Social feeding behavior of *Trichoplax adhaerens.*

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Abstract

Animals have evolved different foraging strategies in which some animals forage independently and others forage in groups. The evolution of social feeding does not necessarily require cooperation; social feeding can be a beneficial individual-level strategy if it provides mutualistic benefits, for example though increasing the efficiency of resource extraction or processing. We found that *Trichoplax adhaerens*, the simplest multicellular animal ever described, engages in social feeding behavior. *T. adhaerens* lacks muscle tissue, nervous and digestive systems – yet is capable of aggregating and forming groups of closely connected individuals who collectively feed. The tight physical interactions between the animals are transitory and appear to serve the goal of staying connected to neighbors during the external digestion of algae when enzymes are released on the biofilm and nutrients are absorbed through the ventral epithelium. We found that *T. adhaerens* are more likely to engage in social feeding when the concentrations of algae are high - both in a semi-natural conditions and *in vitro*. It is surprising that *T. adhaerens* – an organism without a nervous system – is able to engage in this social feeding behavior. Whether this behavior is cooperative is still an open question. Nevertheless, the social feeding behavior of *T. adhaerens*, an early multicellular animal, suggests that sociality may have played an important role in the early evolution of animals. It also suggests that *T. adhaerens* could be used as a simple model organism for exploring questions regarding ecology and sociobiology.

Introduction

In order to maximize their energy gain under the constraints of the environment, animals can forage on a patch of food independently or in groups (Pyke, 1984; Stephens and Krebs, 1987; Giraldeau and Caraco, 2000). Social aggregation around food sources can emerge from the fact that the rate of return is high in a particular patch and so many individuals can successfully forage there. It is also possible that organisms receive some mutualistic benefit from feeding together such as greater success at extracting or processing resources. Generally speaking, social feeding does not require cooperation among organisms (Webster and Fiorito, 2001), although cooperation in social feeding is also possible. For example, animals belonging to the same group can develop complex cooperative or altruistic foraging behaviors (Giraldeau and Caraco, 2000). Cooperation – including cooperative feeding behavior - can evolve as a result of high relatedness and/or ecological constraints. This is thought to be especially the case in the early phases of the evolution of sociality (Hamilton, 1964 a, b; 1972).

Trichoplax adhaerens (Placozoa) is the simplest multicellular animal organism ever described (Schierwater et al., 2010). Placozoa are the most basal non-bilaterian metazoan but their relationship with the other metazoans is controversial (Schierwater et al., 2009; Eitel et al., 2013). *T. adhaerens* is a disk-shaped free living marine organism, 2–3 mm wide and approximately 15 μm high. This organism is composed of only six somatic cell types organized in a three layer tissue anatomy. It lacks nervous and muscle tissues and a digestive system (Schierwater et al., 2009). The cells glide on the substrate by action of the cilia of the lower epithelium. *T. adhaerens* feeds on diatom algae by external digestion, secreting digestive enzymes in the proximity of algae from the ventral epithelium (Smith et al., 2015). In the laboratory, they only reproduce asexually through fission or budding (Schierwater et al., 2010; Eitel et al., 2011; Smith et al., 2014; Schierwater and Eitel, 2015).

Placozoa can be found worldwide in temperate and warm seas (Pearse, 2007; Eitel et al., 2013). At this time, only two species have been described (Eitel et al., 2018). The ecology and behavior of *T. adhaerens* in nature are mostly unknown and a large portion of the available information derives from studies *in vitro*. In this paper, we report new findings regarding the feeding behavior of *T. adhaerens*. In particular, we discovered that *T. adhaerens* engage in social feeding behavior where the animals join together, forming tightly connected groups of individuals that essentially form a pocket over algae, potentially allowing them to concentrate digestive enzymes and more easily process and feed on the algae as a group. *T. adhaerens* has not previously been observed to be social.

