APPENDIX II

TAB A

Federal Rule of Civil Procedure 26
Disclosure of Expert Testimony

of

Michael J. Behe

Professor of Biological Sciences

Lehigh University

Bethlehem, Pennsylvania

1) My qualifications as an expert witness:

PROFESSIONAL EXPERIENCE

9/97-present	Professor of Biological Sciences, Lehigh University
1996-present	Fellow, Discovery Institute's Center for Science and Culture
6/95-8/97	Associate Professor of Biological Sciences, Lehigh University
9/85-6/95	Associate Professor of Chemistry, Lehigh University
7/89-12/89	Visiting Associate Professor of Biochemistry, Hershey Medical Center/Penn State
9/82-8/85	Assistant Professor of Chemistry, City University of New York, Queens College
11/78-9/82	Jane Coffin Childs Fund Postdoctoral Fellow at the National Institutes of Health (Gary Felsenfeld, advisor)
9/74-10/78	National Research Service Award Predoctoral Fellow at the University of Pennsylvania (Walter Englander, mentor)

EDUCATION

Ph. D.	Biochemistry, 1978. University of Pennsylvania, Philadelphia, Pennsylvania.
B.S.	Chemistry, 1974. Drexel University, Philadelphia, Pennsylvania.

PUBLICATIONS IN THE LAST TEN YEARS

Behe MJ, Snoke DW. 2004. Simulating evolution by gene duplication of protein features that require multiple amino acid residues. *Protein Sci 13*:2651-2664.

Behe, M.J. 2004. Rationalism and reform: A review of Who Rules in Science? by James Robert Brown, *First Things*, August/September, pp. 75-79.

Behe, M.J. 2004. "Irreducible Complexity: Obstacle to Darwinian Evolution." In *Debating Design: from Darwin to DNA*, Ruse, M. and Dembski, W.A., eds., Cambridge University Press, pp. 352-370.

Behe, M.J. 2003. "Design in the Details: The Origin of Biomolecular Machines." In *Darwinism, Design & Public Education*, Campbell, J.A. and Meyer, S.C. eds., Michigan State University Press, pp. 287-302.

Behe, M.J. 2003. "Darwin's Black Box." In *Faith, Freedon, and the Future: Religion in American Political Culture*." Dunn, C.W. ed., Rowman and Littlefeld, pp. 87-100.

Behe, M.J. 2003. "The Modern Intelligent Design Hypothesis: Breaking Rules." In *God and Design: The Teleological Argument and Modern Science*, Neil Manson, ed., Routledge, pp. 277-291.

Behe, M.J. 2002. The challenge of irreducible complexity. Natural History **111**, 74.

Behe, M.J. "Molecular Machines: Experimental Support for the Design Inference." In Robert T. Pennock (ed.) Intelligent Design Creationism and Its Critics: Philosophical, Theological and Scientific Perspectives. 2001. Cambridge, MA & London, England: The MIT Press. pp. 241-256.

Behe, M.J. 2001. Reply to My Critics: A Response to Reviews of Darwin's Black Box: The Biochemical Challenge to Evolution, *Biology and Philosophy* **16**, 685-709.

Behe, M.J. 2001. Abstract, Protein Society meeting. A Population Genetics Model for the Number of Generations Needed to Develop a New Protein Binding Site. *Protein Science* **10**, 65.

Behe, M.J. 2001. Review of "Extreme Functional Sensitivity to Conservative Amino Acid Changes on Enzyme Exteriors." *ChemTracts—Biochemistry and Molecular Biology* **14**, 152-158.

Behe, M.J. 2001. A review of *Finding Darwin's God, National Catholic Bioethics Quarterly* **1**, 277-278.

Behe, M.J. 2001. The Modern Intelligent Design Hypothesis: Breaking Rules, *Philosophia Christi* **3**, 165-179.

Behe, M.J. 2001. "Faith and the Structure of Life." In Science and Faith, Gerard V. Bradley and Don DeMarco, eds. St. Augustine's Press, South Bend, Indiana, Chapter 2.

Behe, M.J. 2001. "Darwin's Breakdown: Irreducible Complexity and Design at the Foundation of Life." In *Signs of Intelligence: Understanding Intelligent Design*, William A. Dembski, ed. Brazos Press, Grand Rapids, Michigan, Chapter 7.

