J. Eukaryot. Microbiol., 57(1), 2010 pp. 3–10

DOI: 10.1111/j.1550-7408.2009.00460.x

Using Protistan Examples to Dispel the Myths of Intelligent Design

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ABSTRACT. In recent years the teaching of the religiously based philosophy of intelligent design (ID) has been proposed as an alternative to modern evolutionary theory. Advocates of ID are largely motivated by their opposition to naturalistic explanations of biological diversity, in accordance with their goal of challenging the philosophy of scientific materialism. Intelligent design has been embraced by a wide variety of creationists who promote highly questionable claims that purport to show the inadequacy of evolutionary theory, which they consider to be a threat to a theistic worldview. We find that examples from protistan biology are well suited for providing evidence of many key evolutionary concepts, and have often been misrepresented or roundly ignored by ID advocates. These include examples of adaptations and radiations that are said to be statistically impossible, as well as examples of speciation both in the laboratory and as documented in the fossil record. Because many biologists may not be familiar with the richness of the protist evolution dataset or with ID-based criticisms of evolution, we provide examples of current ID arguments and specific protistan counter-examples.

Key Words. Drug resistance, irreducible complexity, *Plasmodium*, radiation of forms, speciation, transitional fossils.

WITH the publication of ''On the Origin of Species'' in 1859 Charles Darwin formalized a theory that provided a natural explanation of the diversity of living organisms on Earth. This theory is characteristically referred to as ''evolution,'' and in the intervening 150 years, the disciplines of paleontology, genetics, cell biology, and most recently molecular biology have all influenced and helped to refine evolutionary theory. Despite the overwhelming body of evidence that supports the basic tenets of evolution (i.e. common descent of organisms with different forms being the result of natural selection acting upon naturally occurring variation), there is a large proportion of the American population that does not accept the validity of what is perhaps the most rigorously tested scientific hypothesis in history. Many interpret acceptance of evolutionary theory as being incompatible with their deeply held religious beliefs. These individuals have sought to undermine the teaching of evolution through the introduction of religiously motivated and scientifically questionable philosophies into the classroom. In recent years this strategy has been most aggressively pursued by members of the intelligent design (ID) movement, whose stated goal is ''challenging the philosophy of scientific materialism and the false scientific theories that support it'' (Discovery Institute 2003, q.v. Discovery Institute 1999).

A leading figure in the ID movement is Dr. Michael Behe, professor of Biochemistry at Lehigh University and senior fellow of the Discovery Institute, a public policy think tank that advocates for ID. Dr. Behe is the author of two influential books, *Darwin's Black Box* (Behe 1996) and *The Edge of Evolution* (Behe 2007), and he is best known for his concept of ''irreducible complexity'' in which he posits that some biochemical systems are too complex to have arisen through natural, undirected processes. Behe (1996) defined an irreducibly complex system as being ''composed of several well-matched, interacting parts that contribute to the basic function, wherein the removal of any one of the parts causes the system to effectively cease functioning.''

In *The Edge of Evolution* Behe (2007) draws heavily on two examples of what he considers to be irreducibly complex systems: the eukaryotic cilium and chloroquine resistance in the apicomplexan parasite *Plasmodium falciparum*. Creationists, whether affiliated with the ID movement or not, often point to his examples of irreducibly complex systems as proof that modern evolutionary theory is fatally flawed. Other criticisms of evolutionary theory are based on such points as the lack or irrelevance of transitional fossils (Wells 2009), the sudden radiation of biological forms in the Cambrian (Meyer 2004), and the argument that speciation events have not been observed or documented (Skell 2009). Protistan biology provides direct counter-examples to these claims. In many cases protistan examples are actually more compelling than the better known examples of animal or plant evolution, because the large population sizes, short generation times, and easy adaptability of protists to laboratory manipulation often make the biological processes in question more clearly evident. We have chosen four common challenges to evolutionary theory proposed by ID advocates, and provide detailed examples drawn from protistan research to demonstrate why these anti-evolution arguments fail.

THE PRE-PRECAMBRIAN EXPLOSION

> Clearly, we have good reason to doubt that mutation and selection, self-organizational processes or laws of nature, can produce the information-rich components, systems, and body plans necessary to explain the origination of morphological novelty such as that which arises in the Cambrian period.
> (Meyer 2004).

Another senior fellow of the Discovery Institute, Dr. Stephen Meyer, has written extensively on why he believes that the rapid diversification of animal forms, commonly referred to as the ''Cambrian Explosion,'' could not have occurred through natural and undirected processes. The popular press often unwittingly compounds the confusion surrounding the Cambrian Explosion with statements such as ''For some 3 billion years, single-celled life forms such as bacteria dominated the planet. Then, roughly 600 million years ago, the first multi-cellular animals appeared on the scene, diversifying rapidly.'' (Choi 2009). Such oversimplifications create the false impression that metazoa arose de novo, ignoring the fact that protists, which are markedly more complex and more morphologically and genetically diverse than bacteria, had been evolving for approximately 1 billion years before the first multicellular animals appear in the fossil record.

