Cryptosporidium is a leading cause of death from childhood diarrhea, but its biology is poorly understood. A recent study in PLOS Biology reveals hitherto unknown aspects of the parasite’s life cycle that may lead to improvements in ex vivo culture.
Using an elegant set of experiments, English and colleagues [4] start to address some of these important questions, asking first whether extrinsic or intrinsic factors drive *C. parvum* to undertake sexual development. They evaluate this by showing no difference in the timing of the transition to sexual development in media used for prior parasite culture compared to...
fresh media controls. Subsequently, using live-cell imaging with recently developed transgenic stage and sex-specific markers [6], they show that asexual and sexual cycles of *C. parvum* are tightly synchronized. Intriguingly, a transition to sexual commitment and development occurs, almost without exception, after 3 asexual cycles [4].

Finally, using time-lapse imaging, the authors track development of approximately 1,000 individual parasites and show that male and female gamonts (sexual stages) develop directly from 8 nuclei (8N) type I meronts (multinucleate forms that give rise to extracellular merozoites). Interestingly, each type I meront produces both male and female gamonts (slightly more females than males), indicating that sexual differentiation may be determined after type I meront formation in *C. parvum*. These experiments question the existence and need for previously postulated 4N type II meronts for gamont formation in *C. parvum*, despite the inclusion of this stage in the widely accepted life cycle for this parasite (Fig 1). English and colleagues [4] follow up on this question through additional labeling and imaging experiments of 8N and 4N cells. In their experiments, 4N cells largely progressed to 8N type I meronts, leading to merozoites that either continued the asexual phase or developed into gamonts. Although some 4N cells did not form 8N meronts, these were rare and present at stable levels throughout the experiment and expressed none of a series of marker proteins associated with merozoite or gamont formation. The authors do not conclude a role for these 4N cells. They may be the cells that others have, seemingly incorrectly, classified as type II meronts or have an as yet unknown function.

This study generates many intriguing questions. For example, if the highly synchronized and tightly timed asexual cycles are consistent with in vivo development, and there are good arguments for this presented by English and colleagues [4], how is this achieved and maintained seemingly without any extrinsic signaling? Notably, although many apicomplexans are in part driven to sexual differentiation through environmental sensing, at least one species, *Hammondia hammondi*, appears to differentiate into encysted forms through an intrinsically driven program [7]. Furthermore, what happens after 3 cycles of asexual replication to prompt a highly synchronized transition to sexual development? Such a system may include a biological clock, for example, one driven by an unknown molecule that accumulates or is depleted during asexual replication until, after 3 cycles, it reaches a critical threshold that allows sexual development. In addition, what controls the sex ratio within type I gamonts? Further, if 4N cells are not type II meronts and not required for gamont formation, what are the 4N cells observed by English and colleagues [4], and what function do they have? Techniques such as single-cell or bulk RNA sequencing on the discrete generations of meronts appear ripe to start answering some of these questions.

English and colleagues [4] provide valuable contributions to the current understanding of *C. parvum* development, with implications for other apicomplexans. Further, overcoming the intrinsic mechanism triggering differentiation of merozoites to sexual forms may be the key to continuous in vitro culture. As found for other apicomplexans, this differentiation could involve epigenetic factors [8] as well as AP2 [9] or myb-like transcription factors [10]. The study also strongly indicates that a significant layer of regulation, possibly involving a clock-like mechanism, governs the timing and number of asexual replicative phases of *C. parvum* and its transition to sexual development. Understanding the molecular biology of these phases in *C. parvum* has relevance for developing improved control methods. Significant research on sexual development and gametocyte formation in, for example, *Plasmodium falciparum*, a causative agent of malaria, is an important aspect of planned strategies to block parasite transmission [11]. This stage is essential for the parasite’s survival and a key target to reduce transmission. Comparatively, little is known about the mechanisms underpinning these phases in *Cryptosporidium*. 
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Fig 1 was created with BioRender.com

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