a constant level (approximately three times greater than in the normal tunic in autografts), and continue to invade the allograft bed, reaching a frequency three times that of autografts and nine times that of the normal tunic. That is to say, lymphocyte-like cells are responsible for the recognition and destruction of allogeneic tissue (Raftos *et al.*, 1987b). Cooper *et al.* (1992) also show the proliferative activity



Figs. 4-8 - Rejection process between incompatible *Botryllus* colonies shown in thin and ultrathin sections of specimens fixed in glutaraldehyde, buffered with sodiumcacodylate solution, and postfixed with OsO_4. ***Fig. 4***. Hemocytes, principally morula cells, congregating at apex of an ampulla in contact area between two colonies; ×800. ***Fig. 5***. Contact area between two incompatible colonies represented by A and B ampullae; tunics have fused after dissolution of cuticles (remnants marked by arrows). A mass of blood cells has crossed the now fenestrate epithelium of B ampulla, encrusting its external wall; ×350. ***Fig. 6***. Morula cells that have passed or are passing through ampullar epithelium, AE; AL, ampullar lumen; ×3500. ***Fig. 7***. Degenerative changes, involving intercellular junctions, nuclei and endoplasmic reticulum, affect ampullar epithelium crossed by hemocytes; AL, ampullar lumen; ×5600. ***Fig. 8***. Degeneration of cell mass after leaving ampullar lumen: electrondense granules from disintegrating morula cells are scattered among picnotic nuclei and cytoplasmic debris; ×3500.

of these cells in response to allogeneic stimuli in *Styela clava*.

CONCLUDING REMARKS

The three types of allorecognition seen in ascidians – allograft rejection and cell contact reaction in solitary ascidians, and the nonfusion reaction in colonial ascidians – do not seem to be closely related. That is to say, they differ both in genetic control and in the way they take place. A difference of one haplotype prevents both fusion and allograft retention in the solitary *Styela* but not in the colonial *Botryllus*, and does not seem to cause the cell contact reaction in *Halocynthia*. Both the cell contact reaction of solitary ascidians and the nonfusion reaction of colonial botryllids are acute, taking from a few minutes to 1-2 days, respectively. Allograft rejection is a chronic reaction demanding some weeks.

Lymphocyte-like cells are responsible for allograft rejection, which induces specific memory. There is indirect evidence that these cells are also involved in the nonfusion reaction of colonial ascidians, although morula cells are principally affected. Different types of cells take part in the allogeneic cell contact reaction. A similar phenomenon, recalling NK cell activity in mammals, has been suggested but so far not demonstrated, in the nonfusion reaction of some *Botryllus* species.

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