The appearance in the fossil record of many new and diverse phyla of metazoa over a relatively short 20-million-year span of time (550–530 Mya) could be seen as incompatible with classical Darwinian theory, which posits that the accumulation of small and very gradual changes over long periods of time could produce the

Presentation delivered at the workshop: Horizontal Gene Transfer and Phylogenetic Evolution Debunk Intelligent Design, 1st International Society of Protistologists—North American Section Meeting, 11–13 June 2009, Roger Williams University, Bristol, RI, USA

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diversity of animal forms seen in the Cambrian. However, it has long been known that a single innovation can result in what appears to be a greatly accelerated rate of evolution, giving rise to an ''explosive'' radiation of forms (Eldredge and Gould 1972). It is generally accepted that the ancestors of the metazoa were morphologically complex protists, and that diploblastic metazoans, such as the Cnidaria (jellyfish), existed before the Cambrian (Carroll, Grenier, and Weatherbee 2001). The relatively simple innovation of a triploblastic body form with a complete coelom could have allowed for a tremendous diversification of body types that today are recognized as belonging to distinct phyla, but which had their origins among genetically similar acoelomates (Chen et al. 2004). Morphological innovations of this type can be underlain by fairly simple processes, such as changes in cell adhesion properties (Minelli and Fusco 2005). In short, the Cambrian Explosion was likely the result of a few simple innovations that made a vast array of new body plans possible.

It is probable that body fossils of many (if not most) Precambrian organisms are missing from the fossil record, with these organisms represented only by trace fossils (Bengtson et al. 2009). Trackways and other impressions that had been attributed to metazoans (Droser, Jensen, and Gehling 2002; Seilacher, Bose, and Pflüger 1998) date to nearly 400 million years before the Cambrian. However, these fossils may instead be evidence of activity by a highly diverse Precambrian assemblage of protists. For example, Matz et al. (2008) report modern-day tube tracks in the deep waters off the Bahamas that are produced by large gromid protists, and which resemble some Precambrian trace fossils. Such interpretations fit well with current theories that suggest protists first emerged between 1.8 and 1.2 Bya (Knoll et al. 2006), and underwent their own explosive radiation somewhere between 950 and 1350 Mya (Berney and Pawlowski 2006; Douzery et al. 2004).

When one considers the tremendous diversity of protistan forms (see Adl et al. 2005), the array of body plans among the Cambrian metazoa pales in comparison. In terms of biological diversity it can be argued that no group approaches that of the protists, especially if one considers that all the plants, fungi, and animals, including the famously diverse Coleoptera, are merely sub-groups of the protistan clades Archaeplastida and Opisthokonta (Adl et al. 2005). Even with the exclusion of the multicellular ''higher'' eukaryotes, the morphological and physiological diversity among protists is staggering. The major clades of protists contain everything from photosynthetic autotrophs to amitochondriate flagellates and are found in virtually every habitat on Earth (Foissner 2008). The extant diversity of the protists should therefore be seen as the ''background radiation'' of the eukaryotic Big Bang, with the Cambrian radiation of the metazoa being a subsequent event within a specific group.

Analysis of well-conserved genes across the eukaryotes supports the concept of a protistan ''big bang'' in which everything radiated in a short but intense burst of evolutionary innovation (Dacks et al. 2002; Tekle, Parfrey, and Katz 2009), and also provides important clues to the mechanism that produced the incredible variation we see. Current theories of protistan origins focus on the roles played by various prokaryotic partners coming together via symbiosis to form the first proto-eukaryotes (Margulis et al. 2006; Martin and Müller 1998; Moreira and Lopez-Garcia 1998). Events of this type are not slow or incremental; they offer opportunities for radical and novel biochemical and genetic innovations. In addition, there is relatively little ''penalty'' for unsuccessful combinations; because the component organisms are already successful in their own right, a suboptimal symbiosis presents no threat to the survival of either of the parent lineages. While controversy remains over the mechanism(s) that gave rise to the eukaryotic genetic toolkit (e.g. symbiosis and lateral gene transfer, see related symposium article), it is now well supported that the genomes of all protists are chimeras that resulted from single or perhaps multiple unions of bacterial and archaeal ancestors (Rivera 2007; Rivera and Lake 2004).

