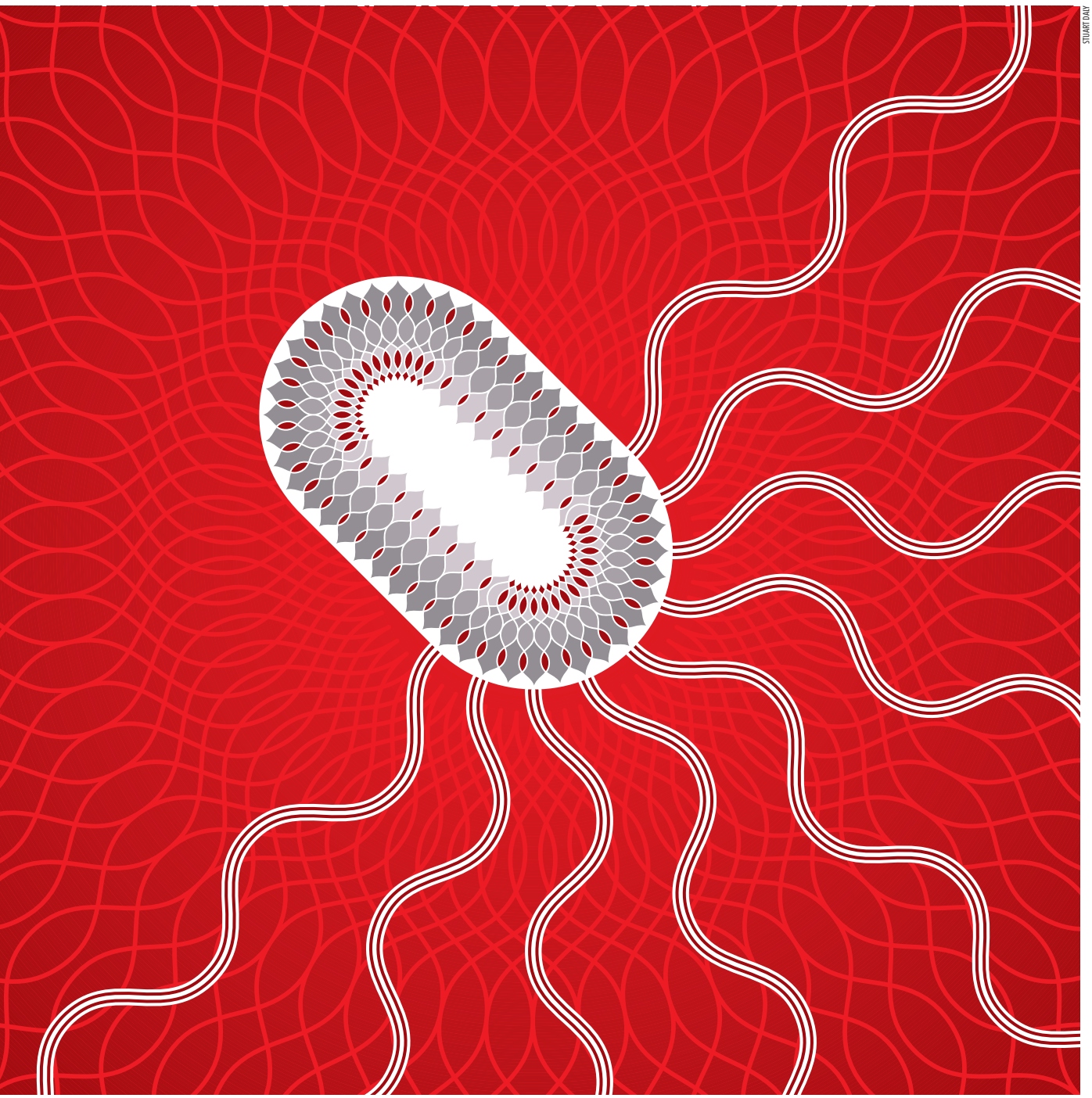


STUART DALY

Engines of **evolution**

The elegant complexity of the bacterial flagellum inspires awe, but its humble origins are becoming apparent, says **Dan Jones**

THREE years before his death in 1805, English philosopher William Paley proposed a now-famous thought eriment. Imagine discovering a watch on heath: how would you explain its intricate angement of parts, its clear design for a rpose? Naturally, you'd conclude that it was lt by a watchmaker, not blown together by nce. By analogy, Paley argued, the natural rld is full of designed complexity which st therefore also have a creator: God.

