Edging Towards Irrelevance

A commentary on recent claims by the Discovery Institute on the evolution of drug resistance in malaria.

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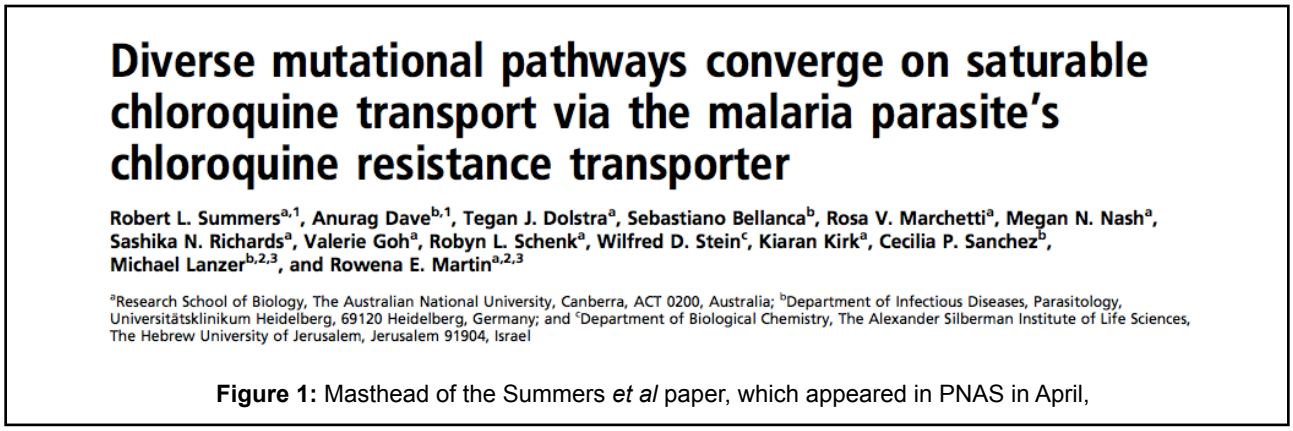
Introduction

Suppose you published a book making a set of very specific claims. Then, after highly critical reviews of your book are published in major scientific journals, an international research team publishes a detailed study in the Proceedings of the National Academy (PNAS) on the very system that was the focus of your book[1]. Great news? Well, maybe, except for one little problem. That research paper shows, in great detail, why the claims at the heart of your book were wrong. Do you walk away quietly, hoping no one notices?



Diverse mutational pathways converge on saturable chloroquine transport via the malaria parasite's chloroquine resistance transporter

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Figure 1: Masthead of the Summers *et al* paper, which appeared in PNAS in April,

Not if you're Michael Behe. Instead, you declare victory, tell everyone who will listen that the research actually vindicates you, and then get your friends at the Discovery Institute to demand *apologies* from those who had criticized your book. In the strange world of "intelligent design" (ID), that's how things seem to work. When new scientific findings support evolution, the ID crowd tries to spin things around by pretending they actually contradict it. They've done this before, and they'll probably do it again.

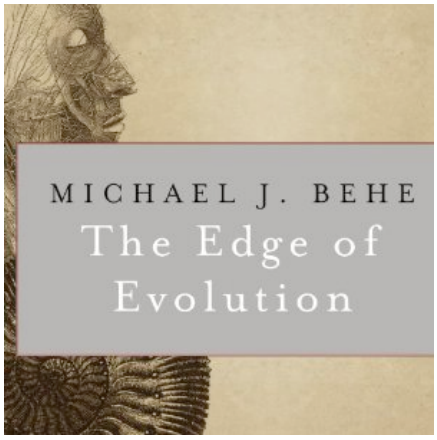
Where does all this start? With *The Edge of Evolution*, a book by Lehigh University biochemist Michael Behe and his portrayal of the evolution of drug resistance by one of the world's most

[1] Summers, R. L., *et al* (2014) Diverse mutational pathways converge on saturable chloroquine transport via the malaria parasite's chloroquine resistance transporter. PNAS 111: E1759-E1767.

deadly parasites — malaria. To explain Behe's argument, as well as the latest research findings, we'll start with his claim of a limit to evolution.

Finding a Limit to Evolution?