It also seems likely that relatively simple events made the radiation of the protists possible, just as simple changes probably underlie the Cambrian explosion. There is good support for the idea that the alpha-proteobacterial ancestor of the mitochondrion formed a union with an archean before the radiation of protists. Much of the evidence for this early symbiosis comes from the study of protists that no longer possess a conventional mitochondrion that functions in aerobic respiration (Embley and Martin 2006). The exact nature of the relationships between very distantly related protists has not yet been completely resolved; the monophyly of some of the larger protistan clades or ''supergroups'' remains controversial (Parfrey et al. 2006) and the predicted branching order of the supergroups within the eukaryotic tree is in even greater flux (Rodriguez-Ezpeleta et al. 2007). Nonetheless the emerging picture is one in which protists arose a single time and then rapidly underwent an explosive radiation of forms that has enabled protists to occupy nearly every ecological niche known to exist on Earth. Such observations are consistent with modern evolutionary theory and do not require the invoking of supernatural explanations to account for the diversity of protists.

ON THE ORIGIN OF SPECIES

One of the most common arguments against evolutionary theory is the assertion that no one has ever witnessed the creation of a new species. It is difficult to even define what is meant by the term ''species'' (Adl et al. 2007), but the common conception of the term usually involves a degree of genetic and reproductive isolation, and at least some definable morphologic or other physical criteria that can be used to distinguish between members of the two species (Mayden 2002). Under this definition, the typical and quite reasonable response by evolutionary biologists is that it takes many generations of genetic isolation under strong selective pressures to bring about the origin of a new species from a parent one. Active speciation events are thought to be quite rare and are predicted to occur over only a tiny fraction of the total ''lifespan'' of a given species. The fact that biologists have only been looking for examples of speciation for less than 150 years and that the generation time of most multicellular organisms is measured in months or even years, it is hardly surprising that relatively few examples have been recognized and documented. The results of a long-term study with *E. coli* suggest that a bacterial speciation event has indeed been observed and well documented (Blount, Borland, and Lenski 2008), but examples from eukaryotic organisms are rarer.

Most examples of eukaryotic speciation contain a significant time component. Some rely on comparisons of morphological or molecular characters shared by two extant species, which are predicted to be more closely related to each other than to any other living organisms. In other cases, identification of the evolutionary sequence is made by comparing fossil forms with living taxa. While these examples are compelling, if well documented, they do not meet the more rigorous standard of actually witnessing a speciation event because the parental strain is no longer available for comparison. What is needed to *completely* answer the objection is a clear-cut example whereby a sub-population of organisms becomes genetically isolated from its parent stock and, in the face of a strong selective pressure, develops traits that were not present in the parent. Furthermore, the genetic isolation must be total and complete, such that should the parent strain and the progeny have the opportunity to interbreed, they would still remain separate and genetically distinct from one another. All of this must be well documented, with both the parent and progeny strains surviving, and it must have been witnessed in a single person's lifetime.

Perhaps the most stunning example of this sort of speciation event in protists comes from the work of Kwang Jeon (Jeon and Jeon 2004). While Jeon was working with a genetically pure culture of strain D of *Amoeba proteus* in the 1960s, some of the culture flasks became contaminated by a bacterium that proved to be lethal to most cells (Jeon and Lorch 1967). Jeon called these uncharacterized pathogens ''X-bacteria.'' They met Koch's postulate in that if isolated from dead or dying *Amoeba*, X-bacteria had the ability to infect and destroy healthy uncontaminated cultures. As despair began to set in, Jeon noticed something remarkable. A few amoebae in the contaminated cultures had survived the infection, and now contained up to 40,000 of the X-bacteria within the cytoplasm of a single cell. These infected amoebae, which Jeon designated as xD amoebae, were able to multiply quickly, but only under very controlled conditions. Compared with their uninfected parents, the xD amoebae were exquisitely sensitive to conditions of overcrowding, food availability, and temperature. Nonetheless, they survived the infection where their more sensitive culture-mates had not.

Then Jeon did something remarkable: using a mixture of antibiotics, he set out to cure the xD amoebae of their bacterial contaminants (Jeon and Lorch 1967). The antibiotics were very effective in killing the X-bacteria, but most unexpectedly the host xD amoebae also perished. The symbiont-free D amoebae, on the other hand, were unaffected by the antibiotics. It seemed that the xD amoebae had in a few short generations become completely dependent on their bacterial symbionts, and could no longer survive without them; the pathogenic killer had become an indispensable partner. Because the X-bacteria are toxic to D amoebae, these xD amoebae could never be recombined with their parent strain. Thus, the two strains of amoebae were now genetically isolated from one another. Quite by chance a new species of *Amoeba* had arisen, and it had done so under the careful eye of a meticulous scientist.