Behe, M.J. 2001 Darwin's Black Box, Navrat Domu, Prague. (The Czech translation of Darwin's Black Box.)

Behe, M.J. 2001 Darwin's Black Box, KCC, Seoul. (The Korean translation of Darwin's Black Box.)

Behe, M.J. 2000. "Evidence of Design in Biology." In M.J. Behe et al., *Science and Evidence for Design in the Universe*, Ignatius Press, San Francisco, Chapter 3.

Behe, M.J. 2000. "Answering Scientific Criticisms of Intelligent Design." In M.J. Behe et

al., *Science and Evidence for Design in the Universe*, Ignatius Press, San Francisco, Chapter 4.

Behe, M.J. 2000. Conservatives, Darwin, and Design: An Exchange. *First Things*, no. 107, November, pp. 28-29.

Behe, M.J. 2000. Evidence of design in biology. *Providence* **5**, 23-32.

Behe, M.J. 2000. Review of "Tolerance of Arc repressor to multiple-alanine substitutions." *ChemTracts—Biochemistry and Molecular Biology* **13**, 409-414.

Behe, M.J. 2000. Self-Organization and Irreducibly Complex Systems: A Reply to Shanks and Joplin. *Philosophy of Science* **67**, 155-162.

Behe, M.J. 2000. Evolution, Religion, and the National Academy of Sciences, *Ethics and Medics* **25**, 1-3.

Behe, M.J. 1999/2000. Darwin's Hostages. *The American Spectator* **32**, December-January, pp. 32-36.

Behe, M.J. 1999. Review of Nature's Destiny by Michael J. Denton, *Persp. Biol. Med.* **43**, 151-153.

Behe, M.J. 1999. Review of God After Darwin: A Theology of Evolution by John Haught, *Metaviews*, December, www.meta-list.org.

Behe, M.J. 1999. Teach evolution without bias. *ExploreZone* webzine, September 7. explorezone.com/archives/99_09/07_opinion_kansas_behe.htm. (This was a companion piece to one written by Bruce Alberts, president of the National Academy of Sciences. explorezone.com/ archives/99_08/30_opinion_kansas.htm)

Behe, M.J. 1999. Darwin's Black Box, Central Compilation and Translation Publishers, Beijing. (The Chinese translation of Darwin's Black Box.) (http://cctpbook.com)

Behe, M.J. 1999. Teach Evolution—And Ask Hard Questions. *New York Times*, August 13, p. A21.

Behe, M.J. 1999. Comment on Denis Lamoureux's Essays. In Johnson, P.E. and Lamoureux, D.O., Darwinism Defeated? Regent College Press, Vancouver, pp. 103-108.

Behe, M.J. 1999. Darwin's Breakdown. *Touchstone* **12**, 39-43.

Behe, M.J. 1999. The God of Science: The Case for intelligent Design. (Review of Tower of Babel: The Evidence Against the New Creationism by Robert Pennock), *The Weekly Standard*, June 7, pp. 35-37.

Behe, M.J. 1999. Review of The Fifth Miracle: The Search for the Origin and Meaning of Life by Paul Davies, *First Things*, June/July, pp. 42-45.

Behe, M.J. 1998. Intelligent Design as an Alternative Explanation for the Existence of Biomolecular Machines. *Rhetoric & Public Affairs* **1**, 565-570.

Behe, M.J. 1998. Intelligent Design Theory as a Tool for Analyzing Biochemical Systems, in Mere Creation, Dembski, W., ed., InterVarsity Press, Carol Stream, Illinois, pp. 177-194.

Behe, M.J. 1998. Tracts of separated, alternating, and mixed adenosine and cytidine residues in the genomes of prokaryotes and eukaryotes. *DNA Sequence* **8**, 375-383.

Behe, M.J. 1998. Tulips and Dandelions: A review of A Case Against Accident and Self-Organization. *Books and Culture*, September/October, pp. 34-37.

Behe, M.J. 1998. Darwin's Black Box, Seidosha, Japan. (The Japanese translation of Darwin's Black Box.)

Behe, M.J. 1998. Darwin's Black Box, Ankara. (The Turkish translation of Darwin's Black Box.)

Behe, M.J. 1998. Embryos and Evolution (letter to the Editor). *Science* **281**, 348.

