

The rise of model protozoa

David Montagnes¹, Emily Roberts², Julius Lukeš³ and Chris Lowe¹

¹Institute of Integrative Biology, University of Liverpool, BioScience Building, Crown Street, Liverpool L69 7ZB, UK

²Department of Biosciences, Swansea University, Singleton Park, Swansea SA2 8PP, UK

³Biology Centre, Institute of Parasitology, and Faculty of Science, University of South Bohemia, 37005 České Budějovice (Budweis), Czech Republic

It is timely to evaluate the role of protozoa as model organisms given their diversity, abundance and versatility as well as the economic and ethical pressures placed on animal-based experimentation. We first define the term model organism and then examine through examples why protozoa make good models. Our examples reflect major issues including evolution, ecology, population and community biology, disease, the role of organelles, ageing, space travel, toxicity and teaching. We conclude by recognising that although protozoa may in some cases not completely mimic tissue- or whole-animal-level processes, they are extremely flexible and their use should be embraced. Finally, we offer advice on obtaining emergent model protozoa.

Protozoan models: a timely tool

This reflection on protozoa as models arises from recognising their versatility and the impinging economic and ethical pressures placed on whole-animal-based experimentation. Consequently, we consider it timely to evaluate the role that protozoa (Figure 1) can play as model organisms. We have been intentionally conservative in our definition of protozoa (i.e. unicellular eukaryotes that are wholly or partially phagotrophic) primarily to limit the review to one of manageable proportions. In addition, we have focused on protozoa that are models, or potentially models, that address broader biological questions and processes, rather than those that are studied as models because they are interesting in their own right (e.g. the malaria parasite *Plasmodium*).

First, to contextualise the issue, we address the term model organism (Box 1, Table 1), which surprisingly has been poorly characterised. The criteria that arise provide the backbone of our review, which then explores question-driven research fields that employ protozoa. In doing so, we introduce the opportunities that microbial eukaryotes offer as models, note their limitations and provide guidance for future directions.

To what extent can protozoa act as model microbes across a range of studies?

Here we pose several key questions and explore how protozoa are used to answer them. This is not an exhaustive set of questions or examples. Rather, through example, we reveal the strengths and weaknesses of the use of protozoa as models.

How do evolution and ecology interact?

Evolution, underpinned by population genetics, is a well-developed biological theory with many studies addressing its mechanisms [1]. Nevertheless, direct observation and manipulation of evolution remain nontrivial: the time scales required to observe multiple generations are often unfeasibly long, and natural systems may be so complex that detection of evolutionary change is challenging [2]. Experiments using microorganisms have circumvented these issues, many of which have focused on bacteria and phage interactions because of their tractability and an interest in evolution within a medical context [3]. However, protozoa, with rapid generation times and extensive trophic diversity, also represent tractable subjects.

Traditionally, ecological and evolutionary processes were regarded as occurring on different time scales, but evidence now suggests that evolution acts sufficiently rapidly to alter population dynamics. A recent study using the ciliate *Tetrahymena* grazing on a bacterium demonstrated that reducing the nutrients available to the prey imposed an evolutionary shift that reduced the ability of the bacterium to defend against grazing [4]. Notably, the predator (ciliate) did not evolve, supporting theoretical arguments that longer generation times, smaller population sizes and the relatively weaker selection on predation efficiency versus prey defence limit the evolutionary potential of the predator [5]. However, given that protozoa often have growth rates similar to their eukaryotic prey [6] there is also scope to investigate how both predator and prey may evolve over tractable experiments.

Similarly, investigations of how interspecific competition shapes evolutionary responses have used protozoan communities. Work based on communities within pitcher plants, consisting of decomposers (bacteria), bacterivores (ciliates) and primary consumers (mosquito larvae), has demonstrated an effect of inter- and intra-specific competition and predation on several traits in ciliates [5,7]. Ciliates were grown for ~20 generations under competition and predation treatments; subsequently, six traits were examined under common conditions. In response to interspecific competition, changes in a range of these traits occurred (cell size, cyst production and growth rate). Informatively, interactions between competition and predation occurred, with trait responses in the opposite direction to that for individual treatments. Such studies illustrate the utility of protozoa in relatively simple experimental designs to detect fundamental direct and indirect effects of ecological interactions on the evolution of life history traits.

Corresponding author: Montagnes, D. (dmontag@liv.ac.uk).