Jeon has devoted much of the past 40 yr to uncovering the mystery of how the xD amoebae survived the initial infection of the cultures in the 1960s, and why they can no longer live without their symbionts. The X-bacteria, which have since been named *Legionella jeonii* (Park et al. 2004), apparently have the ability to inhibit host cell production of an enzyme, *S*-adenosylmethionine synthetase (SAMS), essential for formation of *S*-adenosylmethionine, which is the major methyl group donor in cells (Choi et al. 1997; Jeon and Jeon 2003). This explained why the initial infection was lethal, but not how the xD amoebae survive. More recent research has shown that this was only part of the story. The parental stock of D amoebae have two slightly different copies of the *sams* gene. After infection with *L. jeonii*, gene expression in the amoebae that survived the infection switched from the normal *sams* gene (*sams1*) to a second nuclear-encoded gene, *sams2* (Jeon and Jeon 2004; Jeon 2004). The mechanism of gene switching appears to be mediated by methylation of an internal adenine residue in the *sams1* gene of symbiont-bearing amoebae, which silences the expression of *sams1* and upregulates the expression of *sams2* (Jeon 2008). This alteration of gene expression in the nucleus of xD amoebae appears to be irreversible, thus explaining why the host cells became dependent on their symbionts so quickly.

It is clear that xD amoebae arose as the result of a spontaneous and undirected symbiosis, that they are clearly descended from a parent strain of D amoebae, and that the new strain is permanently genetically different from the parent strain. Because of the required presence of their bacterial symbionts, which are fatal to the parental stock, they are now genetically isolated from D amoebae. Thus, it can rightly be claimed that xD amoebae constitute a new and distinct species, that the speciation event occurred as the result of a specific symbiosis with *L. jeonii*, and that all the steps in the process were carefully observed and documented. It seems that symbiosis with prokaryotes, described in the previous section, remains an important factor in the evolution of eukaryotes even today.

GAPS IN THE FOSSIL RECORD

Another persistent theme in criticisms of evolution is the ''gappiness'' of the fossil record. A recent essay by Jonathan Wells of the Discovery Institute (Wells 2009) stated that ''It turns out that no series of fossils can provide evidence for Darwinian descent with modification.'' His critique is interesting because it includes a diagram illustrating his conception of ''Darwinism'' as a *scala naturae*-like progression of species evolving from one another in discrete steps without branching, which he contrasts with a much more conventional phylogenetic tree that he describes as being a ''better representation of the evidence.'' Because the ''better representation'' is in fact the way evolutionary biologists usually think about taxonomic relationships, it is tempting to consider his argument as hopelessly wrongheaded and leave it at that. However, Wells (2009) uses the diagram to argue that the nodes in the tree are not defined in the fossil record: that we capture only the tips of the branches, not the forks, and that the forks constitute a form of hand-waving. The evolution of xD amoebae just described is one example of the formation of a fork, of course, but there is also a wealth of evidence documenting other branching events that occurred in the past.

Contrary to Wells' (2009) assertion, many evolutionary branchpoints are captured in exquisite detail in the fossil record, and some of the best examples are protistan. Most protists fossilize only under unusual conditions (e.g. Schmidt et al. 2006), but several groups produce morphologically complex hard structures, usually silica or calcium carbonate shells, which are abundantly preserved as microfossils. In living species, the morphological details of these structures correspond well to taxon boundaries as determined by DNA analysis or observed ability to interbreed (Benton and Pearson 2001), so there is good reason to think that similar differences between fossil examples represent similar differences in the organisms that made them. These hard structures are also implicated in important life functions, such as prey processing (Bernhard and Bowser 1999) and improved light harvesting (Jeffryes et al. 2008), and are as biologically significant as the dinosaur bones and trilobite shells that are more familiar to the public.

One great advantage of the microfossil-producing protists, including but not limited to coccolithophores, diatoms, dinoflagellates, foraminiferans, and radiolarians, is that many are extremely abundant and therefore produce a very large number of potential fossils. For example, the great majority of the carbonate formed in the open ocean is produced by two protist groups, coccolithophores and planktonic foraminiferans (e.g. Ziveria et al 2007). In some locales, the incipient microfossils produced by protists when they die are a dominant component of the seafloor: seabeds of this type are called diatom, radiolarian or *Globigerina* oozes, in which more than 30% of the sediment is composed of the tests or frustules of these microorganisms. So abundant are fossil layers of these oozes that they are mined as diatomaceous earth for use as abrasives and pest-control compounds.