Behe, M.J. 1998. Dogmatic Darwinism, *Crisis*, June, pp. 32-36

Behe, M.J. 1998. Randomness or Design in Evolution, *Ethics and Medics* **23**, 3-4.

Behe, M. J. 1998. Darwinism and Design. In Brungs, R. and Postiglione, M., eds. Creation *and* Evolution, ITEST Faith/Science Press, St. Louis, Missouri, pp. 1-25.

Behe, M.J. 1998. "Darwin Controversy: A Commentary on John O. McGinnis's essay", *National Review*, March 9, pp. 53-54.

Behe, M.J. 1998. Darwin's Black Box: The Biochemical Challenge to Evolution, Touchstone- Simon & Schuster, New York (the paperback edition).

Behe, M.J. 1998. A review of Adrian Desmond's Huxley: From Devil's Disciple to Evolution's High Priest, *National Review*, February 9, 54-56.

Mahloogi, H. & Behe, M.J. 1997. Oligoadenosine tracts favor nucleosome formation. *Biochem. Biophys. Res. Comm.* **235**, 663-668.

Behe, M.J. 1997. De Zwarte Doos van Darwin, Ten Have, Netherlands. (The Dutch translation of Darwin's Black Box.)

Behe, M.J. 1997. A Caixa Preta de Darwin, Jorge Zaha Editor, Brazil. (The Portuguese translation of Darwin's Black Box.)

Behe, M.J. 1997. Dragging Darwin In: A review of Matt Ridley's The Origins of Virtue: Human Instincts and the Evolution of Cooperation, *National Review*, June 2, pp. 52-54.

Behe, M.J. 1997. The Sterility of Darwinism, *Boston Review*, Feb-March, p.24.

Behe, M.J. 1996. Darwin Under the Microscope, *New York Times*, October 29, p. A25.

Behe, M.J. 1996. Clueless at Oxford: A review of Richard Dawkins' Climbing Mount Improbable, *National Review*, Oct. 14, 1996, pp. 83-85.

Behe, M.J. 1996. Darwin's Black Box: The Biochemical Challenge to Evolution, The Free Press, New York.

Agarwal, S. & Behe, M.J. 1996. Nonconservative mutations are well-tolerated in the globular region of yeast histone H4. *J. Mol. Biol.* **255**, 401-411.

Behe, M.J. 1995. An overabundance of long oligopurine tracts occurs in the genome of simple and complex eukaryotes. *Nucleic Acids Res.* **23**, 689-695.

Puhl, H.L. & Behe, M.J. 1995. Poly[dA]·poly[dT] forms very stable nucleosomes at higher temperatures. *J. Mol. Biol.* **245**, 559-567.

2) The compensation I will receive for my study, case preparation, and testimony in this matter is $100.00 per hour. All travel expenses will be billed at cost.

3) I have not testified as an expert at trial or by deposition within the preceding four years.

4) **The following includes a complete statement of my opinions to be expressed, the reasons and basis underlying them, the data and other information considered in forming them, and exhibits.**

List of Exhibits

Exhibit 1 —	Abstracts from PubMed which contain the word "theory"
Exhibit 2 —	A drawing of the bacterial flagellum from a biochemistry textbook
Exhibit 3 —	Cover page and Table of Contents of the February 6, 1998 issue of the journal *Cell*
Exhibit 4 —	*New York Times* op-ed summarizing the argument for intelligent design
Exhibit 5 —	"Directed Panspermia" by Francis Crick and Leslie Orgel
Exhibit 6 —	*New York Times* article "Biology Text Illustrations More Fiction Than Fact" (sidebar)
Exhibit 7 —	*Philosophy of Science* journal article
Exhibit 8 —	*Biology and Philosophy* journal article
Exhibit 9 —	My chapter from *Debating Design: From Darwin to DNA* (Oxford University Press)
Exhibit 10 —	*Protein Science* journal article
Exhibit 11 —	journal article on "evolutionary potential" by Barry Hall
Exhibit 12 —	*New York Times* op-ed containing my views on teaching the problems of Darwinian theory

1 Intelligent Design as a Scientific Theory

1.1 How the word "theory" is used in the scientific community

1.1.1 "Theory" as a "well-substantiated explanation"

