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AGGREGATION AND FUSION BETWEEN CONSPECIFICS OF A SOLITARY ASCIDIAN

G. H. SCHMIDT

Department of Zoology, University of Reading, Whiteknights, Reading RG6 2AJ, England

Abstract

Fusion between conspecifics in a solitary ascidian is reported for the first time. *Molgula complanata* Alder and Hancock showed aggregated settlement on Perspex panels in the field, allowing contact between conspecifics after some increase in size. Histological sections of adult animals which were in contact with one or more conspecifics showed that some individuals were fused to others. The frequency of fusion between contacting specimens was 20%. The outer membranes of the tunics were absent between fused animals but present in unfused ones. Fusion was thus characterized by contiguous matrices, which contained cellular elements. No barrier to interchange of tunic cells between fused animals was observed. It is suggested that fusion may oppose inbreeding in hermaphroditic, viviparous ascidians with minimal dispersal.

Introduction

Fusion of animals derived from separate larvae was first described in 1903 in colonial ascidians. It has since received considerable experimental treatment and is of great interest in the context of comparative immunology (Bancroft, 1903; Hildemann and Reddy, 1973; Tanaka, 1975 for references).

Under experimental conditions fusibility is found in many colonial ascidians (Tanaka, 1975). Fused colonies are indistinguishable from those which are derived through asexual budding. This makes it difficult to study the occurrence of fusion in natural populations (Sabbadin, 1978). It was thought that solitary ascidians, which do not bud, might nevertheless show fusion. Aggregative settlement in solitary ascidians would increase the frequency of contacts between conspecifics and thus increase the chances of possible fusions. However, for ascidians no data exist on the pattern of settlement in the field.

Experiments on the genetic control of colony fusion indicated that fusion may be frequent between closely related conspecifics (Tanaka, 1975 for references; Sabbadin, 1978). In the field, viviparity and a short larval life would be expected to increase the chances of closely related individuals settling near each other. It was therefore decided to examine a natural population of *Molgula complanata*, a viviparous hermaphrodite, for aggregated settlement and for fusion in the resulting clusters.

Materials and Methods

The study locality was Langstone Harbor, a fully marine, shallow, natural harbor bordering the northern shore of the eastern Solent (south coast of England).

Perspex settlement panels, 0.25×0.25 m square, were fixed to frames and suspended from a raft within 1.5 m of the surface and about 5 m from the seabed.

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To examine aggregated settlement, three horizontally aligned panels were submerged for four weeks during the spring settlement peak (May). The positions of all attached and metamorphosed individuals on the underside of the panels were then determined with the aid of a grid which was scratched onto a thin sheet of a 0.25 × 0.25 m transparent Perspex and placed directly above the settlement panel. The size of the squares of the grid was 10 × 10 mm; the quadrat sizes chosen to test for aggregation were 20 × 20 mm and 40 × 40 mm. The observed distri-