In his 2007 book, *The Edge of Evolution*[2], Behe argued that evolutionary change has a limit, an "edge," beyond which it cannot go. To put some hard numbers on that edge, he used the evolution of chloroquine resistance in *Plasmodium*, the malarial parasite, as a prime example of the best that evolution can do. Noting that chloroquine resistance has arisen only rarely since the drug was introduced in 1947, Behe estimated that the probability of a single cell becoming resistant to the drug was just 1 in 10^{20}. He called any series of mutations equal to the complexity of chloroquine resistance a "CCC" (chloroquine complexity cluster), and used that 1 in 1 in 10^{20} number to argue that the probability of two mutations of CCC's complexity emerging was the square of that probability, or 1 in 1 in 10^{40}. That, he told us, was beyond the ability of Darwinian evolution to achieve. Therefore, any time we see two or three mutations or adaptations similar to a CCC in an organism, the only possibility is that they were "designed."



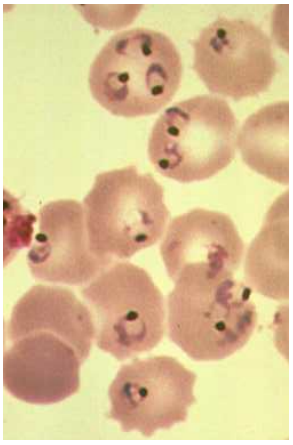


Figure 2: (Left) Michael Behe's 2007 book, *The Edge of Evolution*. (Right) Light micrograph of red blood cells infected with *Plasmodium*, the malaria parasite.

When it appeared in 2007, Behe's book was roundly criticized by reviewers in Science[3], Nature[4], and the New York Times[5]. So why does he now feel he can claim vindication? He does this by

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[2] Behe, M. J. (2007) *The Edge of Evolution: The search for the limits of Darwinism.* The Free Press.

[3] Carroll, S. B. (2007) God as genetic engineer. Science 316: 1427-1428.

[4] Miller, K. R. (2007) Falling over the edge. Nature 447: 1055-1056.

[5] Dawkins, R. (2007) Inferior design. New York Times Book Review, July 1, 2007.

pretending that the criticisms of his book were based on nothing more than that 1 in 10^{20} probability. According to Behe's July 14, 2014 web posting, the new research study shows, "that the need for an organism to acquire multiple mutations in some situations before a relevant selectable function appears is now an established experimental fact." That was, of course, an established experimental fact long before Behe read the PNAS paper, but never mind.

Apparently emboldened a week later, on July 21, 2014 he posted an open letter challenging his critics (myself included) to dispute that 1 in 10^{20} probability for a CCC. As he put it, "Talk is cheap. Let's see your numbers." Such language implies, of course, that these multiple critiques were based on Behe's numbers. But they weren't. The problem was not, as Behe now tries to claim, that anyone disputed the odds of developing resistance to chloroquine. Behe's arguments about an "Edge" to evolution were wrong for a far more fundamental reason. But first, let's look at how badly he misrepresented that PNAS paper in an effort to claim vindication.

Parasites and Drugs

Behe's "Edge" argument rests on two basic points. The first is that a beneficial, selectable trait like chloroquine resistance can arise only after multiple, simultaneous mutations emerge at random. The target for those mutations is the gene for PfCRT, a membrane transport protein. In chloroquine resistant strains, mutant versions of this protein are able to pump the drug out of the cell's digestive vacuole, enabling the parasite to survive.

As Behe puts it, the data argue "that a first, required mutation to PfCRT is strongly deleterious, while the second may partially rescue the normal, required function of the protein, plus confer low chloroquine transport activity."[6] Since that first "required" mutation is so deleterious, it couldn't possibly spread through the population while waiting for the second to appear. Natural selection would weed out the deleterious mutation unless the second one popped up beside it in the same organism. That, according to Behe, accounts for the very low frequency of chloroquine resistance and validates his analysis.

Quite frankly, he must be secretly hoping that nobody actually looks at the details in the PNAS paper.

There is indeed one required mutation in the PfCRT protein, which is a change of an amino acid at position number 76 from lysine to threonine (see Figure 3). In the language of protein chemistry, that's a K76T mutation ("K" stands for lysine, a positively charged amino acid, and "T" for threonine, which is uncharged).

[6] Behe, M. J. (2014) from "An Open letter to Kenneth Miller and PZ Myers," posted on evolutionnews.org and dated July 21, 2014.