Had Paley been in a position to know ut it, he would no doubt have considered markable little device called the bacterial gellum to be an excellent example of igned complexity. With its intricate angement of interconnecting parts, the gellum looks no less designed than a watch, would surely have had Paley reaching for existence of its "maker".

Modern biology, of course, has no need omniscient designers. Evolution – Richard wkins's blind watchmaker – is all that is eded to explain the origin of complexity in ure. Even so, latter-day Paleys continue to rch for evidence of design in the living rld. The bacterial flagellum has become ir cause célèbre – and a focal point in ence's ongoing struggle against unreason.

The bacterial flagellum is one of the most nplex and elegant pieces of biological chinery known. It is the bacterial world's board motor, rotating at high speeds ropel bacteria through their watery vironments. It is made up of about proteins that self-assemble into three ic modules – the basal body, hook and ment (see Diagram, p 43).

The heart of the flagellum is the basal ly; essentially a rotary motor embedded in bacterial cell wall. The motor has a series ings, each about 20 nanometres in meter, with a rod inside that is free to ate a full 360 degrees. Attached to the is a curved "hook" protein linked to a g whip-like filament. This filament is 15 micrometres long and made of thousands epeating units of the protein flagellin. The tor is powered by the flow of sodium or drogen ions across the cell wall. As ions w, the motor kicks into action, causing rod to whizz round at speeds of up to 0 rotations per minute.

Biologists have been interested in the terial flagellum for decades, not least ause it is a prime example of a complex molecular system – an intricate nanomachine beyond the craft of any human engineer. Explaining the origin of such systems is one of the most difficult and important challenges in evolutionary biology.

It isn't just a scientific challenge, though. The study of complex molecular systems has been given added impetus by the "intelligent design" (ID) movement – the intellectual heirs of Paley. To them, such systems are examples of "irreducible complexity", a concept that goes to the heart of their opposition to the theory of evolution.

In an oft-quoted passage from *On the Origin of Species*, Charles Darwin wrote: "If it could be demonstrated that any complex organ existed, which could not possibly have been formed by numerous, successive, slight modifications, my theory would absolutely break down." (In anti-evolution circles, the following line is often omitted: "But I can find no such case.")

Proponents of ID argue that the bacterial flagellum is exactly such a case: each of its interacting components is essential for the system to function, they claim, and if you remove any one of them the whole thing grinds to a halt. ID claims that because of this irreducible complexity, such systems cannot be explained by the stepwise process of natural selection and therefore must be the handiwork of an "intelligent designer".

"The flagellum is complex, but is it too complex to have evolved through natural selection? Until recently it was surprisingly hard to say"

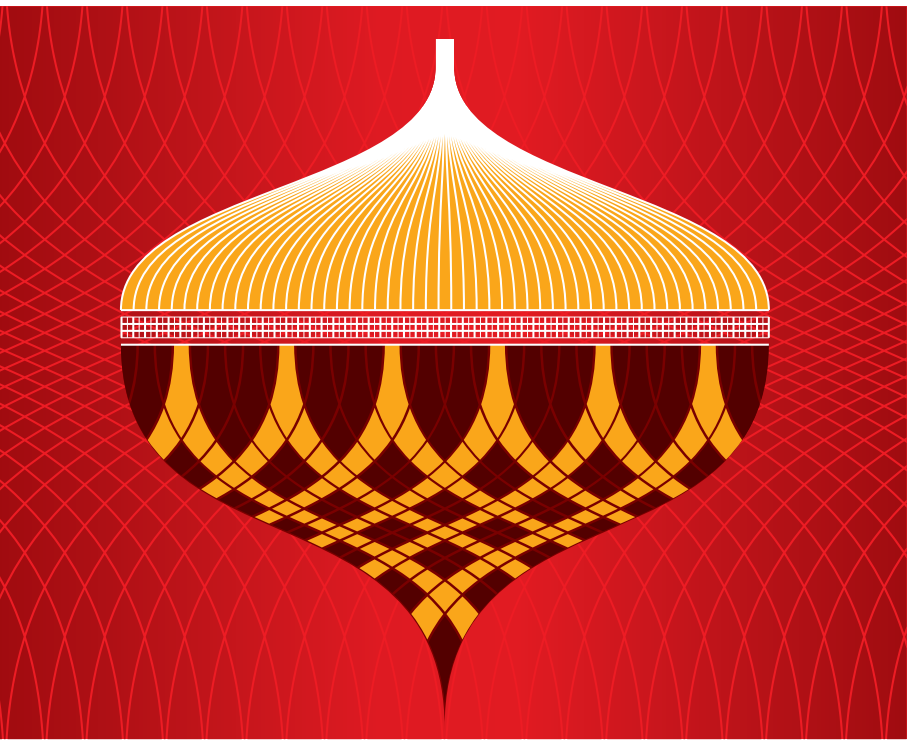
The flagellum is certainly complex, but is it really too complex to have evolved through natural selection? Until recently it has been surprisingly hard for biologists to answer this question satisfactorily. "If you go back just six or seven years, the function of many of the components of the bacterial flagellum were unknown," says Kenneth Miller, a biochemist at Brown University in Providence, Rhode Island. "It's very difficult to work out the evolution of a complex system when you don't understand how the system works." In the absence of this knowledge, biologists all too often fell back on the assertion that "bacterial flagella evolved and that is that", according to Mark Pallen, a microbiologist at the University of Birmingham in the UK.