Because these microfossils are both hyperabundant and morphologically distinctive, it would be very surprising if they did not provide finely detailed examples of protistan species transitions: that is, the forks in the tree that Wells (2009) criticizes as unproven. One beautifully documented transition even represents the origin of a higher taxon: the evolution of the first species in the planktonic foraminiferal genus *Orbulina* from a species belonging to the related genus *Globigerina*. Globigerinid forams create shells, properly called ''tests,'' with successively larger,

bubble-like chambers in which buoyant material is held; these chambers allow them to float near the ocean surface. Modern *Orbulina* tests consist of a small *Globigerina*-like structure completely enveloped by a single, spherical chamber, and molecular geneaologies consistently group the two genera. If they are in fact sister taxa, one would expect fossil evidence of the transition considering how abundant these organisms are today.

Fossils intermediate between *Globigerina* and *Orbulina* were first described in the 1950s, and an accessible study of extremely well-preserved examples was published in the late 1990s (Pearson, Shackleton, and Hall 1997). The parent species, *Globigerina trilobus*, appears throughout the strata embraced by the latter study, but by the end of the Early Miocene additional morphological types appear in which the largest chamber of the test encroaches upon the outer surfaces of the smaller ones, and the chambers become less strongly lobed (Fig. 1). Forms recognizable as *Orbulina* sp. appear during the Middle Miocene. By $\sim$ 14 Mya, the morphologically intermediate forms are gone. This sequence of fossils documents the formation of a node in an evolutionary tree, something that Wells (2009) insists has never been shown. *Orbulina universa* continues to evolve, of course; there is evidence that there are three living species subsumed under this name, which are distinguishable by morphological and molecular criteria (e.g. Morard et al. 2009).

Foraminifera are not the only protists that provide excellent examples of transitional fossils. A much more recent evolutionary transition was documented in the sediments of Yellowstone Lake, home to the endemic diatom *Stephanodiscus yellowstonensis*, which is morphologically similar to the more widely distributed diatom *Stephanodiscus niagarae* (Theriot et al. 2006). The bottom of the core, dating from $\sim$ 13,000 years ago, shows a diatom very similar to modern *S. niagarae*. More recent sediments farther upcore show forms with progressively fewer spines, and also document a size increase about 10,000 years ago; modern *S. yellowstonensis* has a valve diameter 25% greater than *S. niagarae*. The timing of the transition coincides with environmental changes in the area as documented by pollen composition and fire histories (Theriot et al. 2006). Similar smooth transitions are documented for many other protist lineages; indeed, Benton and Pearson (2001) noted that marine plankton often provide excellent examples of lineage splitting, more so than larger and less abundant organisms, such as marine vertebrates.

Later in his critique, Wells (2009) implicitly acknowledges the strength of the fossil evidence for evolution by attempting to reject it on philosophical grounds. ''Imagine finding two human skeletons in the same grave, one about thirty years older than the other,'' he writes. ''Was the older individual the parent of the younger? Without written genealogical records and identifying marks (or in some cases DNA), it is impossible to answer the question. . .With fossils from different species that are now extinct, and widely separated in time and space, there is no way to establish that one is the ancestor of another—no matter how many transitional fossils we find.'' His relative confidence in DNA evidence is encouraging, but is based on a profound misconception. As readers of this journal know, DNA sequences are not like birth certificates, stamping an organism with the time and place of its origin. Ancestries inferred from DNA-based methods are founded on comparison of sequence or genomic data, evidence-based modeling of how DNA changes over time, and calculations of the most credible relationship between genetic sequences or patterns. This is just as true for variable number of tandem repeats (VNTR, or so-called ''DNA fingerprint'') analysis, which is broadly accepted in courts of law, as it is for deep phylogenetic analyses encompassing hundreds of millions of years of evolutionary history. For that matter, acceptance of ''written geneaological records'' requires confidence that the records are not false or misread.