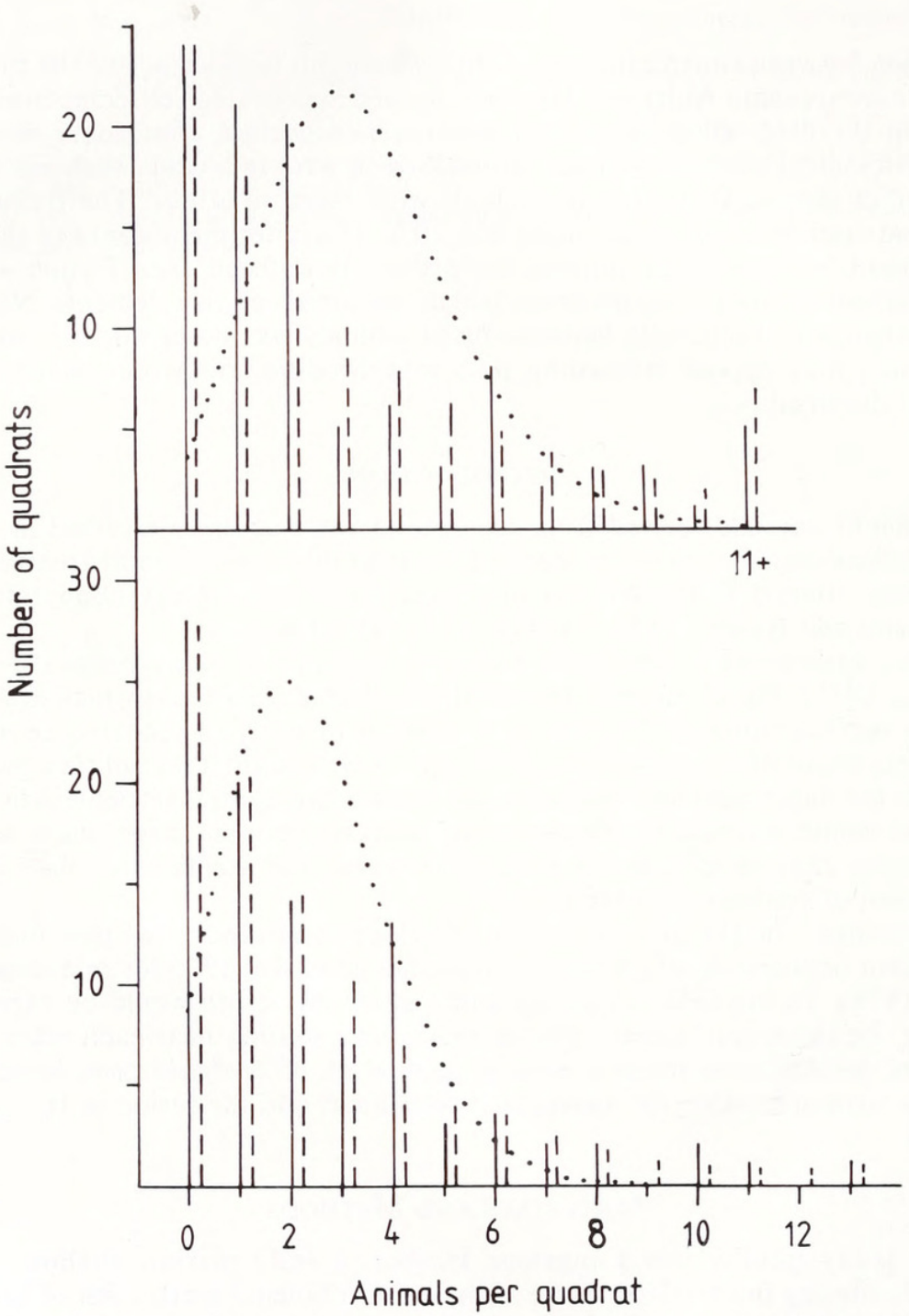

FIGURE 1. Distribution of settled *Molgula complanata* juveniles on 2 replicate panels which had been submerged in the sea for 4 weeks (solid bar). Dotted lines are calculated Poisson distributions; all actual distributions depart significantly from random ($P < 0.001$) but fit well the calculated negative binomial distributions (broken bars).