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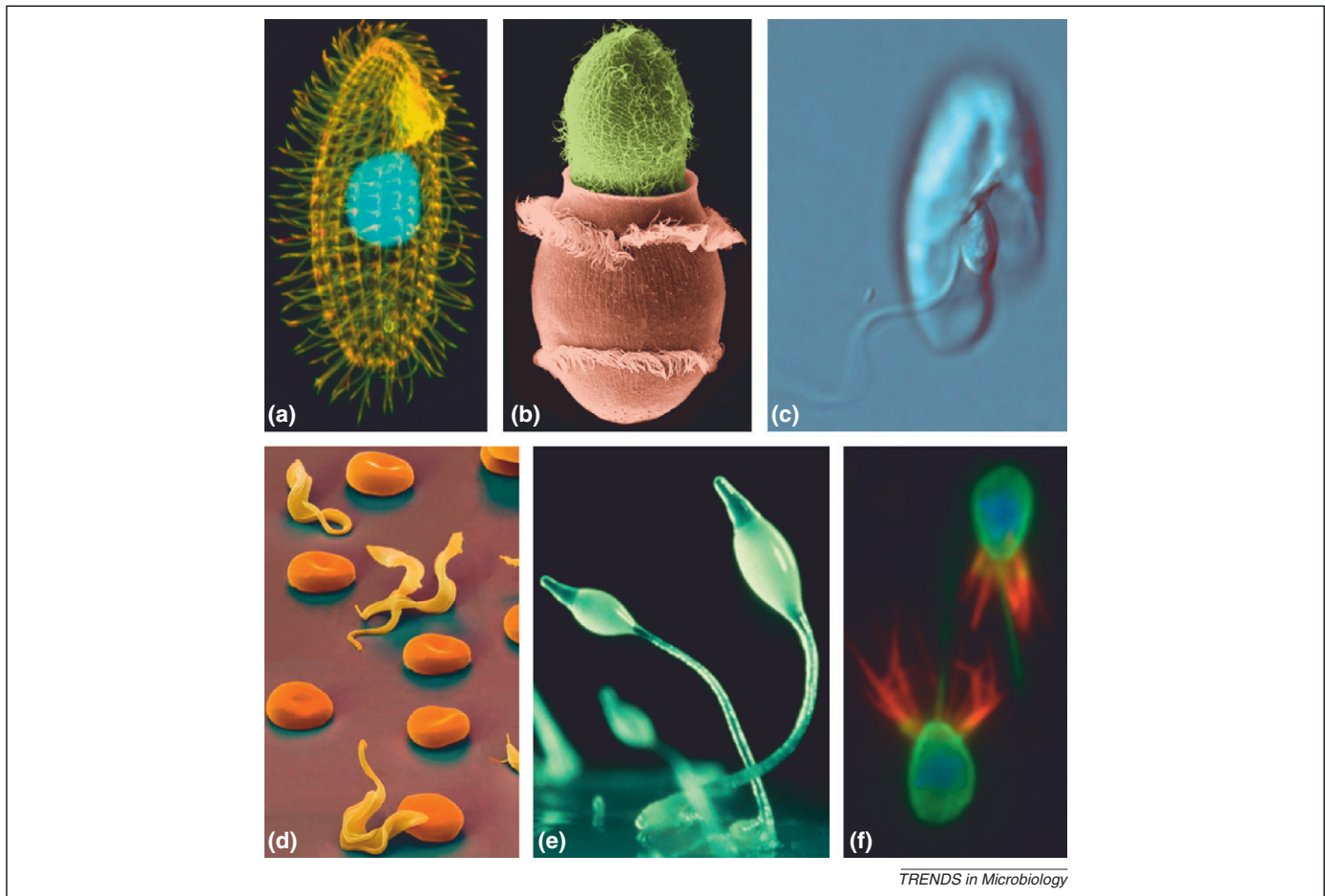


Figure 1. Six examples of protozoa (phagotrophic eukaryotes) that act as models. **(a)** *Tetrahymena*, a ciliate that has a versatile model role (see the text); image from the Open Access source [77]. **(b)** The ciliates *Paramecium* and *Didinium* are used to evaluate predator-prey dynamics among other roles; image kindly provided by K. Hausmann. **(c)** *Oxyrrhis* is a marine flagellate that is emerging as model for a range of topics [61]; image by D. Patterson and provided courtesy of microscope.mbl.edu. **(d)** *Trypanosoma* is a blood parasite that provides a source of mitochondria among other attributes; image rights obtained from Eye of Science. **(e)** *Dictyostelium* is a soil amoeba that is possibly the most important medical-model protozoan; image kindly provided by R.L. Blanton. **(f)** *Monosiga* is a choanoflagellate that is emerging as a model because of its phylogenetic affinity to metazoa [62]; image kindly provided by the King Laboratory, UC Berkeley.

How do populations and communities function?

For almost 100 years, numeric models have evaluated population and community dynamics and predicted events [8]. Most studies use either field data or a very limited number of laboratory data (Figure 2). Microbial microcosms are now

also recognised as useful tools for exploring ecological issues [9], and protozoan-based systems are being appreciated as instructive in both theoretical and predictive terms [10]. Below, we provide two examples of protozoan-based ecological studies that examine model parameterisation.