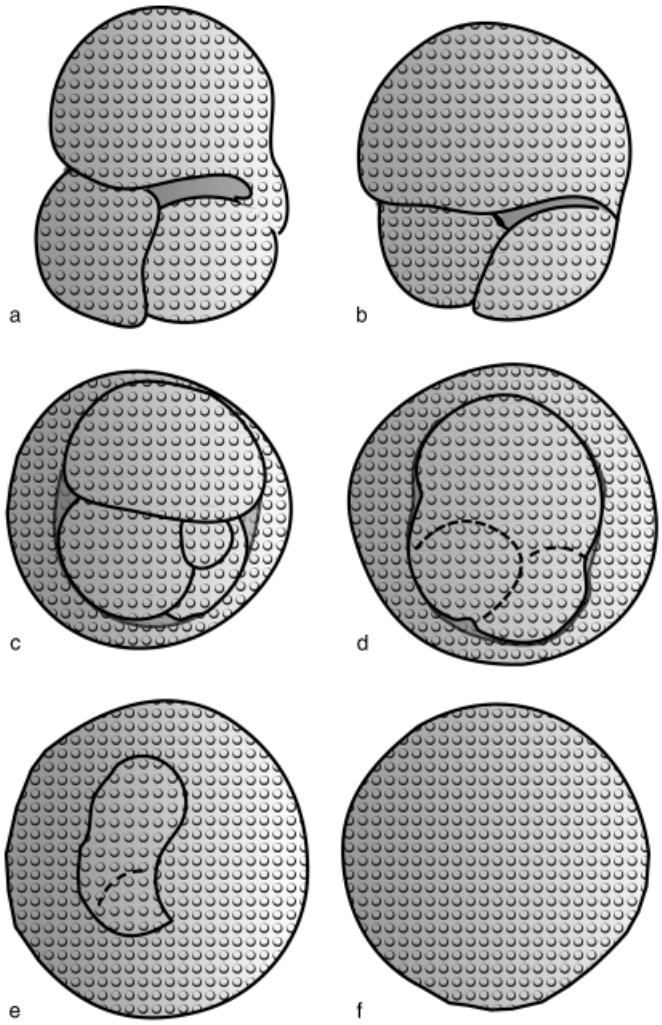

Fig **1.** *Orbulina*, *Globigerina*, and morphological intermediates from Miocene strata. Globigerinid foraminiferans add rounded chambers sequentially to the test in a coiled pattern as they grow. Reticulopodia, the foraminiferal ''pseudopods,'' pass through apertures (gray) and small pores in the test. (**a**) *Globigerina trilobus*. (**b**) *Praeorbulina sicana* in the same orientation. Notice the enlarged final chamber. (**c**) The same specimen rotated 90 degrees on the x axis. The large chamber partially envelops the smaller ones, and additional apertures are now present. (**d**) *Praeorbulina curva* in the same orientation as (c). The final chamber encloses the smaller chambers, which are less strongly lobed. (**e**) *Praeorbulina circularis*, same orientation as (c) and (d). The smaller chambers are almost completely enveloped. (**f**) *Orbulina universa*. *Globigerina trilobus* and *Orbulina universa* survive to the present; the other taxa are extinct. Figures based on Pearson et al. (1997, Fig 1, micrographs 2, 4, 5, 7, 14, and 15).

Therefore, all methods for determining the history of an organism (except for the direct observation described in the previous section) require theory and well-grounded inference, just as stratigraphic analysis does; they stand or fall together. It is difficult to conceive of a line of evidence for *any* past event that could satisfy Wells' criteria, which strongly suggests the objection is specious.

Finally, the spectacular abundance of microfossils also provides evidence for the idea that successive strata were laid down over eons, and reflect changes in the Earth's species composition over time. For example, in today's oceans, coccoliths are mixed freely on the seafloor with foram tests. Both microfossils are composed of calcium carbonate. However, coccoliths do not

appear in sediments older than the Triassic, 251–199 Mya, while the fossil record of calcareous foraminiferans extends into the Carboniferous, 359–299 Mya (see Cavalier-Smith 2006). Even if each species were individually created, the distribution pattern makes no sense based on what we know of modern oceans; a coccolith-free ocean floor is a very rare thing. If geologic strata do not represent a time series, there must be some other mechanism by which the fossils were sorted, but it is difficult to conceive of a way to meticulously segregate minute shells of similar size, composition, and morphology. Some attempts have been made to fit the distribution to ecological zonation, with ''shallow'' layers being stacked on top of ''deep'' ones during a Noachic flood event, but these do not correspond well what is observed in modern ecosystems (Aksu et al. 2002; for an example involving foraminiferans, see Tosk 1988.) If the stratigraphic record is indeed a time series, as generally accepted by geologists and paleontologists, then evolutionary inferences flow naturally from the observed distribution of microfossils in sequential strata.

THE ''IMPOSSIBILITY'' OF EVOLUTION

In his book ''The Edge of Evolution: The Search for the Limits of Darwinism'' Behe (2007) draws heavily upon the example of drug resistance in the malarial parasite *Plasmodium falciparum* as one biochemical pathway that is supposedly too complex to have arisen through natural evolutionary processes. According to Behe (2007), the odds that mutations required to impart chloroquine resistance in *Plasmodium* could arise naturally are so impossibly long that they lie beyond what he considers ''The Edge of Evolution.''