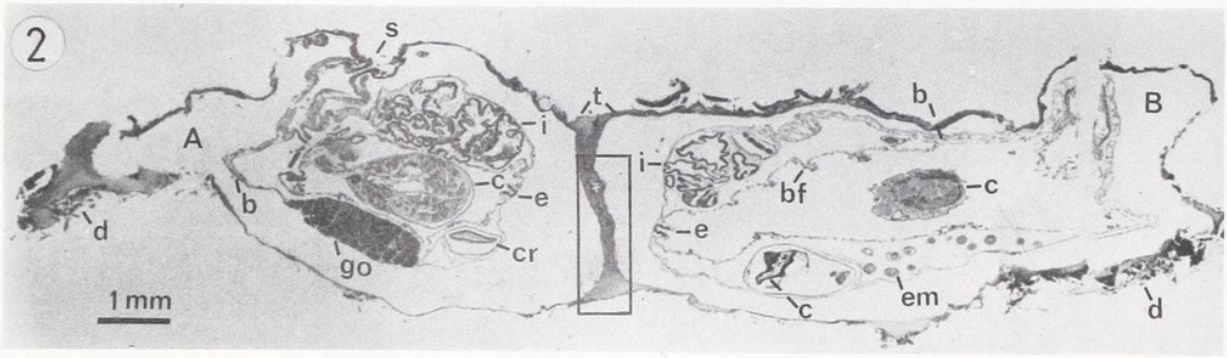

FIGURE 2. Vertical section of *M. complanata* showing extensive fusion over the zone of contact between two individuals, A and B. The framed region is shown at higher magnification in Figure 3. Abbreviations for Figures 2–5: b, body wall; bf, fold of branchial basket; c, associated copepod, *Doropygus pulex* Thorell, in the pharynx; ce, cells in the matrix of the tunic; cr, concretion in renal vesicle; d, debris; e, endostyle; em, embryo; g, gap; go, gonad; i, intestine; om, outer membrane of tunic; s, siphon; t, tunic.

butions were compared with Poisson and negative binomial distributions calculated from the observed data (Ross, 1980).

To examine fusion, aggregated specimens of *M. complanata* were removed from vertically aligned panels which had been submerged for 3–13 months. Individuals in physical contact were carefully separated with forceps, beginning with the side originally attached to the panels. 10 μm vertical sections were made from specimens not separable in this manner. All sections were stained with haematoxylin and eosin.

One side of one panel was analysed for frequency of fusion. Only adult animals $\geq$ 5 mm were considered.

RESULTS

Aggregation

Comparison of the frequency distribution of recently settled *M. complanata* with expected random (Poisson) distributions verified that settling onto the experimental panels was non-random. All distributions, instead, fitted calculated negative binomial distributions, where the defining parameter 1/k indicates that departures from random were due to individuals being aggregated in groups (Ross, 1980). Data for both quadrat sizes are consistent, and examples of two replicate panels are given in Figure 1 for quadrat size 20×20 mm. Although aggregated, the majority of postlarval animals were not in direct physical contact. The percentages of actual contacts between recently settled juveniles were 2%, 2%, and 3% for the three replicate panels.

Other species were present in very low numbers, except the colonial ascidian, *Botryllus schlosseri*, which was as abundant as *M. complanata*. The small colonies of *B. schlosseri*, however, were randomly distributed (*i.e.*, observed distributions fitted expected Poisson distributions) and had apparently not influenced the settlement pattern of *M. complanata*. There was over 95% free space on these panels.

Fusion

The tunics of individuals not separable in the manner described above were fused in the area of contact between animals (Figs. 2, 3, and 4). Cells were abundant in the matrix of the tunics with no evidence of any barrier to cell interchange