Box 1. What is a model organism?

Nanny [64] referred to a select group of ciliates, such as *Paramecium*, as ‘the chosen few’, because they have long acted as successful experimental tools. This contention reflects Krogh’s principle that ‘for such a large number of problems there will be some animal of choice, or a few such animals, on which it can be most conveniently studied’ [65]. However, we must also reflect on the wise words of Box and Draper [60]: ‘Remember that all models are wrong; the practical question is how wrong do they have to be to not be useful’. Thus, although there may be a ‘good’ model for a specific job, it will always fail at being ‘ideal’.

Clearly, many model organisms have been chosen to address specific questions related to particular disciplines, with an emphasis on a handful of taxa; these extend from the prokaryote *Escherichia coli* to the fungus *Saccharomyces cerevisiae*, to the plant *Arabidopsis thaliana*, to the invertebrates *Drosophila melanogaster* and *Caenorhabditis elegans*, and to the vertebrates *Mus musculus* and *Rattus norvegicus*. Indeed, model organisms have been adopted across

countless biological fields, and like any model they are chosen to amalgamate levels of simplicity and complexity needed to address specific questions within a particular discipline [62,66].

There can be no single set of attributes to describe an ideal model that provides a solution to all questions, but there are traits that are useful (Table 1), with the primary truism being that the model (whether it be a process or an organism) must be easier to study than the target it is modelling. Furthermore, each research field will inevitably impose unique criteria when selecting models, with organisms chosen for a specific need [67]. Alternatively, there can be intellectual inertia, as a model originally used for one purpose is adopted for others, at times with serendipitous consequences, e.g. *D. melanogaster*, where the recognition of non-Mendelian inheritance between 1910 and 1920, among other events, stimulated the study of the genetic control of development in the 1970s [68]. It is therefore not our intention to suggest that any specific protozoan can act as a model in an absolute sense. Instead, we address their general and specific versatility.

Table 1. Characteristics of a model species, especially regarding protozoa

A model species should be:	Positive (✓) and negative (✗) level to which protozoa possesses this trait
Easier to study than the target it is modelling	✓ Generally protozoa are easier to study than most targets
Relevant: <ul style="list-style-type: none"> • ecologically • evolutionarily • genetically • behaviourally 	<ul style="list-style-type: none"> ✓ Depending on the question, there is almost always a suitable protozoan to help address issues ✗ Some unusual and unique aspects of protozoan genetics (e.g. the macronucleus of ciliates and the haploid nature of dinoflagellates) require consideration when applying findings to metazoan traits
Economically sensible	✓ Whereas protozoan cultures require a budget of the order of pennies per week to maintain, mammalian cell lines might cost close to \$5 per week, and mammals such as rats cost upwards of \$10 per week per individual. These costs, coupled with the small space (1–100 ml) required to maintain cultures, make protozoa cost-effective to maintain
Small	<ul style="list-style-type: none"> ✓ Easy to store and maintain ✓ Possible to have large population sizes ✗ Difficult to manipulate individuals
Inexpensive to obtain and maintain	<ul style="list-style-type: none"> ✓ Generally cultures can be maintained on simple, inexpensive, and well-defined media ✓ Cultures are available at a number of national and international culture collections and fresh cultures can be easily obtained from nature
Source of required products	<ul style="list-style-type: none"> ✓ Being eukaryotes, protozoa can provide biochemical, genetic and organellar products ✗ Products may be structurally and functionally dissimilar to the target
Low health risk to researchers	<ul style="list-style-type: none"> ✓ Most protozoa will not produce potential irritants or health risks common to some vertebrates and invertebrates ✗ Some protozoa are pathogenic
Not rare or threatened	✓ To our knowledge there are no protozoa defined as endangered
Easy to identify	<ul style="list-style-type: none"> ✓ Present model taxa have been well characterised and can be identified by molecular and morphological traits ✗ Determining the identity of taxa in cultures can take expert molecular and morphological training ✗ It may not be obvious if a single-strain culture is contaminated with another strain or very similar species ✗ Expertise is required to identify taxa when new cultures are established
Easy to manipulate in experiments (e.g. robust to manipulation)	<ul style="list-style-type: none"> ✓ Tend to be robust ✓ Can be studied in difficult environments (e.g. outer space) ✗ Specialist models (e.g. those used to explore ecological issues) may be delicate
Able to survive storage (e.g. cryopreservation)	<ul style="list-style-type: none"> ✓ Storage in culture collections exists, and many species may be cultured with little effort for long periods ✗ Only a few can be cryopreserved and few form cysts ✗ Clonal decline of growing cultures occurs
Rapid generation times	✓ Protozoa are fecund relative to other eukaryotic models
Able to produce clonal lines (to separate environmental from genetic effects)	<ul style="list-style-type: none"> ✓ With little training it is possible to establish clonal lines ✓ Many clonal lines can be obtained from commercial sources and from other researchers ✗ Sex within cultures may result in shifts ✗ Mutations may result in clone changes
Stable in characteristics over many generations	✓ Within the context of most experiments, clones will be stable
Genetically or physiologically variable (where appropriate)	<ul style="list-style-type: none"> ✓ Strains exhibit distinct clonal traits, and mutations can be imposed on clones to create strains ✗ Over extended periods (months to years) clonal traits may shift
Useful for interdisciplinary (e.g. molecular–ecological–numeric modelling) use	✓ A long history of interdisciplinary studies exists, and new techniques and questions are continually applied to standard models
Long-standing, with a history of use and substantial background	<ul style="list-style-type: none"> ✓ Several have been used as models for ~100 years ✓ Some of the older literature has detail on the basic biology that we fail to obtain in modern studies ✗ The older literature may not be as rigorous or detailed as needed to inform modern studies
Unemotive (e.g. not a target for animal rights groups)	✓ There are no restrictions on the care of or experimentation on protozoa