To the casual observer it might appear that he has a valid point. Since its first widespread use in the 1940s, the anti-malarial compound chloroquine has proven to be remarkably effective in treating individuals infected with *Plasmodium*. Chloroquine's mode of action is believed to interfere with the parasite's ability to sequester heme in the biologically inert crystalline form known as hemozoin. By binding to Fe(II)-protoporphyrin IX (FP), chloroquine forms a complex that can disrupt the functioning of the food vacuole membrane of the parasite, ultimately killing it. Beginning in the late 1950s, chloroquine-resistant strains of *P. falciparum*, the most dangerous and therefore most often treated of the four species of *Plasmodium* that infect humans, were separately reported from Thailand and the Colombian–Venezuelan border (Hyde 2007). By the late 1980s, resistance had spread throughout much of Asia, South and Central America, and all of sub-Saharan Africa. Presumably, a new mutation, which conferred resistance to this anti-malarial drug, had arisen in *P. falciparum*, driven by the strong selection pressure of widespread chloroquine treatment.

The ability to resist chloroquine has now been tracked to mutations in a gene that codes for a 49-kDa protein that has been designated as *P. falciparum* chloroquine-resistance transporter (PfCRT). The gene product of *pfcrt* is predicted to have 10 trans-membrane domains (Fig. 2) and to function in the digestive vacuole of the parasite (Martin and Kirk 2004). To date at least 16 resistant strains of *P. falciparum* have been identified, and all have a lysine to threonine substitution at amino acid position 76 (K76T). In addition, most if not all of the resistant strains have a second amino acid substitution at position 220. A number of additional polymorphisms at other positions have been documented, but are not believed to be implicated in drug resistance. The existence of these two mutations in *pfcrt* led Behe (2007) to conclude that both mutations were required in order to confer chloroquine resistance. Based on his calculations of the likelihood of two independent mutations simultaneously occurring in order to confer a new function (Behe and Snoke 2004, 2005) and his estimates of the likelihood of anti-malarial drug resistance (White 2004), Behe (2007) went on to coin the term Chloroquine Complexity Cluster (CCC) to refer to those biochemical changes that would require two simultaneous mutations in order to show any change in protein function. Behe (2007) calculated the likelihood of a CCC event as being in the neighborhood of 1 in 10^{20}, although some of the assumptions he relies on have been challenged (e.g. Lynch 2005). Behe (2007) concluded ''On average, for humans to achieve a mutation like this by chance, we would need to wait a hundred million times ten million years. Since that is many

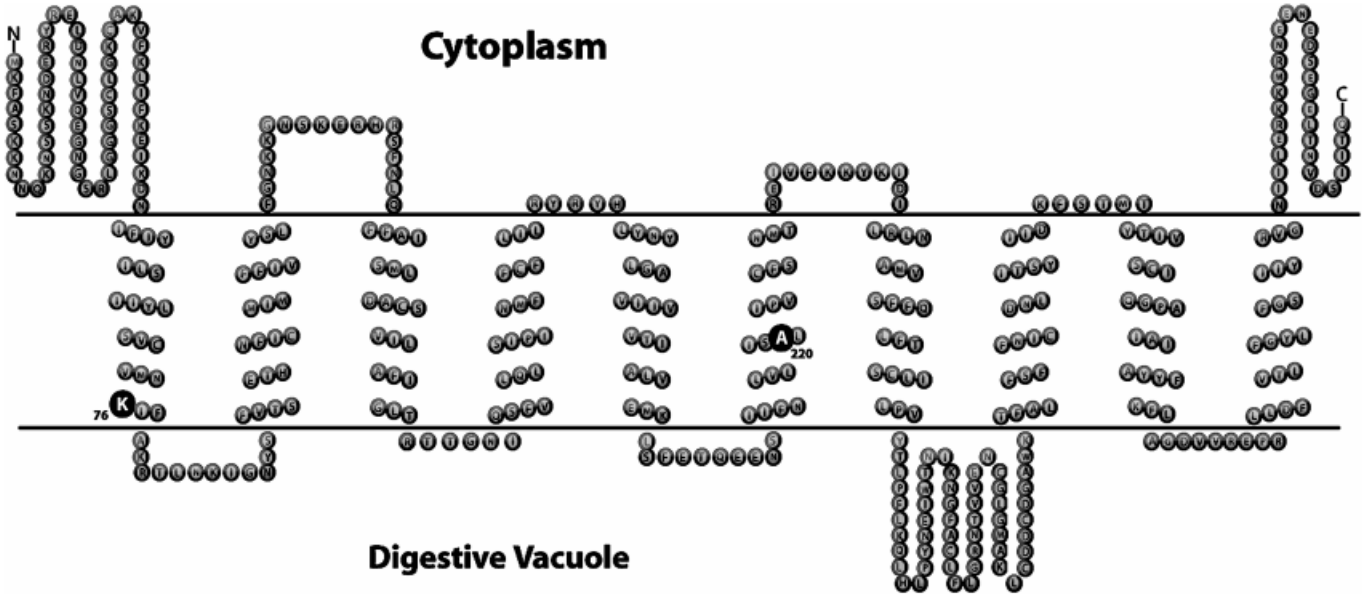

Fig. **2.** Amino acid sequence and predicted folding and distribution of *Plasmodium falciparum* chloroquine-resistance transporter (PfCRT) within the membrane of digestive vacuole of *P. falciparum*. Substitution of amino acids at position 76 (Lysine) and 220 (Alanine) occur in chloroquine resistant forms of PfCRT. Adapted from Martin and Kirk (2004).