Methods

In order to reconstruct an environment similar to the natural habitat of *T. adhaerens* Grell, we set up an aquarium (Red Sea Max Nano, 75 liters) at the Biodesign Institute, Arizona State University, in which we placed rocks collected from the Gulf of Aqaba, Red Sea, where the strain of *T. adhaerens* used in our experiment was isolated (Grell and Benwitz, 1971). These rocks were covered in a variety of marine organisms including algae, bacteria, small invertebrates such as amoebae, nematodes, and vorticella. We filled the aquarium with artificial sea water (ASW) made in laboratory adding 32.5 grams of Instant Ocean® sea salt (Prod. n. SS15–10) per liter of distilled water, pH=8. We kept the water temperature at 23–24 °C. We let the algae and other organisms grow in the aquarium for 8 months, periodically checking to ensure we had not introduced wild *T. adhaerens* or other Placozoa specimens together with the rocks. Each inspection confirmed that no Placozoa from the wild were growing in the aquarium. We then introduced 20 specimens of *T. adhaerens* in the aquarium from *in vitro* culture plates. In order to preserve the original ecosystem we transferred the *T. adhaerens* specimens in a sterile glass dish and starved them for 24 hours before transferring them to the aquarium. Then, we removed any algae through successive washes and we finally transferred the open bottom glass dish containing the *T. adhaerens* specimens near one of the glass walls in the aquarium. After introducing the glass dish with *T. adhaerens* to the aquarium, we observed them move from the glass dish to the aquarium glass where they began feeding on algae in the aquarium. We recorded the movements of *T. adhaerens* on the aquarium wall glass surface of 1650 cm^2 covered with algae in the aquarium for 7 consecutive days by marking the position and the size of the animals and aggregates on transparent plastic sheets attached to the external surface of the aquarium walls. We

estimated the number of animals using ImageJ (https://imagej.nih.gov/ij/). We calculated the average animal size measuring 23 single *T. adhaerens* observed during the first two days of observation. We define a group as an aggregate of two or more individuals that are in physical contact.

In addition to growing and observing *T. adhaerens* in the aquarium, we grew *T. adhaerens in vitro* in 100 mm diameter, 20 mm high glass Petri dishes in 30 ml ASW at a constant and controlled temperature (23°C) and humidity (60%) with a photoperiod of 14 hours/10 hours light/dark cycle in an environmental chamber (Thermo Scientific™, model 3940). The animals were fed *Pyrenomonas helgolandii* algae (Schierwater et al., 2010; Eitel et al., 2011; Schierwater and Eitel, 2015). These animals were used for the *in vitro* experiments.

Two different quantities of algae were used to test the role of algae abundance in triggering social feeding. The low density algae plates had 15 times less (high density plates=30.6×10^4 algae cells; low density plates=2.04×10^4) algae seeded on them. We grew the algae for 7 days on F2 medium (Guillard and Ryther, 1962; Guillard, 1975) then substituted the F2 medium with artificial seawater. The algae were distributed evenly on the surfaces of all plates. High algae content was tested in 7 independent experimental replicates (the average number of *T. adhaerens* was 170.42±7.06 s.e.m. per plate) and low algae content in 6 independent experimental replicates (the average number of *T. adhaerens* was 146.16±22 s.e.m. per plate). The density of the animals was in average 1/49.6 mm^2. *T. adhaerens* were counted under a Nikon SMZ1270 dissection microscope (magnification 10X) at the same time for all plates. A grid of parallel lines were printed on transparent plastic sheets and positioned under the plate to facilitate counting. Because there are two different concentrations of algae (high and low) and the animals could potentially aggregate at different times we used the the highest number of animals counted in the aggregates for each plate for the comparisons between the two algae concentrations.

Results

We observed that the animals congregated in groups of 2–105 individuals while feeding on the algae despite the widespread availability of algae on the surfaces of the aquarium. The aggregated animals formed a 'moving front' which consumed the algae as it moved along the surface of the aquarium (Fig. 1). The number of animals in the same aggregate varied dynamically as they consumed the algae. The groups of animals moved on the solid surface in the direction of the algae biofilm, digesting the algae as they passed (Supp.1). Animals of the moving front were sometimes distributed in multiple lines (Fig.1, Supp.1, 2). The animals, especially the ones in the first lines, were tightly connected to one another (Fig.2). They were observed to be in physical contact with one another other along their margin, which slightly folds over in the ventro-dorsal direction (opposite to the substrate), forming stable but dynamic junctions (Fig.1, Supp.1, 2). Occasionally, *T. adhaerens* would move to fill gaps in the chain of animals by binding themselves to neighbors on either side (Supp.3).