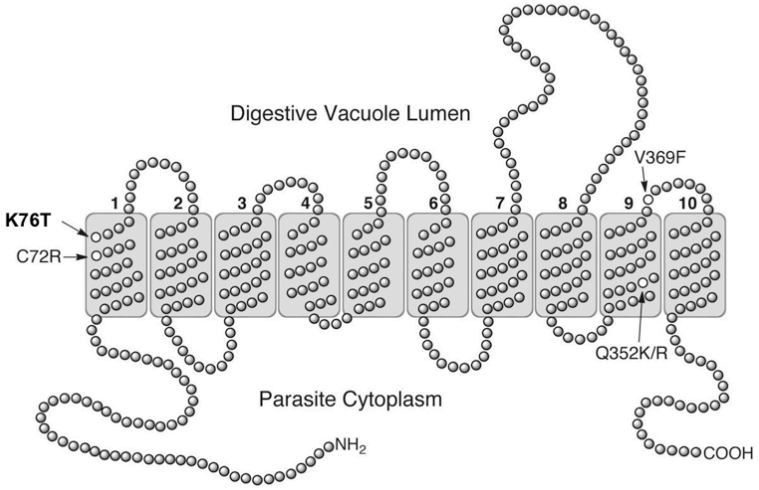

Figure 3: Diagrammatic view of the PfCRT protein, which spans the membrane of the parasite's digestive vacuole. The position of the critical K76T mutation is noted, as are several other mutations known to occur in the protein. (Source: Griffin *et al*, 2012)[7]

But Behe was dead wrong about it being "strongly deleterious." In fact, it seems to have no effect on transport activity at all. A neutral mutation like this can easily propagate through a population, and field studies of the parasite confirm that is exactly what has happened. In fact, a 2003 study[8] recommended against using the K76T mutation to test for chloroquine resistance since that same mutation was also found in 96% of patients who responded well to chloroquine. Clearly, K76T wouldn't have become so widespread if it were indeed "strongly deleterious," as Behe states it must be. This is a critical point, since Behe's probability arguments depend on this incorrect claim.

Directly contradicting Behe's central thesis, the PNAS study also showed that once the K76T mutation appears, there are *multiple* mutational pathways to drug resistance. In most of these, each additional mutation is either neutral or beneficial to the parasite, allowing cumulative natural selection to gradually refine and improve the parasite's ability to tolerate chloroquine. One of those routes involves a total of seven mutations, three neutral and four beneficial, to produce a high level of resistance to the drug. Figure 4, taken from the Summers *et al* paper[9], makes this point in graphic fashion, showing the multiple mutational routes to high levels of transport, which confer resistance to chloroquine.

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[7] Griffin *et al* (2012) Mutation in the Plasmodium falciparum CRT protein determines the stereospecific activity of antimalarial Cinchona alkaloids. Antimicrobial Agents and Chemotherapy 56: 5356-5364.

[8] Vinayak, S.*et al* (2003) Prevalence of the K76T mutation in the pfcrt gene of Plasmodium falciparum among chloroquine responders in India. Acta Tropica 87: 287–293.

[9] Summers *et al* (2014).