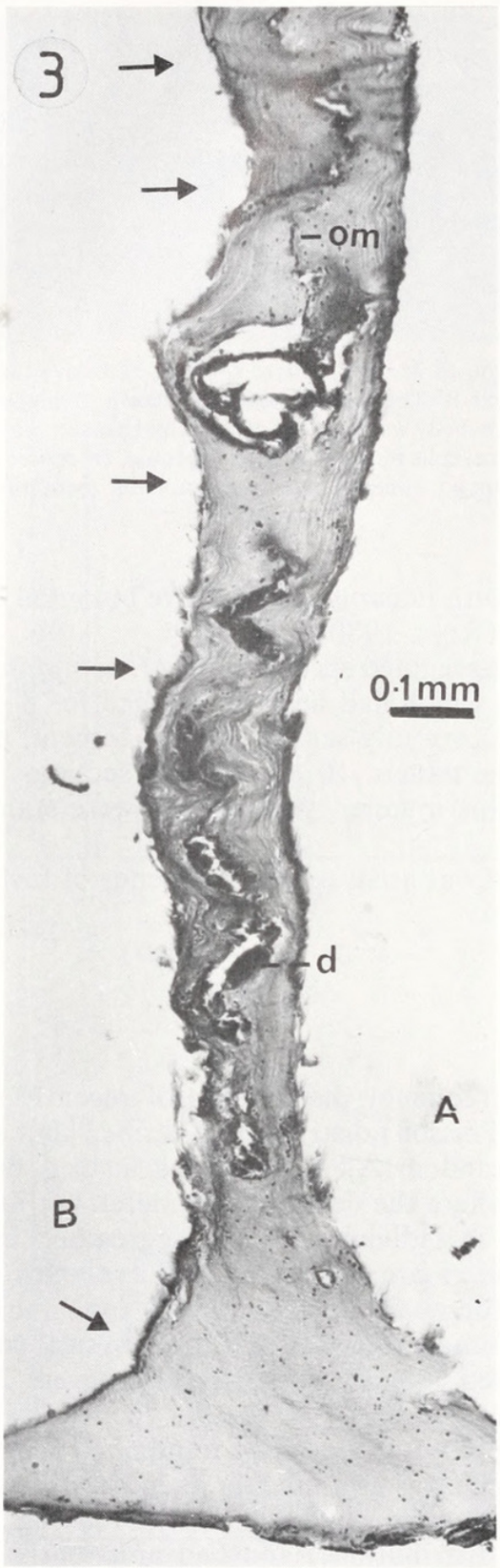

FIGURE 3. Part of the contact region of A and B showing several areas where the tunics are fused (arrows). There are also unfused areas, containing debris and the remains of the outer membranes of the tunics. (Abbreviations: see Figure 2.)

between fused animals. No zones of necrosis were observed. Serial sections showed that fusion could be impeded by debris (Fig. 3). In contrast, Figure 5 shows part of the contact zone between two closely appressed but unfused animals. A line of separation, either as closely adhering outer membranes, or as gaps, is clearly visible.

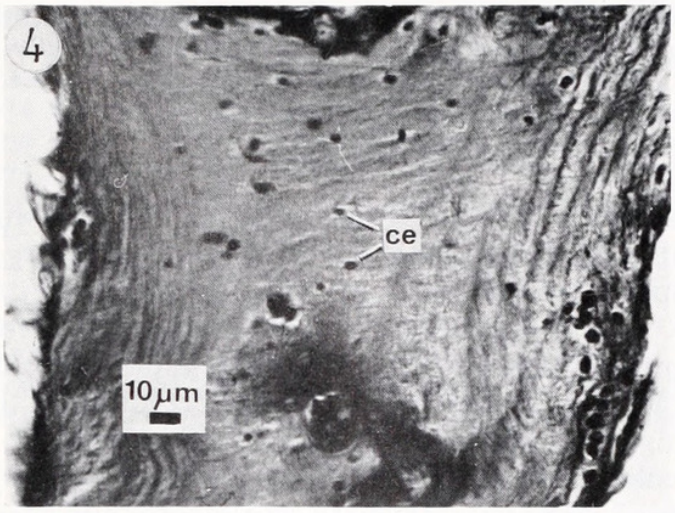

FIGURE 4. Part of Figure 3 at higher magnification. The fibrous matrix is continuous between the fused animals allowing free interchange of the cells contained within the tunic. (Abbreviations: see Figure 2.)

The majority of fusions were between individuals of equal size, but fusion between individuals of several-fold difference in size was also found.