We documented the colonization of a glass surface covered with algae. The first day we identified 6 *T. adhaerens* in close vicinity (maximum distance between two animals 17 mm). After three days we estimated the presence of 347 *T. adhaerens* of which 81.28% aggregated

in randomly distributed groups (largest aggregate = 32 animals). Gradually, the *T. adhaerens* formed an advancing feeding front (Supp. 4, 5) and after 7 days we estimated the presence of 847 animals, of which 56.93% was aggregated (Supp. 4–6). At this stage, the average numberof *T. adhaerens* aggregated per group was 3.52± 0.53 s.e.m. animals and the larger group consisted of 53 animals. The most numerous aggregate observed was composed of 105 *T. adhaerens* and formed after 6 days (Supp.4). Originally, the social behavior was observed in the aquarium, but we have also reproduced and observed social feeding behavior *in vitro* on Petri dishes (Supp. 7)

We varied the concentration of algae in to investigate whether social feeding behavior of *T. adhaerens* is influenced by resource density (controlling for the amount of algae per animal). Each animal had the same quantity of algae available and space on the plate surface to be a solitary feeder or engage in social feeding. We found that a larger percentage of animals engage in social feeding when the concentration of algae is higher (high algae: percentage of animals aggregate= 13.35± 2.41s.e.m; low algae: percentage of animals per aggregate= 3.34± 0.34 s.e.m., $\chi2$= 61.232, df= 1, p<0.0001). We observed - both in the aquarium and *in vitro* - that social feeding behavior ceases, with *T. adhaerens* dispersing after all algae have been consumed.

We also observed that *T. adhaerens* in the aquarium can take a tubular elongated shape and they can move quickly on the substrate (Supp.1–3). This behavior has not previously been described.

Discussion

We observed feeding of *T. adhaerens* under semi-natural condition in the lab, finding that they engage in social feeding behavior as a sort of moving front that collectively digests algae. They establish tight junctions among themselves which seem to create a sort of pocket in which digestive enzymes are concentrated, potentially allowing them to digest the algae more quickly and effectively as an aggregation. We also tested whether resource density would influence the likelihood of social feeding and we foumnd that *T. adhaerens* aggregated with a significantly higher frequency when the concentration of algae was higher (controlling for the among of algae available per *T. adhaerens.*

Many open questions remain about the evolutionary and physiological mechanisms underlying *T. adhaerens* social feeding behavior. For example, it is possible that this social feeding behavior is simply a result of optimal foraging in a resource rich environment. It is also possible that higher concentrations of algae require more *T. adhaerens* to effectively digest them or *T. adhaerens* are able to cooperate by optimizing the quantity of the digestive enzymes released. It is important to underline that *T. adhaerens in vitro* are derived from asexual reproduction and are genetically identical. Relatedness is an important factor for social behavior and cooperation to evolve (Hamilton, 1964 a, b; 1972). This high relatedness would also occur in nature when a *T. adhaerens* colonizes a new source of food and rapidly reproduces asexually, creating a local density of clonal animals.

Also, cooperation, competition and cheating are not mutually exclusive possibilities - they may all play a role in different aspects of social feeding in *T. adhaerens*. The external digestion of food creates opportunities for both cooperation (e.g., for initial digestion through enzyme production) and competition (e.g., during the absorption of the nutrients). Moreover, the food digested by a group of *T. adhaerens* can then become a public goods that might create a vulnerability to cheating strategies (Strassmann and Queller, 2011; Riehl and Frederickson, 2016). Cheaters that join a group and absorb or scavenge the nutrients left behind by other animals without secreting digestive enzymes themselves could have an evolutionary advantage over producers, leading to selection for cheating. These alternative or concomitant explanations need to be tested in future work.

Another interesting aspect of our findings is our observation that *T. adhaerens* are able to engage in this social feeding behavior without a nervous system. Interestingly, cells capable of expressing neuronal proteins have been found in the margins of *T. adhaerens* (Smith et al., 2014). Therefore it is plausible that communication may be occurring among *T. adhaerens* as they interact during social feeding. They may also be capable of detecting the presence of algae and/or factors produced by other *T. adhaerens* that may be facilitating collective digestion. Future work should explore the sensing and communication capacities of *T. adhaerens* and how these operate during social feeding.