Many theoretical and applied population models assume that specific mortality is independent of prey availability [8]. Recent empirical analysis of ciliates indicates that the predator (*Didinium*) mortality rate decreases exponentially with increasing prey (*Paramecium*) abundance, and inclusion of this variable alters model outcomes [11]. These empirical data and the protozoan-based model reflect modelling of large herbivore populations that include a similar function [12]. Thus, work on protozoa supports and reveals fundamental processes that are possibly common to many systems.

Clearly, other basic processes, such as variable assimilation efficiency [13], will continue to be addressed by using protozoa.

Another focus of protozoan models is to assess food web theory. A series of studies using protozoa revealed that inclusion of omnivores in simple food webs provides outcomes that differ from those predicted by theory: rather than destabilising the system, stability arises [14,15]. Thus, protozoa provide a means to assess controlled food web dynamics [10] and direct our study of less tractable large-scale systems.

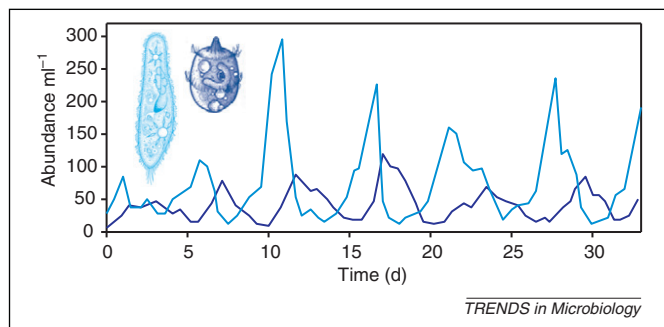


Figure 2. The classic data set for population dynamics used by a number of recent studies [11], as developed by Luckinbill [63]. These data show population cycles of the ciliates *Paramecium* (prey, light blue) and *Didinium* (predator, dark blue) over ~30 days. Such data continue to act as an empirical test for assessment of theoretical models of population dynamics. Although instructive, these data have not been replicated, and there is a need to improve on them. The images of the two ciliates were kindly provided by D. Patterson.

How do we fight infectious diseases?

As part of the human innate immune response, phagocytic cells (e.g. macrophages and neutrophils) find pathogenic microbes using chemotaxis and remove them through phagocytosis. The study of such responses in mammalian cells is problematic because of the complex and costly experimental approaches required and the ethical implications associated with animal experimentation. Protozoa also use chemotaxis and phagocytosis to locate and ingest microbes, providing an alternative model to mammalian cells. In particular, the amoeba *Dictyostelium discoideum* is a powerful system for studying how we recognise, destroy and are sometimes overcome by pathogens [16]. In addition, *D. discoideum* is recognised by the National Institutes of Health as a model organism for biomedical research (<http://www.nih.gov/science/models/>).

Signal transduction pathways controlling chemotactic activity are highly conserved across eukaryotes [17]. Consequently, mechanisms that mediate chemically induced directional sensing and motility in mammalian cells can be determined using *D. discoideum* [18]. *D. discoideum* has also proved invaluable for establishing how actin functions in the cytoskeleton during phagocytosis [19]; for example, the actin-binding protein coronin was first isolated in *D. discoideum* [20] before being recognised in mammals [21].

The study of host–pathogen interactions within mammalian cells remains technically challenging. Consequently, *D. discoideum* has served as a useful model for investigating mechanisms of pathogenesis for human pathogenic bacteria including *Legionella pneumophila*, *Mycobacterium marinum* and *Salmonella Typhimurium* [16]. Certain bacteria use similar mechanisms to evade and proliferate inside the phagolysosomes of both protozoan and mammalian phagocytes, making *D. discoideum* an excellent tractable model for studying the pathogenesis of opportunistic human pathogens [22]. Similarly, the diversity of bacterial associations within ciliates (from mutualism to parasitism) may offer models of host–symbiont interactions [23].