Frequency of fusion

One panel was examined for frequency of fusion. Of 190 animals, 48 were single; occasionally they had unscored juveniles attached to their tunics. The remaining 142 individuals were in direct physical contact with one or more conspecifics, yielding groups of 2–10 animals. As 29 individuals in these groups were unseparable by the method described above, a subsample of 13 of these was analysed histologically. These were fused. It was concluded that about 20% of those which occurred in groups were fused to conspecifics. Fusion was, with one exception, between two ascidians; one group of three fused individuals was found. Although

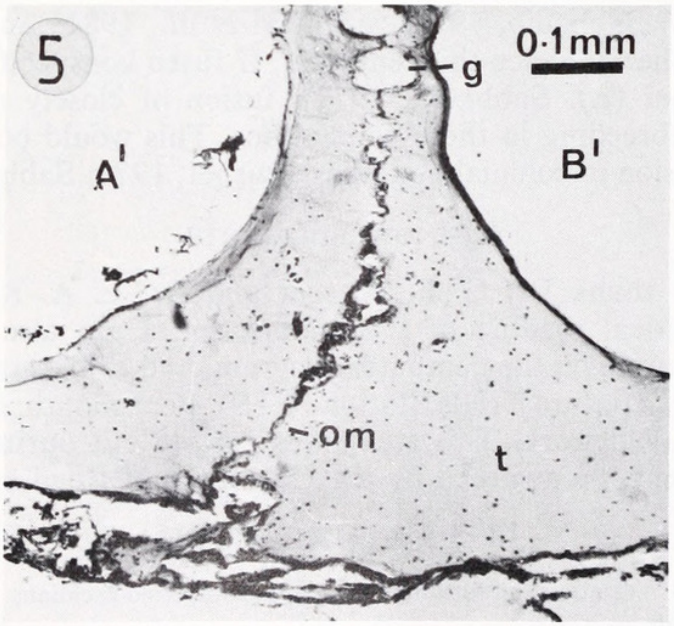

FIGURE 5. Basal part of contact zone of closely appressed but unfused individuals (A′ and B′). A line of division, either as gaps or adhering outer membranes, separates the two animals. (Abbreviations: see Figure 2.)

only one panel was quantitatively sampled for fusion, fused animals were also found on all other panels (11 altogether).

Discussion

This is the first report of aggregated settlement of a solitary ascidian in a natural, field population. There is one account of a laboratory study for another solitary ascidian species which demonstrated aggregative settlement (Young & Braithwaite, 1980). The results indicate that *M. complanata* recognizes conspecifics and settles close to them. The formation of aggregations would favor cross-fertilization in contrast to possible self-fertilization (inbreeding) in this hermaphroditic ascidian. Also, it might have reduced the chances of juvenile *M. complanata* being overgrown by the colonial *B. schlosseri*.

In clusters of adult animals both fused and closely appressed but unfused individuals occurred. Fusion appears to be a relatively frequent, naturally occurring event. This suggests that self versus not-self recognition may exist. This idea successfully explains colony specificity in colonial ascidians (Burnet, 1971; Tanaka, 1975 for references).

Fused animals were apparently compatible. Fusion followed disappearance of the outer membrane of the tunic which is believed to be proteinaceous (Goodbody, 1974). The fibrous matrix of the tunic was continuous between fused individuals, so the cells found in the tunic may have interchanged unimpeded. The tunic cells of ascidians are amoeboid cells and blood cells (Goodbody, 1974). An invasion of tunic cells of an allogeneic animal after tunic fusion might mobilize immune responses, such as those between incompatible colonial ascidians or the cellular reactions between incompatible coelomic cells in solitary ascidians (Tanaka and Watanabe, 1973; Manning and Turner, 1976; Fuke, 1980). But no evidence of any such reactions was found in the present material. For solitary ascidians it has been difficult to obtain information on histocompatibility in laboratory experiments (Hildemann, 1974; Tanaka, 1975).

The life history parameters of *M. complanata* make it likely that closely related individuals, *e.g.* larvae released from the same parent, will occasionally settle in close proximity (*cf.* Sabbadin, 1978; van Duyl *et al.*, 1981) and aggregative settlement would further enhance this tendency. If fused conspecifics were unable to fertilize one another (*c.f.* Sabbadin, 1979), fusion of closely related individuals would promote outbreeding in these populations. This would be of selective value as suggested for fusion in colonial ascidians (Burnet, 1971; Sabbadin, 1978, 1979).

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