Considering that Placozoa are the simplest animals ever described (Schierwater et al., 2010), the discovery that they possess a social behavior is potentially the most primitive case of social behavior ever described in non-colonial animal. *T. adhaerens* do not have a nervous system, yet they engage in complex social feeding behavior. This is consistent with work showing that nervous systems – despite playing an important role in sociality (Blumstein et al. 2010) - are not required for it. Social behavior has been described in prokaryotic (Crespi et al., 2001) and eukaryotic unicellular organisms like dictyostelids (Fortunato et al., 2003; Queller et al., 2003). These organisms are able to achieve complex multicellular and social structures with neuronal specialization (Kessin, 2001). The social feeding behavior of *T. adhaerens* suggests that sociality may have played an important role in the early evolution of animals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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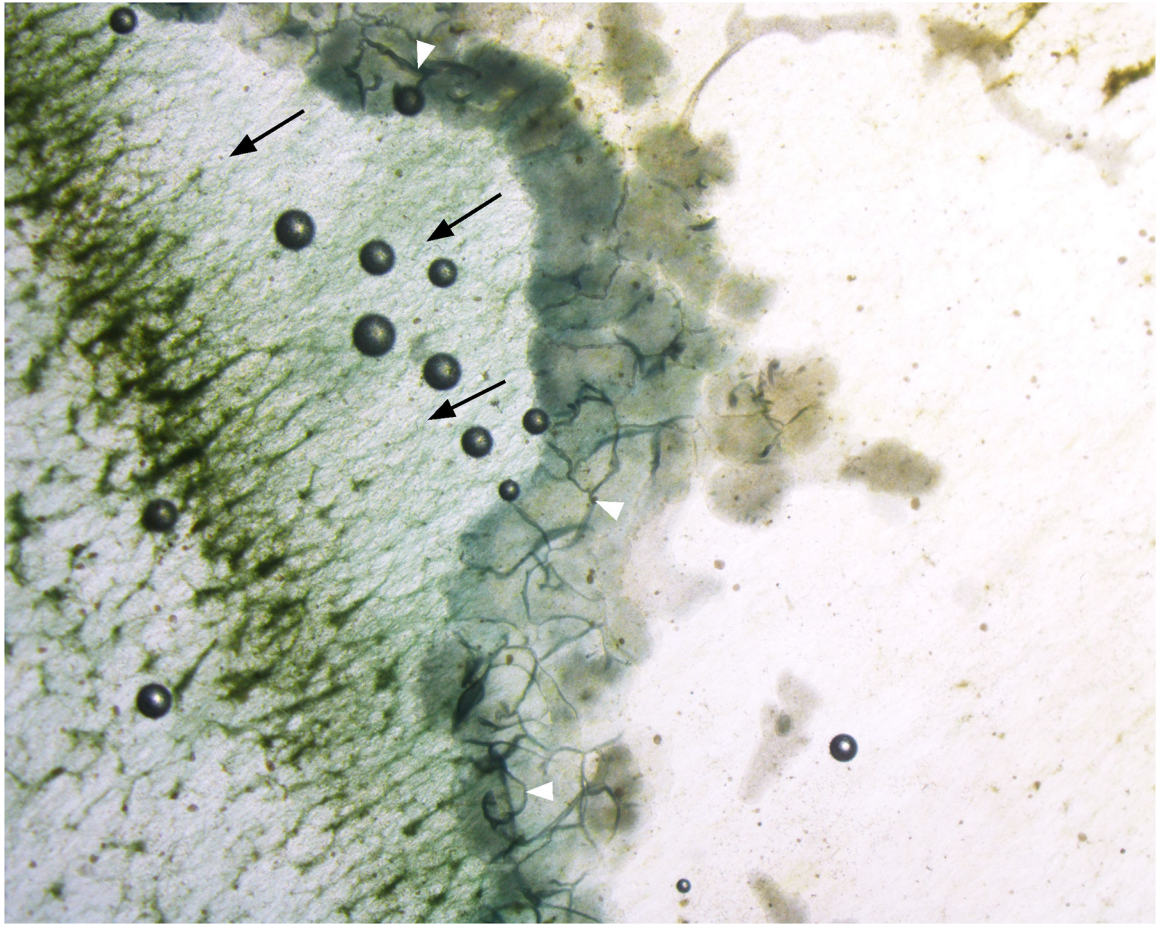Webster SJ. and Fiorito G (2001). Socially guided behaviour in non-insect invertebrates. Animal Cognition. 4, 69–79