In both common parlance and scientific practice the word "theory" has several meanings[1]. The word "theory" is sometimes used in science to indicate, in the words of the National Academy of Sciences, "a well-substantiated explanation of some aspect of the natural world that can incorporate facts, laws, inferences and tested hypotheses."[2] It is important to remember, however, that even when used in the sense of "well-substantiated" a "theory" is not necessarily either complete or correct. For example, Newton's theory of motion and gravity was superseded by Einstein's theory of relativity.[3] Newton's theory had been "well-substantiated" for centuries for bodies of medium size moving at medium speeds (such as cannonballs flying through the air), but did not fit well with bodies moving at very fast velocities, near the speed of light. Another example is the ether theory of the propagation of light, which the eminent 19th century physicist James Clerk Maxwell confidently supported because he thought his equations for electromagnetism required it. The theory proved to be entirely wrong — physicists no longer believe there is such an entity as the "ether".[4] Thus, as the history of science richly shows, even a "well-substantiated" theory can later be shown to be incomplete or incorrect.

1.1.2 "Theory" as a word that applies to a limited area of science that may be wrong

The word "theory" is also used in scientific practice to apply to proposed explanations that are neither broad in scope nor well-substantiated, which deal with quite limited phenomena that have varying amounts of evidence in their favor, which sometimes are weak or even counterindicated by the data. For example, a search of the online biological database PubMed (which is maintained by the National Library of Medicine, a division of the United States National Institutes of Health) for the word "theory" in abstracts of scientific articles published during or after the year 2000 shows the word is used in varying ways. One article in the *Journal of Theoretical Biology* states that "The membrane pacemaker theory of aging is an extension of the oxidative stress theory of aging."[5] A paper in the *Journal of Urology* states that "This study does not support the previous theory that urethral sphincter overactivity ... leads to work hyperplasia..."[6] A paper in the journal *Breast Cancer Research* states their results "do not support the estrogen hypothesis as a unifying theory for the influence of this period."[7] The point is that the word "theory" is used to indicate a wide range of ideas, some supported by results but limited in scope, some contradicted by results.

Ten abstracts of articles found in the PubMed database are included as Exhibit 1. In each, I have circled the word "theory" in red.

1.1.3 "Theory" applied to each of rival explanations

The word "theory" is also sometimes used in science for each of the opposing explanations which seek to account for the same set of facts. For example, speaking of the continuing

lack of an accepted Darwinian account of the origin of sexual reproduction (which I discuss at more length in section 3.3) an article in the journal *Science* remarked, "Biologists have come up with a profusion of theories since first posing these questions a century ago."[8] Notice that the word "theories" is used here to indicate competing explanations. Another, more pertinent example is "complexity theory" in biology, which posits that some complex systems can self-organize. Complexity theory has been proposed as an explanation for the origin of such disparate systems as cellular metabolic pathways and the number of different cell types which an organism contains. A leading complexity theorist, Dr. Stuart Kauffman of the University of Calgary, specifically sees aspects of complexity theory as rival explanations to Darwinian theory. In his book *Origins of Order: Self-Organization and Selection in Evolution* (Oxford University Press, 1993) Kauffman wrote:

> Darwin and evolutionism stand astride us, whatever the mutterings of creation scientists. But is the view right? Better, is it adequate? I believe it is not. It is not that Darwin is wrong, but that he got hold of only part of the truth. For Darwin's answer to the sources of the order we see all around us is overwhelmingly an appeal to a single singular force: natural selection. It is this single-force view which I believe to be inadequate, for it fails to notice, fails to stress, fails to incorporate the possibility that simple and complex systems exhibit order spontaneously.[9]

Kauffman and other complexity theorists defend their view against both intelligent design theory and Darwinian theory in the recently published book *Debating Design: From Darwin to DNA* (Cambridge University Press, 2004). Thus in scientific usage different "theories" can be rival explanations for the same data.

1.1.4 "Theory" as a singular word applied to a body of multiple, distinct claims

It is critical to realize that in science the word "theory", in the singular, may be applied to a body of multiple, logically-separable claims, some of which may turn out to be true and others false, which can vary widely in the strength of the evidence supporting them and the ease with which they can be tested. In his book *One Long Argument*, in a chapter entitled "Ideological Opposition to Darwin's Five Theories", the eminent, recently-deceased Harvard biologist Ernst Mayr, one of the founders of the "neo-Darwinian synthesis" in the middle of the 20th century, stressed that what is commonly called "Darwin's Theory" actually contains at least five distinct claims.

