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Filter-feeding in Colonial Protists

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Many protists form cell colonies. Among them several are filter-feeders depending on suspended food particles such as bacteria. It has been suggested that the formation of colonies enhances feeding efficiency and implied that – in the case of colonial choanoflagellates – it was an adaptive trait that led to the evolution of metazoans. Here it is shown experimentally – for a colonial peritrich ciliate and for a choanoflagellate – that colony-formation does not enhance the efficiency of filter-feeding relative to solitary cells and that the adaptive significance of cell colony-formation must have some other explanation.

Key words: Cell colonies; suspension-feeding; peritrich ciliates; choanoflagellates.

Introduction

Many protists form cell colonies: cells do not separate following mitotic division, but remain attached in close contact or via branching stalks. The colonies reproduce by forming motile cells that detach from the colonies to form new colonies. It is clear that colony-forming protists potentially represent a step towards true multicellularity; that is, with a germline consisting of potentially immortal cells (the germ line) and somatic cells that are specialized for different functions, but have a limited lifespan thus allowing formation of tissue with special functions (e.g., Woodland 2016). With respect to some phototrophic lineages among the eukaryotes such as green algae and rhodophytes the evolution to multicellularity in this sense is well understood. With respect to the origin of metazoans it was more than a century ago suggested that choanoflagellates, among which several are colonial, represent the ancestors of sponges and by implication other metazoans due to the similarity of these flagellates to the choanocytes of sponges. More recently, molecular evidence and some structural details at the cellular level have shown that the choanoflagellates do, in fact, constitute a sister group to the metazoans and so the evolutionary role of choanoflagellates has drawn renewed interest (Leadbeater 2015). However, even in colonial choanoflagellates all cells retain the potential to form new colonies and are not "multicellular" in the above sense.

In this context it has been suggested that the efficiency of suspension feeding is enhanced if the cells form colonies and so – by implication – that this was a selective force that resulted in colony-formation among a number of choanoflagellate species (Cavalier-Smith 2017; Roper et al. 2013) although this result was based only on theoretical considerations without experimental evidence. In the present contribution this is examined for a colonial peritrich ciliate (*Zoothamnium dublicatum*

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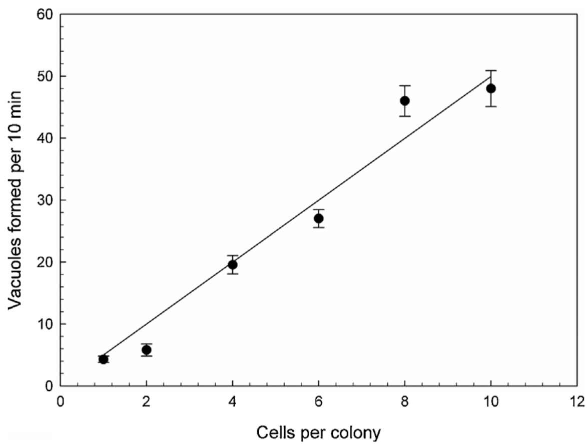

Figure 1. Formation of feeding vacuoles in colonies of *Z. dublicatum* as a function of cell numbers per colony after 10 min exposure to a 0.2 μm latex bead suspension (concentration 2×10^6 ml^{-1}) for ten minutes. The graph is based on the vacuole numbers of 7 colonies with a total of 51 cells and the error bars represent the standard deviation of the mean values ($r^2 = 0.953$).

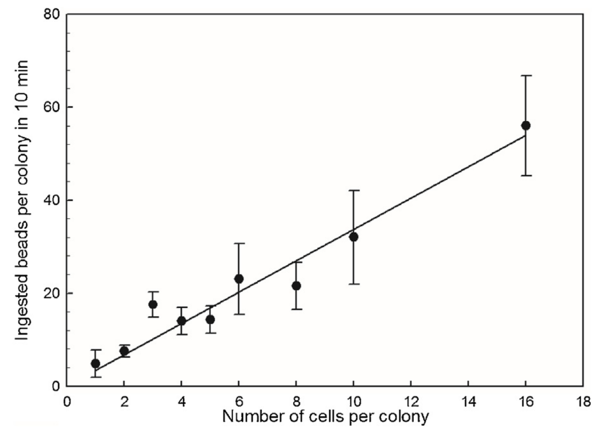

Figure 2. Uptake of particles (0.5 μm) during 10 min by *Codosiga botrytis* colonies as function of number per cells per colony after exposure to a particle concentration of 4×10^6 ml^{-1}. The data represent the vacuole contents of 21 colonies with a total of 69 cells and the error bars represent the standard deviation of the mean ($r^2 = 0.946$).

Kahl) and a species of colonial choanoflagellates: *Codosiga botrytis* Ehrenberg.

Results

Peritrich Ciliates

Zoothamnium dublicatum forms stalked, branching colonies where the cells form a single slightly curved layer (canopy) with up to 15–20 cells. Since the spherical food vacuoles have a very uniform volume (115.0 μm^3, SD = 14.2) they can, for comparative purpose, be used as a measure of feeding rate. Figure 1 shows the formation of food vacuoles formed in colonies with different cell numbers incubated in a suspension of 0.2 μm latex beads with a concentration of 2.1×10^6 ml^{-1}. It is seen that the number of vacuoles per colony is a simple linear function of the number of cells. Using 0.2 μm beads they were so densely packed within the food vacuoles that individual beads could not be counted. Incubation in a suspension of 2 μm beads (2.2×10^6 beads ml^{-1}) provided an estimate of clearance per cell to be 3.86×10^{-4} ml h^{-1}.