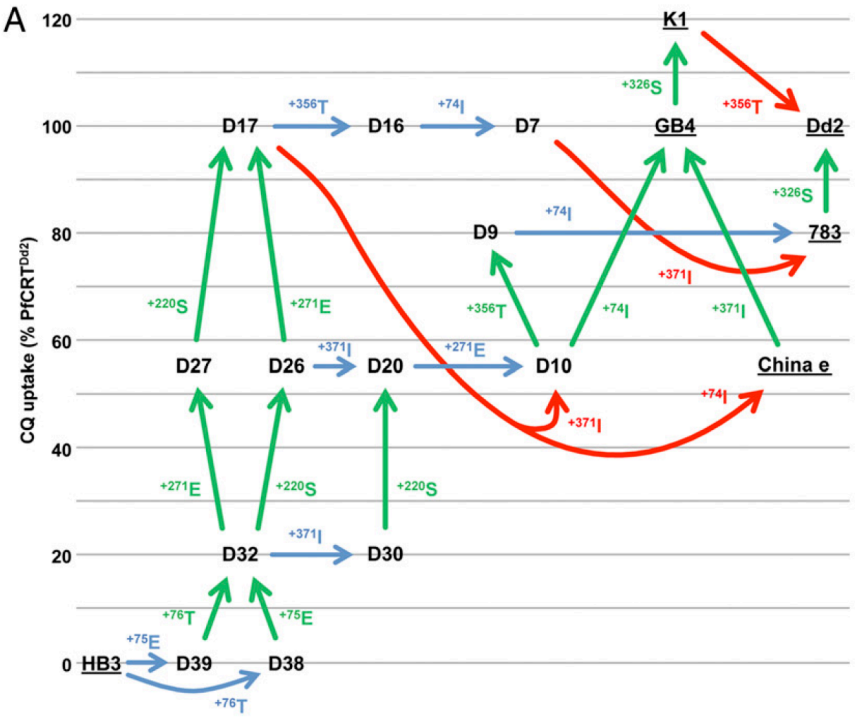

Figure 4: Mutational pathways from the unmodified PfCRT protein (strain HB3) plotted against their ability to transport chloroquine. Neutral mutations are shown in blue, those that decrease transport in red, and the beneficial mutations that increase transport are shown in green.

Pathways of this sort, involving sequential mutations, are exactly what Behe had tried to rule out, as I wrote in my own review of his book in 2007:

> Behe obtains his probabilities by considering each mutation as an independent event, ruling out any role for cumulative selection, and requiring evolution to achieve an exact, predetermined result. Not only are each of these conditions unrealistic, but they do not apply even in the case of his chosen example. First, he overlooks the existence of chloroquine resistant strains of malaria lacking one of the mutations he claims to be essential (at position 220). This matters, because it shows that there are *several mutational routes* to effective drug resistance. Second, and more importantly, Behe waves away evidence suggesting that chloroquine resistance may be the result of sequential, not simultaneous, mutations.[10]

We now know, courtesy of the PNAS paper, that such criticisms were right on target. There are indeed *several mutational routes* to drug resistance, and they are indeed the result of sequential, not simultaneous mutations. This matters because the assumption of simultaneous mutations is at the very heart of Behe's math. That's how he justifies multiplying one probability times another times another to conclude that complex traits are beyond the reach of the evolutionary process. To put it clearly, the problem with the logic of *The Edge* isn't the specific figure of one chance in

[10] Miller, K. R. (2007).

10^{20}, but the way in such probabilities are multiplied. In fact, it doesn't really matter if chloroquine resistance emerges at a probability of one chance in 10^{20}, one in 10^{15}, or even one chance in 10^{10}. The problem is the logic that Behe uses to calculate the chances of evolution producing two or more CCCs. As we will see, that's the most critical part of his argument — and it's wrong.

Rigging the Odds

Cells are filled with protein-to-protein binding sites, which play vital roles in signal transduction, gene expression, and biosynthetic pathways. According to Behe, these sites are so specific that "generating a new protein-to-protein binding site is of the same order of difficulty or worse than the development of chloroquine resistance in the malarial parasite." [11] In other words, roughly one in 10^{20} or worse. He goes on:

> "Now suppose that, in order to acquire some new, useful property, not just one but two new protein-binding sites had to develop. … So, if other things were equal, the likelihood of getting two new binding sites would be what we called in Chapter 3 a 'double CCC' — the square of a CCC, or one in ten to the fortieth power. Since that's more cells than likely have ever existed on earth, such an event would not be expected to have happened by Darwinian processes in the history of the world. .. And the great majority of proteins in the cell work in complexes of six or more. Far beyond that edge." [12]

Pretty conclusive, eh? And even if 10^{20} isn't the right number for a CCC, even if that probability is one in 10^{15} or 10^{10}, once you string together three or four such binding sites the odds of "Darwinian processes" getting there vanish into nothingness. Sort of makes you wonder why mathematical biologists haven't thought it before, doesn't it? Well, if they have, they've dismissed it at once, because such reasoning is built around a statistical trick. That trick is demanding a fixed set of particular, highly specific outcomes for a series of unrelated events.