> In both scholarly and popular literature one frequently finds references to "Darwin's theory of evolution," as though it were a unitary entity. In reality, Darwin's "theory" of evolution was a whole bundle of theories, and it is impossible to discuss Darwin's evolutionary thought constructively if one does not distinguish its various components. The current literature can easily leave one perplexed over the disagreements and outright contradictions among Darwin specialists, until one realizes that to a large extent these differences of opinion are due to a failure of some of these students of Darwin to appreciate the complexity of his paradigm.[10]

Mayr lists those five separate claims as:

(1) *Evolution as such.* This is the theory that the world is not constant nor recently created nor perpetually cycling but rather is steadily changing and that organisms are transformed in time.

(2) *Common descent.* This is the theory that every group of organisms descended from a

common ancestor and that all groups of organisms, including animals, plants, and microorganisms, ultimately go back to a single origin of life on earth.

(3) *Multiplication of species.* This theory explains the origin of the enormous organic diversity. It postulates that species multiply, either by splitting into daughter species or by "budding," that is, by the establishment of geographically isolated founder populations that evolve into new species.

(4) *Gradualism.* According to this theory, evolutionary change takes place through the gradual change of populations and not by the sudden (saltational) production of new individuals that represent a new type.

(5) *Natural selection.* According to this theory, evolutionary change comes about through the abundant production of genetic variation in every generation. The relatively few individuals who survive, owing to a particularly well-adapted combination of inheritable characters, give rise to the next generation.[11]

The strength of evidence showing that, say, "the world is not constant" (the first claim) is disputed by virtually no one. On the other hand, the strength of evidence showing that natural selection is the sole or principle mechanism of that change in all cases (the fifth claim) is much weaker — necessarily so, since one has to recognize that change has occurred before one attempts to explain what has caused the change. Mayr writes that in the years after Darwin published his theory, although most scientists accepted change in the world and common descent, few scientists thought that the mechanism of natural selection was convincing, as shown in the table below reproduced from Mayr's book.

TABLE I[12]

The composition of the evolutionary theories of various evolutionists. All these authors accepted a fifth component, that of evolution as opposed to a constant, unchanging world.

	Common descent	Multiplication of species	Gradu-alism	Natural selection
Lamarck	No	No	Yes	No
Darwin	Yes	Yes	Yes	Yes
Haeckel	Yes	?	Yes	In part
Neo-Lamarckians	Yes	Yes	Yes	No
T. H. Huxley	Yes	No	No	(No)[a]
de Vries	Yes	No	No	No
T. H. Morgan	Yes	No	(No)[a]	Unimportant

a. Parentheses indicate ambivalence or contradiction.

In terms of the various usages of the word "theory" discussed in section 1.1, the Darwinian claim that change has occurred on the earth is "well-substantiated". On the other hand, *the altogether separate Darwinian claim that natural selection drove all the major changes on earth, or built all the complex biochemical and cellular systems, is a "theory" in the much weaker sense of a "hypothesis" or "proposed explanation", with much less hard evidence in its favor.*

1.2 How intelligent design theory fits the definition of a scientific theory

1.2.1 The basic claim of intelligent design theory

Intelligent design (ID) theory proposes that the origin of some aspects of living organisms is best explained as the result of deliberate intelligent design, rather than as the result of such unintelligent processes as the self-organization proposed by complexity theory or the natural selection proposed by Darwinian theory. As such, it is crucial to keep in mind that, much like complexity theory, ***intelligent design theory focuses exclusively on the proposed mechanism of how complex biological structures arose***. In other words, ***ID focuses exclusively on the fifth claim of Darwinism (Natural selection) in Ernst Mayr's list on the preceding page***, and does not concern ***any*** of the other claims.

No matter what some people in the general public may say or hope, ID theory does not concern the age of the earth or common descent or any other claims of Darwinian theory except its proposed mechanism. Rather, ID theory exclusively focuses on the question of whether the complex features of organisms are best explained as the result of intelligent or unintelligent causes.