What is the function of mitochondria?

Mitochondria are involved in most processes of the eukaryotic cell [24] and exist in an array of forms, modifications

and derivations in different protozoan lineages. Comparative analyses of protozoan mitochondria and mitochondrion-derived organelles provide insight into molecular pathways in typical metazoan organelles. For example, they aided in explaining the emergence of protein import machineries [25] and revealed that the only function shared by all these organelles is the synthesis of iron–sulfur clusters [26]. Below we highlight the value of protozoa to biomedical research and as sources of mitochondria for experimentation, and describe how comparative studies involving protozoan models have revealed novel mitochondrial functions.

Within metazoan tissues, mitochondria differ in their protein composition, activity, size and abundance [27], confounding analyses. By contrast, protozoa can provide organelles of just one type [28]. Furthermore, when mitochondria undergo changes during the life cycle [29], synchronisation of a protozoan population allows purification of identical mitochondria from a given stage. Thanks to short generation time and, in comparison to mammalian cell lines (Table 1), cheap cultivation of model protozoa such as *Trypanosoma brucei*, *Leishmania tarentolae*, *Chromera velia* and *Tetrahymena pyriformis*, large-scale mitochondrial purification methods are possible [30]. Consequently, some protozoan mitochondria belong to organisms with the best-known proteomes [31,32].

The discovery of RNA editing in the kinetoplastid mitochondrion of *T. brucei* [33] was instrumental in detecting similar phenomena inside and outside the organelle in metazoa. Furthermore, integrative genomics is extensively used to identify novel mitochondrial protein functions by comparing proteomes from distantly related organisms [34]. For instance, the absence of respiratory complex I in trypanosomes has allowed novel subunits of this complex to be identified in humans [35], and it was the presence of a putative mitochondrial calcium uniporter in the same protozoa that aided its identification in humans after a decades-long search [34].

Why do we age?

Protozoa probably cannot act as models for metazoan senescence, because metazoa are partitioned into somatic and germ cells, whereas protozoa are unicellular. Thus, the use of protozoa to assess tissue ageing is tenuous, but ageing at the cellular level may be elucidated using protozoa; two examples are outlined below.

Muller's ratchet is the accumulation of deleterious mutations, such as in an asexual protozoan population [36], a strictly clonal metazoan population [37] and somatic tissue [38] and in plastids such as mitochondria [39]. Concomitant with the increase in mutations, there will be a decrease in fitness and function [38]. Protozoa resolve Muller's ratchet through sex [36], a process that metazoan tissues are incapable of, and thus the utility of the model is reduced, but the fundamental process may apply to senescence of somatic tissue [38]. Furthermore, there are protozoa that are apparently immortal or at least have reduced senescence [40]; these too may be useful models in assessing ageing (through its absence).

The ciliate *Tetrahymena thermophila* is undoubtedly the most prestigious recent model protozoan, playing a

major role in a 2009 Nobel Prize [41]. Its unique feature is that, similar to all ciliates, *T. thermophila* possesses a macronucleus composed of unusually high quantities of minichromosomes, each with accompanying telomere ends, a sequence protecting the chromosome from deteriorating or fusing with other chromosomes. It was this unique abundance of telomeres that allowed their initial characterization. Over the past three decades, telomere quality has been linked to cell and organism senescence, cancer and other diseases [41]. Clearly, protozoa were instrumental models in these discoveries.

What physiological affect will space travel have?

The absence of gravity influences cells and organisms and effect include muscle atrophy, impaired bone formation and depressed lymphocyte activity [42]. Gravity is perceived at the tissue level, and metazoan structures such as statoliths and the inner ear have developed to this end, but cellular-level gravity receptors also exist. Reduced gravity experienced in space may therefore have deleterious effects in both uni- and multicellular organisms, and we undoubtedly require an understanding of how cells perceive and respond to gravity.

There are two main response mechanisms that the study of gravity in protozoa has revealed. Taxa such as the ciliate *Loxodes* employ organelles to recognise gravity; such structures are analogues of statoliths and inner ears, and may offer insight into the function of multicellular structures. Alternatively, the entire protozoan cell may perceive gravity by recognising differential tension and compression between the plasma membrane and the extracellular matrix [43]. Although these mechanisms may seem of little direct relevance to metazoan behaviour, the processes associated with responses may be homologous (e.g. movement, orientation, and particle recognition and capture). Furthermore, subcellular processes may occur within tissues, and there are physiological and ultrastructural changes that alter the growth and development of protozoa that may provide insights into the subcellular and tissue-level processes of metazoa [42]. Finally, protozoa make useful models for evaluating evolutionary processes (see *How do evolution and ecology interact?*), so they may be used to assess evolutionary responses to reduced gravity.