Here's how it works. Let's accept Behe's number of 1 in 10^{20} for the evolution of a complex mutation like his CCC. As he admits, CCC's have arisen multiple times in the malaria parasite population since the drug was first introduced in 1947. In fact, resistance to the drug appeared in the late 1950s and early 1960s, within just 15 years of its widespread use. So it only took a decade and a half for one of Behe's CCC's to emerge in the parasite population. Now, suppose that another drug, equal in effectiveness to chloroquine, were to come into wide use. According to Behe, resistance to both drugs would require two CCCs, and the probability of double resistance arising would be a CCC squared. That's 1 in 10^{20} x 10^{20} or one chance in 1 in 10^{40}. According to Behe's math, that's such a large number that we can call it impossible:

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[11] Behe, M. J. (2007) p. 135.

[12] *Ibid,* p. 135.

> "…throughout the course of history there would have been slightly fewer than 10^{40} cells, a bit less than we'd expect to need to get a double CCC. The conclusion, then, is that the odds are slightly against even one double CCC showing up by Darwinian processes in the entire course of life on earth."[13]

Wow! Not even once in the history of life on earth? Pretty impressive. But the math is wrong, and it's easy to see why. Chloroquine resistance arose in just a decade and a half, and is now common in the gene pool of this widespread parasite. Introduce a new drug for which the odds of evolving resistance are also 1 in 10^{20}, and we can expect that it will take just about as long, 15 years, to evolve resistance to the second drug. Once you get that first CCC established in a population, the odds of developing a second one are not CCC squared. Rather, they are still 1 in 10^{20}. Behe gets his super-long odds by pretending that both CCCs have to arise at once, in the same cell, purely by chance. They don't, and I pointed this out in my Science review when Behe attempted to apply his reasoning to human genetics:

> Behe, incredibly, thinks he has determined the odds of a mutation "of the same complexity" occurring in the human line. He hasn't. What he has actually done is to determine the odds of these two exact mutations occurring simultaneously at precisely the same position in exactly the same gene in a single individual. He then leads his unsuspecting readers to believe that this spurious calculation is a hard and fast statistical barrier to the accumulation of enough variation to drive darwinian evolution.
>
> It would be difficult to imagine a more breathtaking abuse of statistical genetics.[14]

Interestingly, Behe's kind of math would apply only in one very special situation, and that would be if both drugs were applied in similar doses at exactly the same time, so that the emergence of resistance to one would be useless without the simultaneous appearance of resistance to the other. That, in fact, is the reason that multiple drug therapy can be effective against HIV and other diseases. By manipulating the doses of several anti-viral drugs at once, it's possible to prevent the emergence of resistant strains of the virus. But this situation only prevails under carefully designed therapeutic conditions. You might say, ironically, that it takes "intelligent design" to produce conditions favoring the long odds he demands, conditions that don't exist in nature.

But that's not the only problem with Behe's math. When he turns his attention to protein binding sites, he uses the extremely tight and specific fit between antibody and antigen as his model. On that basis, he feels justified in telling his lay readers that "…one way to get a new binding site

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[13] Behe, M. J. (2007) p. 63.

[14] Miller, K. R. (2007).

would be to change just five or six amino acids in a coherent patch in the right way.[15]" Not surprisingly, the odds of getting "just" five or six specific, *predetermined* point mutations to occur together in a single genome are too long to be within the bounds of probability. But that's because, just as before, Behe has stacked the statistical deck in a completely unrealistic manner. Sean Carroll was quick to point this out in his review of *The Edge*:

> He insists, based on consideration of just one type of protein structure (the combining sites of antibodies), that five or six positions must change at once in order to make a good fit between proteins—and, therefore, good fits are impossible to evolve. An immense body of experimental data directly refutes this claim. There are dozens of well-studied families of cellular proteins (kinases, phosphatases, proteases, adaptor proteins, sumoylation enzymes, etc.) that recognize short linear peptide motifs in which only two or three amino acid residues are critical for functional activity [reviewed in (*7*–*9*)]. Thousands of such reversible interactions establish the protein networks that govern cellular physiology.[16]

Needless to say, nothing in the PNAS study supports Behe's mistaken view of how new protein binding sites must evolve. Behe insists that each such site must include five or six specific amino acids, which is not correct, and calculates his probabilities by insisting on predetermined results, which unrealistically stacks the deck. As Carroll wrote in his review:

> Behe has quite a record of declaring what is impossible and of disregarding the scientific literature, and he has clearly not learned any lessons from some earlier gaffes. [17]

What about those "earlier gaffes?" They have been plenty of them, but some of the most telling have involved Joe Thornton's groundbreaking work on protein evolution.