1.2.2 Definition used here for "scientific theory"

Is intelligent design a "scientific theory"? As shown in section 1.1 the word "theory" has multiple meanings. It is my opinion that the relevant dictionary definition for that term in the present context is "the analysis of a set of facts in their relation to one another".[13] In other words, a "theory" is a proposed explanation for a set of facts. I have argued in the philosophy of science journal *Biology and Philosophy* that a "scientific" theory is a theory which is constructed solely on the foundation of empirical facts about the natural world and logical inferences.[14] Since it is supposed to be based solely on empirical facts and logical inferences, a "scientific" theory should not tailor its claims to agree with authoritative sources, such as the scriptures of any religion or the statements of any religious or governmental leaders, nor should it tailor its claims to disagree with them. Neither should a "scientific" theory deliberately adjust its claims to agree with prevailing expectations among scientists in general of what sorts of phenomena should exist in the universe, nor should it adjust its claims to disagree with them. Rather, a scientific theory should be developed in utter disregard of any factors other than the physical, empirical evidence.

1.2.3 Why ID is a scientific theory

1.2.3.1 The appearance of design in biology

ID is a "theory" because, as discussed above in 1.2.2, it is a proposed explanation for a set of facts. It is a "scientific" theory because, as in 1.2.2, it is based entirely on empirical, observable facts about biology plus logical inferences. The fact that design is indeed based on empirical, observable facts about biology is evident in the writings of some biologists who are not proponents of intelligent design. For example, Francis Crick, the recently deceased Nobel laureate and co-discoverer of the shape of DNA, wrote that, "Biologists must constantly keep in mind that what they see was not designed, but rather evolved".[15] Apparently they must strive so hard to do this because the appearance of design in life is so strong. Brandeis University biologist David DeRosier, a scientist who does research on

the bacterial flagellum, which is a structure that many bacteria use to swim through liquid, remarked in the science journal *Cell* that "More so than other motors, the flagellum resembles a machine designed by a human."[16] (A copy of a drawing of the bacterial flagellum which appears in the university textbook *Biochemistry* by Voet and Voet is included as Exhibit 2.) In 1998 a special edition of *Cell* was devoted to the topic of "Macromolecular Machines" — that is, structures found in the cell which are literally machines made out of molecules. Articles in the journal had titles such as: "The Cell as a Collection of Protein Machines"; "Polymerases and the Replisome: Machines within Machines"; and "Mechanical Devices of the Spliceosome: Motors, Clocks, Springs, and Things". Commentary on the page containing the Table of Contents effused:

> Like the machines invented by humans to deal efficiently with the macroscopic world, protein assemblies contain highly coordinated moving parts. Reviewed in this issue of *Cell* are the protein machines that control replication, transcription, splicing ... —the machines that underlie the workings of all living things.[17]

In other words the cell — the foundation of life — contains systems that function like sophisticated, designed machinery. A copy of the cover of the issue of *Cell*, as well as a copy of its table of contents, is included as Exhibit 3.

1.2.3.1.1 Richard Dawkins on the appearance of design in biology

That biology exudes the appearance of design is insisted upon by one of the foremost proponents of Darwin's theory, Oxford University biologist Richard Dawkins. On the first page of his book *The Blind Watchmaker* Dawkins bluntly observes, "Biology is the study of complicated things that give the appearance of having been designed for a purpose."[18] As a Darwinist, Dawkins thinks that in reality natural selection accounts for the appearance of design. Nonetheless, he states strongly that the appearance of design in life is overpowering:

> Natural selection is the blind watchmaker, blind because it does not see ahead, does not plan consequences, has no purpose in view. Yet the living results of natural selection overwhelmingly impress us with the appearance of design as if by a master watchmaker, impress us with the illusion of design and planning.[19]

Dawkins writes that design can easily be recognized from the physical attributes of a system:

> We may say that a living body or organ is well designed if it has attributes that an intelligent and knowledgeable engineer might have built into it in order to achieve some sensible purpose, such as flying, swimming, seeing ...[20]

Dawkins further points out that a system does not have to be perfect to have the marks of design:

> It is not necessary to suppose that the design of a body or organ is the best that an engineer could conceive of. ... But any engineer can recognize an object that has been designed, even poorly designed, for a purpose, and he can usually work out what that purpose is just by looking at the structure of the object.[21]

1.2.3.1.2 The lack of rigorous, detailed Darwinian explanations for the appearance of design in biology

Proponents of Darwin's theory such as Richard Dawkins are convinced that natural selection can account for the strong appearance of design in biology. However, like proponents of complexity theory, proponents of intelligent design theory are skeptical of the Darwinian claim, and deny that random mutation and natural selection have been shown to account for some complex aspects of life.