What are the physiological and genetic effects of toxins?

Animal models are used to assess the impact of potentially harmful substances [44,45], as are protozoan models [46,47]. Animal experiments, however, have shortcomings, not the least of which is their emotive nature [48]. Below we provide two examples using the model ciliate *Tetrahymena*, one that supports their utility and a second that does not.

To examine the deleterious effects of the street drugs cocaine and crack on DNA, researchers focused on the easily observable macronucleus of *Tetrahymena* [49,50]. This work revealed genomic instability and carcinogenic effects of the drugs and indicated a dose-dependent, aneuploidic effect of cocaine and a dose-independent, aneuploidic effect of crack; the study also suggested that the observations may in part aid in understanding the mechanism associated with the highly addictive nature of crack.

Likewise, antidepressants can have deleterious aspects and are associated with accidental poisoning and suicide attempts [51], necessitating assessment of their toxic effects. Cytotoxicity assays using *Tetrahymena* were carried out using 13 antidepressants [51]; the results regarding their toxicity correlated with other *in vitro* metrics (rat blood, human neutrophils and rat kidney cells), but there was a poor predictive relationship between the protozoan metrics and a whole-organism metric (deaths per million prescriptions issued). Thus, it was concluded that protozoa may not be useful for predicting the toxic effect of antidepressants on humans and, as others have suggested [45],

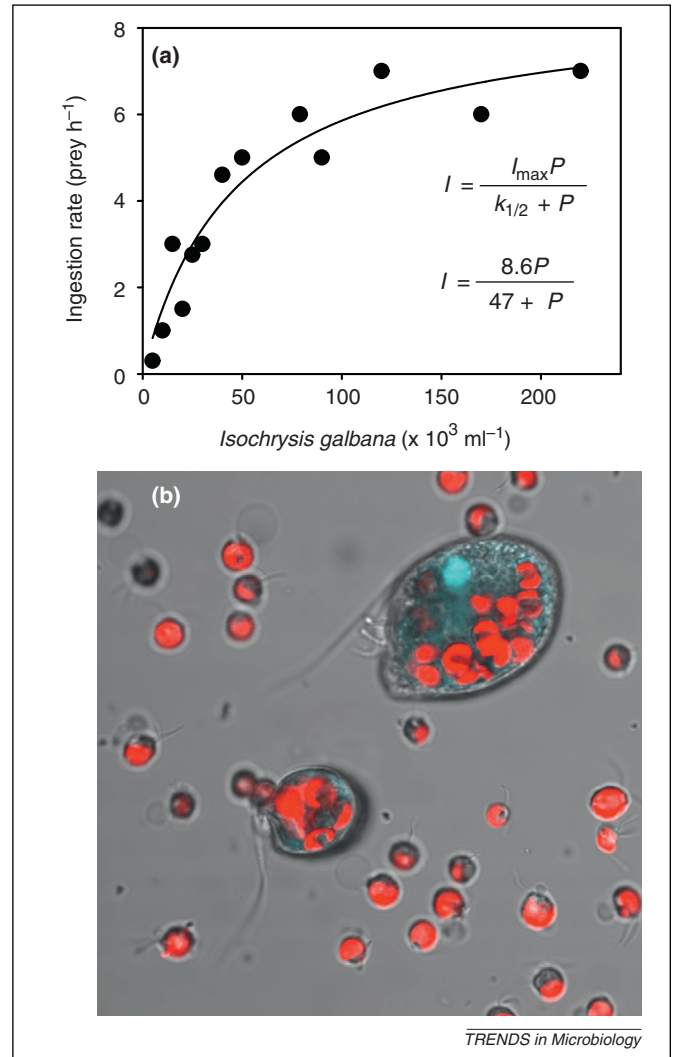


Figure 3. Use of protozoa to mathematically explore mechanistic behaviours. **(a)** The relationship between ingestion rate and prey abundance typically follows a rectangular hyperbolic response (often referred to as a Holling type II response [8]), which is mechanistically homologous to Michaelis–Menten enzyme kinetics; the two equations represent the general version and the version parameterised by the presented response, respectively. I is the ingestion rate, I_{\max} is the maximum ingestion rate obtainable, $k_{1/2}$ is the prey concentration that elicits half I_{\max} , and P is prey concentration. **(b)** By observing cultures of the protozoan *Oxyrrhis marina* (the large cells) grazing on the prey *Isochrysis galbana* (revealed by their red autofluorescent chloroplasts), students can appreciate that the ingestion rate is dependent on encounter and processing. Then, by determining the ingestion rate (the number of prey within a predator) at a range of prey concentrations, it is possible to establish a response curve as in **(a)**; finally, a mechanistic function can be fitted to the data and students can use this function to estimate the handling time and rate of encounter. The attractiveness of this experiment is that it can be conducted in half a day and is relatively inexpensive. Given the importance of the functional response in ecology and Michaelis–Menten kinetics in physiology, the utility of such an experiment should be clear.

in vitro culture of cells cannot approximate the complexity of multicellular organisms.