That all started to change in the 1990s, however, when microbiologists discovered so-called "type III secretion systems" (T3SSs), a class of molecular machine used by disease-causing bacteria such as *Salmonella*. A typical T3SS is a complex made up of 15 to 20 proteins embedded in the cell wall that shuttles toxic proteins from inside the bacterial cell into a needle-like structure on the outside, which the bacterium uses to inject toxins into its victim.

The relevance to flagellum evolution? Variants of at least seven T3SS proteins are also found in the flagellum, within a subsystem called the protein export system. This sits within the basal body and funnels replacement flagellin subunits to the filament, using a mechanism remarkably similar to the T3SS. In fact, the two systems are so similar that the flagellar protein export system is now considered to be a subclass of the T3SS (*Trends in Microbiology*, vol 14, p 157).

Such similarities, or "homologies", are strong evidence that the two systems evolved from a common ancestor – analogous to the way that the arrangement of bones in the limbs of horses, bats and whales reveal their common ancestry despite their very different outward appearance and function. Similar homologies can be seen in the DNA sequences of genes, and in the amino acid sequences and 3D structures of proteins – all are clear evidence of shared descent.

The evolutionary events linking flagella and T3SSs are not clear, but the homology between them is a devastating blow to the claim of irreducible complexity. This requires that a partial flagellum should be of no use whatsoever – but clearly it is. "The T3SS is a useful model of how a 'partial flagellum' could function in protein export," says Nicholas Matzke of the University of California, Berkeley, a prominent defender of evolution and author of a number of academic articles on the flagellum. Miller adds: "The notion that these proteins can only be used in flagella simply falls apart." This argument helped swing the outcome of the "ID trial" in Dover, Pennsylvania, in 2005, in which ▶



Why would an intelligent designer go to all the trouble of reinventing the flagellum over and over again?"

reducible complexity formed a key plank of he ID movement's failed bid to have ID taught school science classes.

So how exactly is the flagellum's protein xport system related to the T3SS? One ossibility is that the T3SS evolved first and as later co-opted as part of the flagellum. A econd is that the flagellum evolved first and s protein-export system gave rise to the T3SS. is also possible that both evolved in parallel om a common ancestor.

Most researchers think the best options are agellum-first or parallel evolution. One fact favour of the flagellum-first view is that acteria would have needed propulsion before ey needed T3SSs, which are used to attack lls that evolved later than bacteria. Also, agella are found in a more diverse range f bacterial species than T3SSs. "The most arsimonious explanation is that the T3SS rose later," says biochemist Howard Ochman the University of Arizona in Tucson.

Resolving which of these systems evolved first is just one part of the flagellum evolution problem. Another is reconstructing the sequence of events by which the individual proteins in the flagellum arose.

Again, homology is the key. By scouring a large number of bacterial genomes looking for genes that encode flagellar proteins, you can see which genes are related and the nature of their connection. It is rather like compiling a family history using DNA samples from its living members.

One thing these genome scans have underlined is that there is no such thing as *the* bacterial flagellum: although all are built to roughly the same specifications there are many variations in aspects of form and function. This is additional strong evidence that the flagellum evolved, as it is exactly what you would expect to see if today's flagella had diversified from a common ancestor. It also raises the question of why an intelligent designer would go to all the trouble of reinventing the flagellum over and over and over again (apparently reinventing the basic design twice more for good measure: flagella are also found in the other two domains of life, Archaea and Eukaryotes, but neither resemble bacterial flagella).