times the age of the universe, it's reasonable to conclude the following: No mutation that is of the same complexity as chloroquine resistance in malaria arose by Darwinian evolution in the line leading to humans in the past ten million years.'' (Behe 2007). In reaching this conclusion he commits several errors of logic, most significantly with his initial assumption that mutations at both positions 76 and 220 are required to confer chloroquine resistance.

At the time of the publication of *The Edge of Evolution*, there was already evidence that this assumption was deeply flawed. Lakshmanan et al. (2005) had already demonstrated the essential contribution of the K76T substitution to chloroquine resistance by showing that wild-type levels of chloroquine susceptibility could be obtained by using allelic exchange between mutant and wild-type strains. The defects in the probability estimate by Behe (2007) became even more apparent when, less than a year later, Jiang et al. (2008) demonstrated that the single amino acid substitution at position 76 was both necessary and sufficient to confer chloroquine resistance. They conclude that ''Mutations in PfCRT . . . particularly the substitution at amino acid position 76 (a lysine to threonine substitution) confer chloroquine (CQ) resistance in *P. falciparum*. Point mutations in the homolog of the mammalian multidrug resistance gene (pfmdr1) can also modulate the levels of CQ response.'' (Jiang et al. 2008). While the substitution at position 220 enhances resistance, it is not essential. Thus, the idea that two mutations in *pfcrt* had to occur simultaneously in order to confer a significant selective advantage for *Plasmodium* was simply not true. This was known in 2005, as noted above, yet Behe (2007) did not cite Lakshmanan et al. (2005). Indeed, this pattern of cumulative effectiveness in drug resistance mutations in *Plasmodium* is the rule, not the exception. For example, resistance to the anti-malarial cycloguanil is partially conferred by several independent single amino-acid substitutions (Le Bras and Durand 2003). These substitutions are often additive if present in the same organism, but each is sufficient to confer resistance in isolation as well. Because mixed infections involving more than one resistant strain would be fairly common in areas with a high incidence of malaria, it would be startling from an evolutionary standpoint if strains containing multiple protective mutations did *not* arise in relatively short periods of time.

What is most troubling is that the ID community continues to hold up Dr. Behe as a shining example of a credentialed scientist even as he demonstrates extraordinarily bad scholarship (Forrest and Gross 2007). While he remains a professor of biochemistry who is clearly capable of conducting rigorous research, he has largely abandoned the peer-review process that guides the scientific community. The flaws in Behe's model of simultaneous vs. two-step mutations have been pointed out by many (Carroll 2007a, 2007b; Durrett and Schmidt 2008), yet he repeatedly brushes aside such criticisms (Behe 2009) and fails to acknowledge other examples of step-wise drug resistance in *Plasmodium* that were published nearly a decade before his book (Sirawaraporn et al. 1997). Rather than proving to be an example of an irreducibly complex system that could not have arisen through evolutionary processes, chloroquine resistance is instead revealed to be yet another example of how a naturally occurring mutation can confer selective advantage and quickly become fixed in the genotype of a population of protists (Nagesha et al. 2003).

CONCLUSION

Darwin's idea of common descent of all life on Earth, the result of natural selection acting on naturally occurring variation in biological forms, has been hailed as one of the two great stages in scientific thinking, the other being the Copernican concept of heliocentrism (Ayala 2007). In the 150 yr since the publication of the ''On the Origin of Species'' (Darwin 1859), the theory of evolution has undergone many changes and modifications as data from fields as diverse as genetics, paleontology, and molecular systematics have contributed to our understanding of the basic concept of descent with modification. While much of this evidence comes from the study of extant metazoa and their fossilized predecessors, the average lifespan and developmental complexity of multicellular organisms often makes it more difficult to use them to demonstrate many basic principles of evolution. Likewise, a general unfamiliarity with prokaryotes and their classification among the general public can make them less than ideal for demonstrating some evolutionary concepts. Protists combine the advantages of both groups for this educational purpose and avoid many of the disadvantages of each. A detailed understanding of protistan biology, therefore, offers scientists and lay persons alike the ability to address current attacks on evolutionary theory, and to refute the claims of ID creationists who insist on invoking supernatural explanations to account for observable phenomena.

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Received: 09/10/09, 10/19/09; accepted: 10/19/09