How can we best teach biology?

With growing concerns regarding animal welfare, coupled to reduced funding for teaching, protozoa are ideal experimental organisms for education from children and the public to graduate students. Many of the attributes that make protozoa so tractable for research (Table 1) are equally if not more important in the practical use of protozoa in the classroom (Figure 3).

The use of protozoa in teaching is possibly best exemplified by the book *Exploring the World Using Protozoa* [52], which covers topics ranging from succession to symbiosis. However, there are more specific examples: Cruzan illustrates the point that by using microcosms (heavily dominated by protozoa), an entire ecology course can be taught [53]. There is also a wide range of websites that encourage the use of protozoa as tools. Ciliates in the Classroom (http://www3.wooster.edu/biology/Ciliates/Ciliates_in_the_Classroom.html) argues that ciliates are beneficial for a range of reasons, including that a friendly, international research community supports work on them and that they are intrinsically fascinating for students to watch. Teaching Tools Using *Dictyostelium discoideum* (http://dictybase.org/teaching_tools/index.html) also guides teachers to resources, and an overview of educational resources (including support for teachers and students and even children's songs and stories) is available (<http://www.uga.edu/protozoa/about.html>).

Finally, a range of resources providing protozoa for teaching is available. Large and small biological supply companies and national and international collections offer cultures for use by teachers (<http://www.uga.edu/protozoa/about.html>). Furthermore, there are commercial kits available for growing protozoa and it is equally easy to collect model species, such as *Paramecium*, from field samples using relatively simple methods [54]. Thus, protozoa are simple to use and inexpensive to obtain and maintain, making them ideal teaching models.

Concluding remarks

Clearly, by providing examples and through synthesis (Table 1), we have indicated the versatility and tractability of employing protozoa as models from subcellular to ecosystem levels. Admittedly, protozoa can fail to mimic processes that occur at either tissue or whole-animal levels [45]. For instance, although protozoa age and die, they do not exhibit tissue or organ-level process that lead to death; consequently, their application is limited to cell-based process such as apoptosis (programmed cell death) [55] and macromolecular damage, a key mechanism in aging [56]. Another example of the fallibility of protozoan models is indicated by the revelation that although toxins act at molecular, genetic and cellular levels, their impact may only be appreciated when tissue or organ damage occurs. Similarly, specific processes associated with metazoan behaviours may be poorly resolved in studies of single cells. This was illustrated above; although we suggest that protozoan recognition of gravity may be analogous to animal mechanisms, it differs both in function and complexity.

However, authors who study protozoa in space remind us that unicellular organisms are ideal for examining relatively short signalling pathways, with all stages occurring in the one protozoan [43], and protozoa are an ideal source of subcellular structures. Likewise, although not outlined above, ciliates are instrumental in the study of secretory processes, a fundamental function in human physiology [57], and are ideal models for the assessment of epigenetic effects [58]. Thus, studies using protozoan models must be cautious in distinguishing between relevant intracellular and potentially less relevant intercellular phenomena; the exception to this is in cases such as the model colonial protozoan *D. discoideum*, which exhibits cell–cell interactions [16], and possibly the search for emergent models (Box 2) should focus on this attribute.

Box 2. A strategy for choosing a model protozoan

Our first recommendation is to take advantage of extant models, such as those mentioned in this review: the ciliates *Didinium*, *Paramecium*, *Tetrahymena* and *Loxodes*; the flagellates *Trypanosoma brucei* and *Leishmania tarentolae*; and the amoeba *Dictyostelium*. These, and hundreds of others, are available through commercial and non-commercial culture collections. Methods are well established for their maintenance and there is substantial background information on them; a good starting point might be *Portal to Protistology* (<http://www.uga.edu/protozoa/portal/portal.html>). Thus, these 'chosen few' (*sensu* Nanny [64]) should be the researcher's first port of call for pragmatic and economic reasons [69]. There are now, however, a number of emergent models that provide tools for focused studies, such as the choanoflagellate *Monosiga*, and that may be instrumental in recognising properties associated with early metazoan evolution [62]. Thus, the 'chosen few' are increasing.

New models have emerged primarily through the application of molecular technologies that identify and may characterise key processes [62,66,70,71]. These molecular traits may then be searched for, specifically in the context of this review, in poorly characterised protozoa, of which there are ~8000 species catalogued, with a potential of almost 30 000 remaining to be recognised [72]. Note that strain differences in single species (e.g. for the model species *Oxyrrhis* [73]) will undoubtedly increase this diversity several-fold. Consequently, this approach is extremely attractive for fields ranging from medicine to ecology. However, molecular-based exploration is not a panacea. Depending on the focus of study, it might be pragmatic for researchers to screen potential protozoan candidates using other means, such as ecophysiological metrics (e.g. growth rate) or morphological traits (e.g. nuclear size).