Despite this variation, it is clear that all bacterial flagella have much in common – again, exactly what you would expect if they shared a common ancestor. The best studied flagella are from the bacterial species *Escherichia coli* K12 and *Salmonella enterica* LT2, each of which is made up of about 40 proteins. In a recent review, Pallen and Matzke compared the flagella proteins in these two with those of 13 other bacterial species. They found that 23 of the proteins were present in all of them, albeit with slight variations (*Trends in Microbiology*, vol 13, p 143).

Deep evolutionary descent

This suggests that all of these systems evolved from an ancestral "core" flagellum, probably made up of about 20 proteins. These core proteins include elements of the motor, rod, hook, filament and flagellin protein-export machinery – all the components required for a fully functional flagellum.

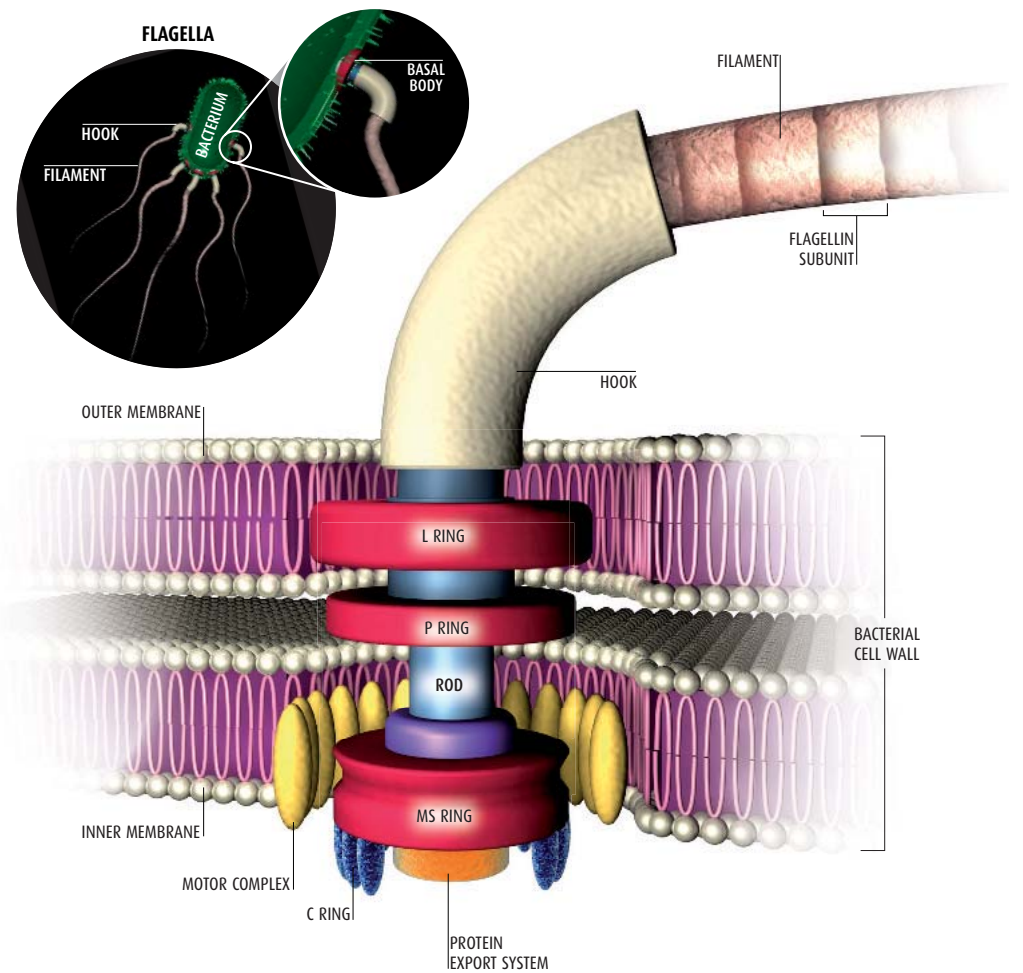
Such an ancestral core flagellum is still a complex molecular system in need of explanation, but yet again sequence homology helps. When Pallen and Matzke looked for homology among their 23 modern core-proteins, they found patterns of deeper evolutionary descent. Flagellin, for example, is homologous to the protein FlgL, which joins the hook to the filament. Furthermore, the rod and hook contains six proteins that are homologous to one another. These homologies suggest that the rod, hook and filament evolved from just two ancestral proteins – a proto-flagellin and a proto-rod/hook protein. These proto-proteins were likely to have been components of a putative flagellum that is ancestral to all flagella, dubbed the "ur-flagellum" (*Nature Reviews Microbiology*, vol 4, p 784).