For comparison with solitary peritrichs *Vorticella elongata* Fromentel has a clearance of 1.6×10^{-4} ml h^{-1} using similar methods for quantification of clearance (Fenchel 1986). But *Z. dublicatum* has a cell volume that is about 2.5 times larger than that of *Vorticella elongata* (Fenchel 1986). There is therefore no evidence to suggest that colony formation, which is frequent among peritrich ciliates, enhances the per capita rate of suspension feeding.

Choanoflagellates

Codosiga botrytis forms stalked colonies with up to 20 cells. The main stalk is attached to a solid substrate and at the end of the stalk cells are attached to shorter branching stalks to form a curved canopy. Eventually, when the maximum number of cells is reached the canopy forms a hemisphere. The water currents generated by the cells are directed upwards and the flow lines converge just above the colony so that the water current is directed towards the colony all along its passage.

Figure 2 shows the total particle (0.5 μm) uptake as a function of the number of cells per colony. The relation is linear and corresponds to 20.3 beads cell^{-1}h^{-1} so that the filtration efficiency per cell is invariant with respect to colony size (Table 1).

Discussion

The present results show that colony formation does not enhance the efficiency of filter feeding as previously suggested by Cavalier-Smith (2017) and by Roper et al. (2013). It is in all cases only a question of a number of pumps that work in parallel. A somewhat similar conclusion was drawn by Kirkegaard and Goldstein (2016) on a theoretical

Table 1. Filtration rates per cell of solitary and colonial choanoflagellates. The values for *Diaphanoeca* derive from *Diaphanoeca* derive from Andersen (1988/89) and from Fenchel (1982) and were derived from growth kinetics and flow through the collar respectively. Data on *Monosiga* derive from Fenchel (1986) and were obtained from growth kinetics. Data on *Codosiga* derive from the present study.

	Cell volume (μm^3)	Clearance ($ml\,h^{-1}$)	Volume specific clearance (h^{-1})
Diaphanoeca	66	2.80×10^{-6}	1.13×10^5
–	–	2.79×10^{-6}	1.12×10^5
Codosiga	130	1.04×10^{-5}	8.0×10^4
–	–	1.30×10^{-5}	1.0×10^5
Monosiga	20	2.0×10^{-6}	9.8×10^4

basis. The essential problem is that the cell colonies must have a structure so that a given parcel of water is not strained of food particles by more than one cell. Thus the architecture of the colonies is in all cases that of a single layer of cells that either point in the same direction or are arranged on a convex surface. For suspension feeders it applies that attachment to solid surfaces, or if suspended, that they possess some sort of drift anchor, will increase the efficiency of suspension feeding. In this way water currents will converge towards the filter or – in the case of colonial forms – converge to the colony. This is in contrast to non-thrust swimming in which case the organism experiences parallel flow lines (Christensen-Dalsgaard and Fenchel 2003). A suspended colonial organism – such as the colonial form of *S. rosetta* (Dayel et al. (2011) in principle pumps water outwards in all directions and this will function as a sort of drift anchor.

Colony formation is a widespread phenomenon among different groups of protists – phagotrophs as well as phototrophs – and there may not be a universal explanation for this phenomenon. In some cases colony formation is induced in the presence of predators (Boraas et al. 1998; Jakobsen 2002; Kapsetaki and West 2019). So predator avoidance is perhaps also a more general explanation for the formation of cell colonies.

Methods

Zoothamnium dublicatum was collected on the thallus of the rhodopyte *Ceramium virgatum* growing in shallow water in Aarhus Bay (East coast of Jutland, Denmark) and the algae were maintained in aerated seawater (salinity: ca. 20 ppt). *Codosiga botrytis* was collected on filamentous green algae growing in a freshwater tropical fish tank.

The measure of feeding efficiency referred to as clearance is the volume of water cleared of food particles per unit time. At low particle density this is equivalent to the volume of water passing through the filter, that is, the array of cilia (ciliates) or microvilli (choanoflagellates). A direct method to quantify this was to measure the flow rate through the filter using suspended particles (latex beads or heat-killed bacteria) as markers and to quantify the water flow from video recordings (25 fps). I also used the uptake of suspended fluorescent latex beads with sizes 0.2, 0.5 or 2.2 µm (Polysciences) over a known time period and counting the ingested particles in the fluorescence microscope in formalin fixed cells. In the choanoflagellates it was thus possible to count individual particles in the cells. With respect to *Zoothamnium* using 0.2 µm particles the individual particles could not be resolved within the vacuoles. However, feeding vacuoles were spherical and of uniform sizes so for a comparative purpose the rate of vacuole formation could be used.

With respect to estimating clearance from particle uptake or growth kinetics it has to be kept in mind that clearance is a function of ambient particle concentration because there is a maximum rate of processing captured particles and the measured clearance can be described in terms of Monod-kinetics as a function of particle density (Fenchel 1986). Therefore only very low particle concentrations, where particle uptake is almost linearly proportional to ambient particle concentration, must be applied. Alternatively it is necessary to estimate clearance for several different particle concentrations so that it is possible to extrapolate back to zero-concentration, i.e. the water flow through the filter. This then provides a measure of the competitive ability at low particle concentrations (Fenchel 1986). All rates recorded in the present paper refer to a temperature of 20 °C.

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