Regardless of the method used to characterise potential taxa, our next recommendation relates to the ubiquity, high diversity, wide range of habitats, and ease of culture of protozoa (<http://www.uga.edu/protozoa/portal/portal.html>). Water and soil samples will yield tens if not hundreds of species. Similarly, protozoa reside in a number of extreme environments, providing taxa with potentially unique attributes such as anaerobic metabolism. Thus, when in search of a model, the next step is to choose your environment.

Finally, we suggest a pragmatic approach: do not search for the proverbial needle in a haystack. Rather, we suggest a simple method for finding new models: take large samples from the target environment; apply to subsamples a range of enrichment methods that will be feasible to continue culture maintenance (e.g. enrichment with bacteria); and then ignore the samples for an appropriate time (hours to days). Some taxa will increase to sufficient numbers so that they can easily be observed; these are the 'weeds' that will make good models (Table 1). Next, survey the samples and isolate individuals (with fine-drawn Pasteur pipettes or automated cell-sorting methods) into the chosen enriched media. This will produce a collection of potential models.

Paradoxically, the weakness of protozoa as models, their lack of multicellular complexity, is also a strength; their simplicity allows exploration of animal to ecosystem scenarios that cannot otherwise be pragmatically addressed. Protozoa have provided significant insights into large-animal processes despite lacking age-structured dynamics and sex-driven reproduction. Furthermore, the great range of trophic complexity exhibited by protozoa (bacterivores, herbivores, carnivores and parasites) allows whole food webs, containing multiple trophic levels, to be studied in a beaker [59]. Similarly, protozoa provide unique tools for assessing cellular and subcellular metazoan process.

A commonality arises from our examples: the use of protozoa circumvents arduous requirements for animal experimentation, with their associated emotive and ethical issues and the excessive costs for housing and manipulation. For instance, protozoa may mimic phagocytic blood cells and this is without doubt beneficial, because human blood cells are problematic for a range of reasons: safety concerns, vaccination, sourcing and storing [51]. Protozoa are also generally less expensive to maintain (Table 1), are easier to obtain (Box 2) and have fewer ethical implications than mammalian cell lines, which are the unicellular counterpart to protozoan models. Furthermore, one reason why *D. discoideum* dominates medical research as a model largely relates to its genetic tractability; this amoeba has a small (34 Mb), haploid, sequenced and annotated genome that contains many homologues to vertebrate genes. Many medical questions can thus be addressed by applying molecular techniques including gene knockout, restriction enzyme-mediated mutagenesis, RNAi and inducible gene

Box 3. Cautionary recommendations for the search for and application of protozoan models

- It will undoubtedly be sensible to use multiple models to explore specific questions and to use a range of models to explore various questions; such an argument has been made for plants, with *Arabidopsis* seen as a useful but far from comprehensive model [74], and this view seems to be supported at a more general level [75]. Given the tractability for rapid testing of hypotheses using protozoa and the diversity of these organisms at any number of levels (genetic, biochemical, physiological and ecological), we suggest that researchers continue to explore their potential in a comparative and interdisciplinary manner.
- There is a caveat to recommending unrestrained establishment of emergent models; Slack [69] cautions that we must critically consider our models, not least in terms of the practicality of maintaining and obtaining them. This should not preclude exploration of new taxa and their ultimate rejection, and again, given the ease of collection and culture of protozoa, this seems a sensible direction. We therefore support the pursuit of exploratory research and plumbing of the depths of protozoan diversity. Similarly, we strongly support the better characterisation of established model protozoa, which, we argue, continue to reveal new insights.
- Finally, in this age of integrative biology, Satterlie *et al.* [76] argue a need for emphasis on organismal biology, with the organism being the essential link between fields as disparate as genomics and field ecology. We could not agree more and propose that for protozoa to act as useful models there is a need to understand them as organisms, and not simply as factories for generating products or bags of genes. The corollary to this is that protozoologists are required, and we conclude by strongly supporting collaboration between protozoologists and the wider scientific community in the pursuit of better models.

expression (<http://dictybase.org>). Thus, at some levels protozoa may be used to replace human and animal experimentation.

Finally, we recognise that protozoa are excellent teaching tools, especially because students shy away from animal experimentation, and in this sense they provide ideal models, not for research but for training the new generation of researchers. In conclusion, with the caveat that no model will be perfect [60], we strongly support, following Krogh's principle (Box 1), the utility of protozoa as models, and argue for their continued use across research and teaching disciplines (Box 2). We end with three cautionary recommendations in Box 3.

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