Been There, Done That

There are two ways in which Michael Behe and his supporters have misrepresented the meaning of the Summers *et al* PNAS study. First, and most fundamentally, they have misstated criticisms of *The Edge* to make it appear that the book's many critics had argued that the evolution of malaria resistance did not require multiple mutations and was much more probable than one chance in 10^{20}. In reality, *none* of the criticisms took issue with that number at all. That's why Behe's "Show me your numbers" challenge is meaningless. Second, they have ignored the study itself, which does indeed show that resistance to chloroquine evolves along multiple pathways,

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[15] Behe, M. J. (2007) p. 134.

[16] Carroll, S. B. (2007).

[17] Ibid (2007).

each of which involves multiple steps of increasing resistance to the drug. This matters, because it shows that the pathways to resistance are sequential, not simultaneous, and therefore can be favored by natural selection.

Far from offering vindication, the PNAS paper actually cuts the legs out from under Behe's claims about evolution and the malaria parasite. How could he and his supporters get it so wrong? It may help to know that this is not the first time they've done something like this.

One of the leaders in the study of protein evolution is Joe Thornton, now at the University of Chicago. Over the past decade, Thornton and his colleagues have patiently teased out the evolutionary pathways that gave rise to a handful of important proteins. One of these is the glucocorticoid receptor (GR), a protein that binds to hormones like cortisol and then alters patterns of gene expression in response to the presence of the hormone. In working out these pathways, Thornton's lab has shown that the modern GR protein has its roots in an ancient protein that is the common ancestor of a number of other receptors, including those for testosterone, estrogen, and progesterone. As their studies continued, they explored the specific pathways leading from that common ancestral receptor to the modern GR protein, and found that chance events, many of them highly improbable, played key roles in GR evolution.

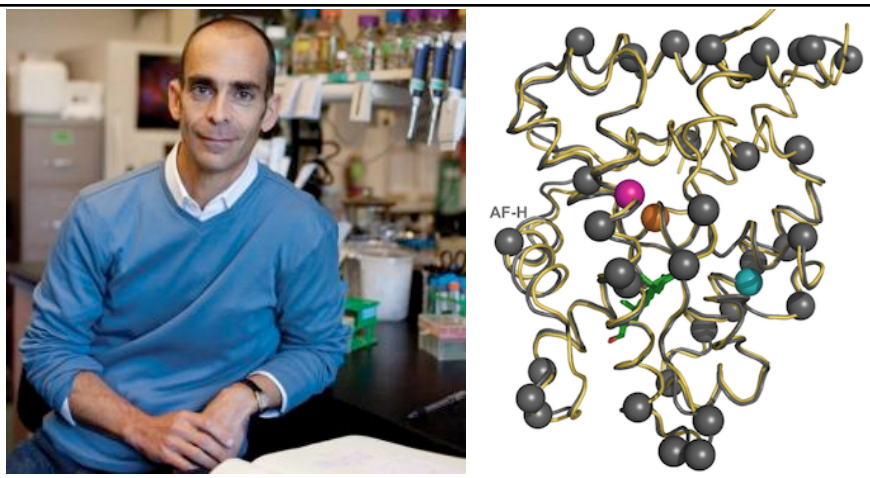

Figure 5: Joe Thornton (left) and his lab's calculated structure for the ancestral glucocorticoid receptor protein (right).

This is hardly surprising. The role of contingency in evolutionary processes has been long appreciated (see Stephen Jay Gould's book "*Wonderful Life*" for a popular treatment of this theme). However, the work of the Thornton lab has given contingency a much more specific biochemical and biophysical basis. In one of their most recent studies[18], Thornton and coauthor Michael J. Harms showed evidence that a specific series of "permissive" mutations were necessary for the evolution of the modern GR protein. They summarized the contingent nature of

[18] Harms, M. J., and Thornton, J. W. (2014) Historical contingency and its biophysical basis in glucocorticoid receptor evolution. Nature 512: 203-207.

these mutations by pointing out that the identical series of mutations would be highly unlikely to evolve a second time:

> If evolutionary history could be replayed from the ancestral starting point, the same kind of permissive substitutions would be unlikely to occur. The transition to GR's present form and function would probably be inaccessible, and different outcomes would almost certainly ensue. Cortisol-specific signaling might evolve by a different mechanism in the GR, or by an entirely different protein, or not at all; in each case, GR—or the vertebrate endocrine system more generally—would be substantially different.[19]

Almost immediately, Michael Behe pounced on the work, proclaiming "that severe problems face even relatively minor Darwinian evolution of proteins." According to the title of Behe's web posting, Thornton's recent work provides "More Strong Experimental Support for a Limit to Darwinian Evolution."[20] He writes, "The paper's conclusion is that, of the very large number of paths that random evolution could have taken, at best only extremely rare ones could lead to the functional modern protein."

Now, Behe is right that only a few rare paths could lead to *the* modern protein, meaning the *exact* form of the GR protein we see today, but he is completely mistaken in seeing this as a "problem" for evolution. Thornton has been the victim of Behe's distortions so many times before that back in 2009, at the urging of science writer Carl Zimmer, he responded with in detail (The complete letter is available here [21]). Here are a few highlights from that letter to Zimmer:

> Thanks for asking for my reaction to Behe's post on our recent paper in Nature[22]. His interpretation of our work is incorrect. He confuses "contingent" or "unlikely" with "impossible." He ignores the key role of genetic drift in evolution. And he erroneously concludes that because the probability is low that some specific biological form will evolve, it must be impossible for ANY form to evolve.
>
> A path to a new function that involves neutral intermediates is entirely accessible to the evolutionary processes of mutation, drift, and selection. Our work showed

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[19] Harms, M. J., and Thornton, J. W. (2014) p. 206.

[20] Behe, M. (2014) at this URL: http://www.evolutionnews.org/2014/06/more_strong_exp087061.html

[21] http://blogs.discovermagazine.com/loom/2009/10/15/the-blind-locksmith-continued-an-update-from-joe-thornton/#.VE5uQ0skC3A

[22] Bridgham, J. T., *et al* (2009) An epistatic ratchet constrains the direction of glucocorticoid receptor evolution. Nature 461: 515-519.

> that these classic neodarwinian processes are entirely adequate to explain the evolution of GR's new function.
>
> Behe erroneously equates "evolving non-deterministically" with "impossible to evolve." He supposes that if each of a set of specific evolutionary outcomes has a low probability, then none will evolve. This is like saying that, because the probability was vanishingly small that the 1996 Yankees would finish 92-70 with 871 runs scored and 787 allowed and then win the World Series in six games over Atlanta, the fact that all this occurred means it must have been willed by God.
>
> Behe's argument has no scientific merit. It is based on a misunderstanding of the fundamental processes of molecular evolution and a failure to appreciate the nature of probability itself. There is no scientific controversy about whether natural processes can drive the evolution of complex proteins. The work of my research group should not be misinterpreted by those who would like to pretend that there is.

I'm not sure that anyone could put it more succinctly than Thornton did in his 2009 letter, and yet five years later neither Behe nor his friends at the Discovery Institute get it. The misunderstanding (or is it misrepresentation?) of protein evolution and probability continues. Clearly it serves the purposes of the ID movement to pretend that the odds are hopelessly stacked against evolution when all the evidence indicates otherwise.

In a 2012 interview with Nature[23], Thornton expressed weariness with the way in which ID proponents continue to take issue with the clear implications of his work. "I'm sort of bored with them," he told the journal. In truth, I am, too. Time after time, they take work that devastates their key claims, like the PNAS study on drug resistance in malaria, and pretend to their willing adherents that science is trending their way. As it misrepresents one study after another, the ID movement continues on its steady and certain downward slide to irrelevance.

Time to Apologize?

In July of this year, Casey Luskin, professional spokesman for the Discovery Institute, demanded that Behe's critics apologize to him. I certainly do agree with Mr. Luskin that an apology is in order, but it's not the one he's been demanding. The real apology, which is long overdue, should be promptly sent out to all of those who have been taken in by Luskin's and Behe's continuing misrepresentations and distortions of the science of protein evolution. Knowing the Discovery Institute, however, I'm not holding my breath waiting for it.

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[23] Pearson, H. (2012) Prehistoric Proteins" Raising the dead. Nature 483: 390-393.