The process by which a single protein or gene can give rise to whole suites of homologues is called gene duplication and divergence. Occasionally, errors in DNA replication cause entire genes (or even larger stretches of DNA) to be duplicated. This is an opportunity for some serious evolutionary innovation: the "redundant" duplicate escapes the selection pressure acting on the original gene and is free to accumulate new mutations and will sometimes, by pure chance, evolve useful new functions. The result is two homologous genes in the same genome. The process can repeat many times, creating many variations on a common theme. This is what has happened with the flagellum.

Flagellum-like proteins also turn up in non-flagellar systems, such as the enzyme complex called F1-ATPase that manufactures the chemical unit of energy ATP (*Proceedings*

NATURE'S OUTBOARD MOTOR

Despite the intricacies of the bacterial flagellum, biologists are unravelling its workings and making great headway in understanding how the nanoscale appendage evolved



he National Academy of Sciences, vol 104, 85). Flagellar homologues have also been nd in bacteria that do not have flagella. Taken together, this abundance of mology provides incontrovertible evidence t bacterial flagella are cobbled together m recycled components of other systems – l vice versa – through gene duplication and ersification. In other words, they evolved.

The million-dollar question now is this: to at extent is it possible to reconstruct the ire sequence of evolutionary events that to the flagellum? Last year Ochman and his eague Renyi Liu made the most ambitious h yet in this direction.

They compared the genomes of 41 species acterium, hunting flagellum homologues. t led them to a set of 24 core genes, similar he core set derived by Pallen and Matzke *oceedings of the National Academy of ences*, vol 104, p 7116). But when Liu and hman compared the genes with one other, they found evidence of homology ong all 24. Further analysis led them to clude that the entire flagellum evolved ough stepwise gene duplication and ergence from a handful of ancestral genes – haps even a single gene.

Liu and Ochman even inferred the order in ich these genes evolved. Duplication and ergence, they argue, initially created the proteins, which in turn gave rise to the ok proteins and, finally, the filament.

Yet biologists have been quick to point out ential problems with these conclusions. e is that Ochman assigned homology based gene sequence even when the proteins the es code for were completely different pes. "This is strong evidence against mology," says Pallen. "The prevailing dom is that [protein] structure is a better de than sequence." Another criticism is the phasis Liu and Ochman gave to gene lication as the principle source of genetic velty. While the majority of new bacterial es arise in this way, bacteria also pick up es by lateral gene transfer from unrelated cies. Previous studies suggest that lateral e transfer has played an important role in gellum evolution, says Uri Gophna, a eticist at Tel Aviv University in Israel. thermore, geneticists W. Ford Doolittle l Olga Zhaxybayeva of Dalhousie University Halifax, Canada, argued that Liu and hman probably overlooked some cumented events of lateral gene transfer (*Current Biology*, vol 17, p R510).

Whatever the outcome of this new debate, its very existence is another two-fingered salute to the opponents of evolution. "Critics of evolution argue that Darwinian evolutionary theory has become a dogma that no one dare question," says Miller. "Yet who tore into the study? Other scientists."

Thanks to all the recent work, the big picture of flagellum evolution is much clearer than it was just a few years ago, and getting better all the time. "This work is just getting started," says Matzke. Ultimately, though, it is unrealistic to hope to unravel every twist and turn of the bacterial flagellum's 3-billion-year-plus evolutionary journey. "That is impossible," says Doolittle. But he argues that the scientific imperative is not to reconstruct the entire process but simply to prove that the evolution of the flagellum is plausible using well-established natural processes.

That won't be enough for some opponents of evolution, of course. But just as it wasn't good enough for biologists to say "bacterial flagella evolved and that is that", neither is it good enough for defenders of ID to say "bacterial flagella are designed and that is that". Evolutionary biologists have put their house in order. It's time for their opponents to do the same. ●

Dan Jones is a freelance writer based in Brighton, UK