////Title: Exploring Symbiotic Relationships Using Flow Cytometry

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Symbiosis is the interaction between two species that offers benefits to one or both of the organisms involved. One important type of symbiosis that has been instrumental in advancing evolution is endosymbiosis, where one of the organisms lives inside the other. Endosymbiosis has typically been studied using microscopy, but Dr Toshiyuki (Tosh-ee-you-key) Takahashi (Tak-ah-hash-ee) from the National Institute of Technology in Miyazaki (Me-yah-zak-ee), Japan, proposes that a technique called flow cytometry can offer more detailed insights into endosymbiotic relationships and advance our understanding of these important associations.

//// Main Text

One of the most significant examples of endosymbiosis occurs in plants and algae. Plants and algae are primary producers and support various ecosystems by providing food and producing oxygen. To do this, they need to carry out photosynthesis – a process whereby they convert light energy into chemical energy. Chloroplasts, which absorb the sunlight that plants use in photosynthesis, evolved from a single-celled photosynthetic bacterium that had been engulfed by a primitive algal cell, forming an endosymbiotic relationship.

In aquatic ecosystems, phytoplankton, including microalgae, are the primary producers. Some microalgae exist in endosymbiosis with other organisms and are amazing examples of evolution. Aside from their crucial role in aquatic environments, algae are used in industry for producing biofuels. Environmental scientists can also survey microalgae populations to assess pollution levels.

Studying the interactions between a host and the organism within it – or 'endosymbiont' – is difficult, as most endosymbionts can no longer survive outside the host cell. One exception to this is algae that have been isolated from *Paramecium bursaria* (para-MEE-see-um burr-SAh-ree-ah), a single-celled organism found in freshwater. This type of algae can survive away from its host and can even multiply independently. This makes *Paramecium bursaria* an excellent model for studying endosymbiosis in the laboratory.

Dr Toshiyuki Takahashi at The National Institute of Technology, Miyakonojo (Me-yak-oh-no-joe) College, focuses on the interactions between host cells and endosymbiotic algae. In his recent paper, entitled 'Life Cycle Analysis of Endosymbiotic Algae in an Endosymbiotic Situation with *Paramecium bursaria* Using Capillary Flow Cytometry', he presents an approach for studying the endosymbiosis of *Paramecium bursaria* using a technique called capillary flow cytometry.

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In his paper, Dr Takahashi first describes what is known about the cell cycles of the host Paramecium cells and its endosymbiotic algae. He explains how algae inside Paramecium cells have adjusted their cell cycle so that they undergo cell division at the same time as the host cells.

Paramecium bursaria has developed mechanisms that allow it to regulate the number of endosymbionts within the cell during the cell cycle. The algae are continually moved around the host cell by a process known as cytoplasmic streaming. While these algae cells are continuously moved around, they do not divide. Shortly before a Paramecium cell proliferates, cytoplasmic streaming stops, which is the cue for the algae to divide. The new algae cells are then shared between the new Paramecium cells, ensuring that the endosymbiotic relationship continues.

Paramecium bursaria has traditionally been studied using microscopy, but its complex relationship with endosymbionts is challenging to monitor using these methods. Dr Takahashi proposes that flow cytometry should be used to monitor the life cycle of the endosymbionts to understand this relationship further.

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Flow cytometry is a useful tool that is used worldwide in scientific research and diagnostics. It can conduct several procedures, including cell counting, cell cycle analysis, and cell sorting. In flow cytometry, cells are passed through a laser beam, and information is recorded on the way the light bounces of each cell.

Flow cytometry records the light scattering pattern of each cell as well as any fluorescence, if present, and this information is converted to electrical signals. Different measurements can be made from the same signal, which can provide a whole host of information about the characteristics of the cell. Flow cytometry has been used to study the cell cycles of many different types of cells, including microalgae.

Paramecium bursaria cells are too large for the most commonly used method of flow cytometry. In his paper, Dr Takahashi advocates the use of a different type of flow cytometry, called capillary flow cytometry, for studying endosymbiotic relationships in this species. Most importantly, capillary flow cytometry can be used to detect intact *Paramecium bursaria* cells, allowing researchers to observe the algae within them and examine their symbiotic relationship.

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Capillary flow cytometry can also be used to detect difference stages in the cell cycle of the Paramecium host cells. *Paramecium bursaria* goes through three stages within the cell cycle, and this technique can differentiate between these stages based on the size of the host cell. In the first stage, the cells are small, as this is shortly after cell division has taken place. In the second stage, the cells are beginning the process of separating into two daughter cells, and so are larger in size. The third stage consists of medium-sized cells. These are either in a stationary phase or in the process of dividing their DNA.

Finally, Dr Takahashi describes how capillary flow cytometry can be used to assess the behaviour of endosymbiotic algae in Paramecium cells. This technique can detect algae cells at different stages of their life cycle and has shown how tightly correlated their life cycle is with that of their host cell. For example, when the host cells are dividing rapidly, so do the algae cells within. However, when the host cells have low rates of division, the algae cells divide very slowly too, demonstrating that the algae can hasten or slow its cell cycle schedule to match that of the host cell.

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The main take-home message from Dr Takahashi's paper is that capillary flow cytometry is a technique that detects intact *Paramecium bursaria* cells, and can discriminate between subtle differences in the host cells. He explains how this technique can also be used to evaluate the life cycles of the algae endosymbionts in the host cell simultaneously, making it a valuable tool for exploring such endosymbiotic relationships. In future, this technique may be able to provide invaluable insights into these relationships, which may hold the key to answering questions that have previously eluded scientists.

This research was partly supported by Grants-in-Aid for Exploratory Research from the Japan Society for the Promotion of Science (KAKENHI Grants Nos. 17K955 and 19K06347).

This SciPod is a summary of the paper 'Life Cycle Analysis of Endosymbiotic Algae in an Endosymbiotic Situation with *Paramecium bursaria* Using Capillary Flow Cytometry' from *Energies*.
https://doi.org/10.3390/en10091413

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