Figure 1:

T. adhaerens aggregated on the aquarium wall biofilm. Contact points between Placozoa are visible and dark (white arrowheads). Black arrows indicate the Placozoa march direction. The surface after the passage of the *T. adhaerens* front appears depleted of algae (asterisks, right side of picture). Magnification: 10X.
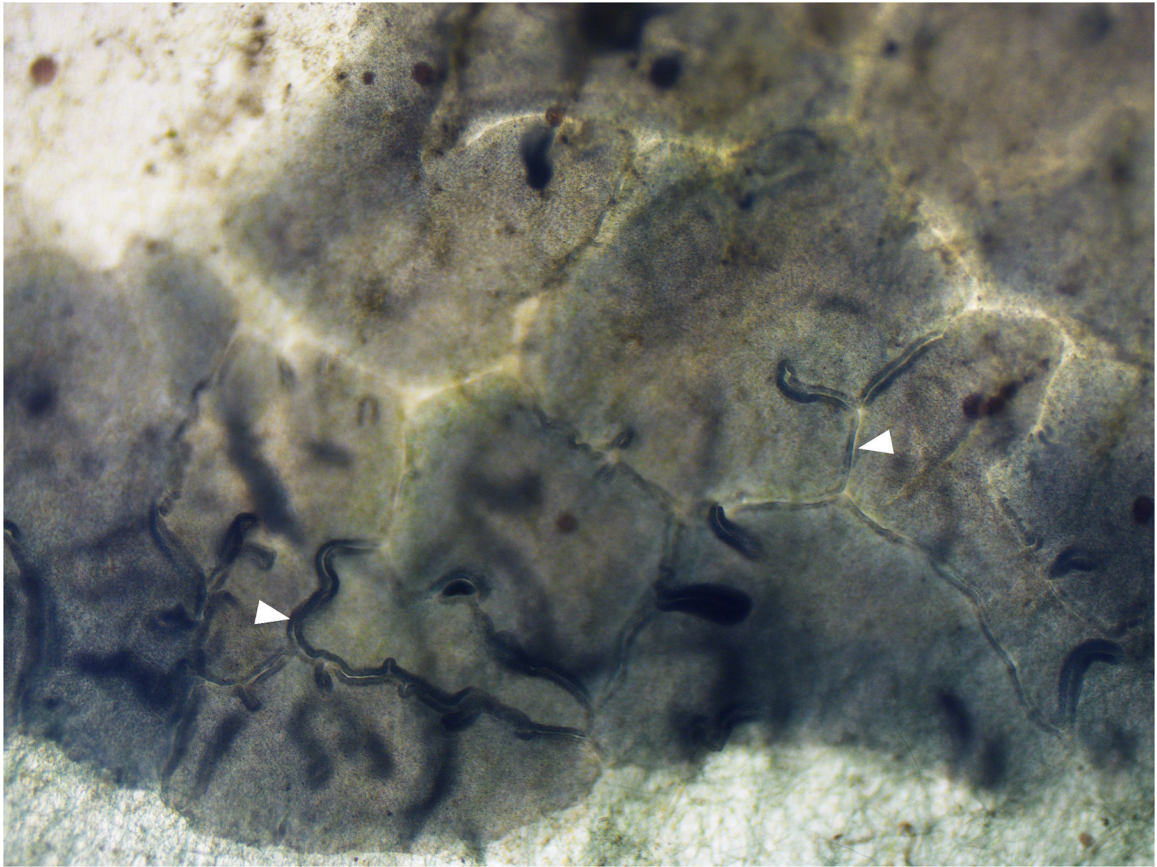
Figure 2:
Magnification of *T. adhaerens* aggregate on the aquarium wall biofilm. White arrowheads indicate the contact points between Placozoa. Magnification: 40X.