Some scientists who are not proponents of intelligent design freely admit that Darwinian theory has so far been unable to give rigorous, detailed explanations for the complex biochemical machinery discovered in the cell by modern science. For example, in *The Way of the Cell*, published by Oxford University Press in 2001, while considering the claims of intelligent design proponents Colorado State University emeritus microbiologist Franklin M. Harold wrote that:

> [W]e must concede that there are presently no detailed Darwinian accounts of the evolution of any biochemical system, only a variety of wishful speculations.[22]

When reviewing my book, *Darwin's Black Box: The Biochemical Challenge to Evolution*, which argued that Darwinian explanations have not yet been given for complex biochemical systems, for the science journal *Nature*, University of Chicago evolutionary biologist Jerry Coyne wrote:

> There is no doubt that the pathways described by Behe are dauntingly complex, and their evolution will be hard to unravel.... We may forever be unable to envisage the first proto-pathways.[23]

The point is that some scientists who are not at all sympathetic to ID nonetheless admit that Darwinian theory has not given detailed, testable explanations for the Darwinian evolution by random mutation and natural selection of complex biochemical systems in the cell. *Thus the path is open to alternative explanations.*

1.2.3.2 Intelligent design reasoning

Intelligent design proponents start from ***the same*** observable facts as do other scientists such as Richard Dawkins, and notice ***the same*** strong resemblance of some biological systems, such as the molecular machinery of the cell, to systems we know to be designed. ID advocates also notice that other theories, such as complexity theory or Darwinian natural selection, have not yet given satisfactory accounts, as many scientists freely admit. Thus, ID proponents see a situation where science has discovered that life contains many systems that strongly appear to have been designed and for which no non-design explanation is in hand. ID then advances the plain, straightforward proposal that perhaps the complex structures and molecular machinery of the cell appear designed because they actually were designed by an intelligent agent. I summarized the intelligent design argument in a recent New York Times op-ed, which is included as Exhibit 4.

Although saying that parts of life were actually designed strikes many scientists and other people as unexpected and unsettling, the logic of the design argument is a simple inductive argument (inductive arguments are common and important in science[24]): Whenever we see functional systems of a certain degree and type of complexity in our everyday world, we have always found them to be designed. Now we have discovered such complex systems in the cell. Since we currently have no other explanation for the origin of such systems, we are justified in extending the induction to the cell, and concluding that there may have been real design involved in its construction.

1.2.3.2.1 An analogy between intelligent design theory and the theory of the Big Bang

The logic of the argument for intelligent design in biology is in some ways similar to that used when the Big Bang theory was first proposed in the earlier 20th century. In the 19th and early 20th century many physicists thought that the universe was eternal and unchanging. Then it was unexpectedly observed that the light from many galaxies was shifted in wavelength toward the red end of the spectrum. This meant that the galaxies were rapidly moving away from the earth and away from each other. Because this is the pattern observed after an explosion in our common experience, by using inductive reasoning the data could be interpreted to be pointing to the aftermath of a huge explosion of the universe itself. This further suggested that the universe was not eternal and unchanging, but may have had a beginning.[25]

The Big Bang theory struck some scientists as having religious overtones — perhaps the Big Bang was initiated from outside of nature, perhaps it was even a supernatural creation event — and because of this some scientists disliked the theory.[26] But the Big Bang theory was not justified by any religious text or dogma. Rather, it was justified by the strong pattern of an explosion suggested by the data. I have written in the philosophy of science journal *Biology and Philosophy* that I think intelligent design theory is similar to the Big Bang theory in the following respect.[27] Some people may worry that the theory has religious overtones, but science should ignore any extra-scientific overtones and focus exclusively on the data. Like the Big Bang, intelligent design is justified by the pattern of the physical data.

