Gregarine apicomplexans – model organisms to uncover the evolutionary path to obligate intracellular parasitism?

Every organism is supposed to be infected by at least one parasite in its lifetime. While we normally treat parasites as foes, could they actually be our friends? For most people in our society, it might sound odd that it could be beneficial to have a parasitic infection. Especially if we look at organisms that belong to the phylum Apicomplexa, a group described as obligate parasites. Included in this group are the causative agents of potentially fatal infectious diseases in humans such as malaria and toxoplasmosis. Nonetheless, scientists have shown that the presence of some apicomplexan species can have either no or beneficial effects on their hosts. This is especially true for the gregarine apicomplexans, which can be found on the whole spectrum of symbiosis from mutualism to parasitism.
In this article, we are going to introduce gregarine apicomplexans and discuss how they can help us to understand the evolution of parasitism in this phylum.

Gregarine apicomplexans

Gregarines infect a broad range of freshwater, marine, and terrestrial invertebrates and some species have a wide geographic distribution. Most gregarines are host specific, but some species are capable of infecting multiple host organisms. There are around 1800 gregarine species described from various hosts. Gregarines have been described from a fraction of existing invertebrates leaving many to still be discovered.

Gregarines belong to the class Conoidasida and are still often lumped into three major groups, the archigregarines, eugregarines, and neogregarines, as the taxonomy is in steady flux. Neogregarines infect only terrestrial, primarily insect hosts and the majority of these hosts belong to the Diptera. Neogregarines can be found in their host's fat bodies, haemocoel, Malpighian ducts, and intestines. Archigregarines exclusively infect marine invertebrates and mostly their intestines. Eugregarines contain most of the known species and are found in marine, freshwater, and terrestrial habitats. Eugregarines inhabit the intestines, coeloms, and reproductive vesicles of their invertebrate hosts.

What differentiates gregarines from other apicomplexa is the sexual association, a process called syzygy, between gregarine gamonts. The gamonts form connections with each other and secrete a gamontocyst around them. In eugregarines (and most archigregarines), gamonts contained within the gamontocyst undergo gamogony which results in the formation of gametes. At this stage, the cyst is called gametocyst. Gamete's fuse and form zygotes. These zygotes then secrete an oocyst wall and go through zygotic meiosis to form haploid infective sporozoites in a phase called sporogony, which are then released from the oocyst. A gametocyst can produce several thousand oocysts, each containing about eight sporozoites. Some archigregarine species are also capable of asexual replication — a process called schizogony or merogony.

Friends or foes?

Symbiotic relationships can be either mutualistic (beneficial), commensalistic (no effect), or parasitic (harmful). They can lead to behavioural changes in the host, which could affect the host's ability to escape predation or compete for space and resources. A recent review by Rueckert *et al* (2019) has proposed that gregarine apicomplexans can be found across the entire symbiotic spectrum. A few examples will be presented below:

In earwigs it was observed that food-deprived insects survived longer when they had a gregarine species colonising their gut compared to those without, showing a beneficial relationship between the gregarine and its earwig host. In pseudoscorpions high prevalence and infection levels of gregarines were reported to be neither beneficial nor harmful. Dragonflies, however, show a decrease in fat content when infected with gregarines. This results in lower muscle power output which negatively effects motility leading to lower mating success.

Our understanding of the biological processes that are associated with mutualism commensalism, and parasitism in the various gregarine species is currently limited. However, the utilization of a combination of traditional methods and state-of-the-art -omics technologies can help to pinpoint major steps along the symbiotic spectrum that led to the evolution of parasitism.

The evolutionary path to obligate intracellular parasitism…

Despite being of medical and ecological importance, a lot is still to be discovered in the evolution of parasitism in the apicomplexans. It is known that apicomplexans evolved from algal ancestors, but the processes that drove the evolution from a free-living photosynthetic organism to an intracellular parasitic lifestyle need further exploration. Gregarines are early branching Apicomplexa potentially having undergone an extraordinary radiation along with their marine and terrestrial hosts.

So far molecular information in gregarines has mainly been used to differentiate species and describe their phylogenetic relationships. Studies over the past decades have provided evidence of a remnant plastid (small organelles usually found in photosynthetic organisms), the so called 'apicoplast', in many apicomplexan species. The presence of this organelle in gregarines has been proven with molecular techniques only recently, adding to the evidence of a common photosynthetic origin. These findings support the loss of photosynthesis in the evolutionary path of gregarines in the transition to a symbiotic lifestyle. This evolutionary process has happened multiple times resulting in multiple lineages of similar symbionts. Some gregarines, likewise with *Cryptosporidium*, seem to have lost the apicoplast, which is essential to many other Apicomplexa. It is important to understand how gregarines and other closely related organisms have coped with the complete loss of the apicoplast and how their metabolic and cellular machinery has adapted over evolutionary time. While recent transcriptomic and genomic studies have provided first ideas about these transitions, the use of genetic and cell biological techniques in organisms across the symbiotic spectrum will provide the answer to our questions.

Gregarine apicomplexans – a useful experimental model?

Our limited knowledge regarding gregarines is in a large part due to the lack of available culturing techniques. Currently there are no in vitro culture methods to culture gregarines in a laboratory environment. The current culture methods are limited to the culturing of the host organisms which can be restricted due to seasonality (for collection of hosts), costs (to maintain complex host life cycles) and labour-intensiveness (for regular feeding, cleaning, and maintenance of host culture systems).

In a Gordon & Betty Moore Foundation funded project co-ordinated by Edinburgh Napier University (Scotland), with partners at the University of Kent (England), the University of Rhode Island (USA), and the Institute of Parasitology at the Biology Centre CAS (Czech Republic), we are working on the development of an *in vitro* culture platform for gregarines. The ability to culture gregarines in a laboratory environment would allow a consistent and host free supply of gregarine material. This in turn would enable the scientific community to not only generate transcriptomic and genomic data more easily but utilize the gregarines in *in situ* environments to explore their biological functions (using -omics) and symbiotic roles. The latter could be achieved by integrating novel microfluidic devices along with specialised polymers; these technologies have been used in the past to investigate microbiome host interactions in humans and other animals.

Rueckert S, Betts EL, Tsaousis AD. The Symbiotic Spectrum: Where Do the Gregarines Fit? Trends in Parasitology. 2019 Sep;35(9):687-694. doi: 10.1016/j.pt.2019.06.013.