1.2.3.3 Scientific theories, intelligent design, and falsifiability

Some philosophers of science have argued that one mark of a scientific theory is that it is falsifiable.[28] On the other hand, other philosophers of science strongly disagree that falsifiability is a necessary mark of a scientific theory, or that other simple criteria reliably demarcate science from non-science.[29] Some scientists have claimed that a theory of intelligent design is unfalsifiable and therefore is not scientific.[30] On the other hand, other scientists have actually advanced scientific claims intended to falsify ID, showing that they think ID is indeed falsifiable.[31] (I have rebutted their claims that ID had been shown to be wrong, arguing instead that the science behind their claims was either mistaken or not pertinent.)[32] I have argued in an article in the philosophy of science journal *Biology and Philosophy* that intelligent design theory is falsifiable.[33] The point is that the necessity for

a "scientific" theory to be falsifiable is disputed, but that, in any event, ID is indeed falsifiable.

2 Intelligent Design is not "Creationism"

2.1 Definition of "creationism"

Is ID "creationism"? To answer that question we must first decide what "creationism" is. Some people use the word "creationism" very broadly to indicate any belief that a supernatural being has affected nature in any way. For example, in 1987 John Maddox, then the editor of the prominent science journal *Nature*, wrote an editorial in *Nature* entitled "Down with the Big Bang" that argued that the Big Bang theory was "philosophically unacceptable", partly because it gave succor to "creationists":

> Creationists and those of similar persuasions seeking support for their opinions have ample justification in the doctrine of the Big Bang. That, they might say, is when (and how) the Universe was created.[34]

Nonetheless, the common usage of the word "creationism" indicates "the literal belief in the account of creation given in the Book of Genesis".[35] Thus in popular usage a "creationist" is a person who thinks the world is relatively young, on the order of ten thousand years, and that the major categories of organisms were created ex nihilo by a supernatural being, God.

2.2 ID requires none of the presumptions of creationism

Intelligent design theory is not creationism because intelligent design theory does not require belief in any tenet of creationism. As discussed in section 1.2.1, intelligent design theory focuses ***exclusively*** on the question of whether biological systems, such as the molecular machinery found in cells, exhibit features consistent with actual intelligent design. As interesting as they may be, the topics of when such designing occurred, who did the designing, why it was done, how it was done, and so forth, are further, additional questions — beyond the basic question of whether there is design present — for which the scientific evidence is yet insufficient to draw a firm conclusion.

Interestingly, a "creationist" does not at all have to think that biology shows physical, empirical signs of design. A "creationist" can simply believe in creation based on faith in a religious text, private religious experience, or some other source, without consideration of nature at all. Although he was not a "creationist" in our modern sense of the term, the 19th century Englishman Cardinal John Henry Newman exemplified that attitude. In his *Letters and Diaries* he wrote, "I believe in design because I believe in God, not in a God because I see design."[36] In other words, strong religious faith does not require that biology show any physical evidence of design of the kind of which the Darwinian evolutionary biologist Richard Dawkins writes (see section 1.2.3.1.1), or that Brandeis University biologist David DeRosier sees in the bacterial flagellum (sections 1.2.3.1).

One can be a "creationist" in the popular sense of that word and think biology does show signs of design, but one certainly need not be. For example, as I discussed in my book *Darwin's Black Box*[37] and in an article in the philosophy of science journal *Biology and Philosophy*,[38] one is free to suppose, based on nonscientific reasons, that the designer was a natural being, such as a time traveler or space alien. (Francis Crick, the Nobel laureate, once proposed in an article entitled "Directed Panspermia" in the science journal *Icarus* that the origin of life on earth may have been the result of the deliberate seeding of life here by spores sent by intelligent space aliens.[39] A copy of Crick's article is included as Exhibit 5.) Indeed, there is at least one group (the "Raelians") who profess to believe that humans were designed by space aliens.[40]

One can also hold that the designer is some other, yet unexplained, natural entity. If one decides, on the basis of nonscientific reasons, that the designer is a supernatural being, then one can hold that the designer is a subordinate supernatural being such as the "demiurge" of Plato.[41] If one supposes that the designer is a supreme supernatural being, God, then it may be the God of any religion, such as that of Christianity, Islam, Hinduism, Native American religions, or others. Or one can simply keep an open mind, and think that the question of the identity of the designer has yet to be resolved with any firmness.

One can also think, unlike a "creationist", that a designer set up the universe to unfold in a planned way over immense times, to give rise to the complex structures science has discovered in life, without any discernible exception to natural laws. I discussed this view in a letter to the editor published in the July 2001 edition of the NCSE Reports. The newsletter is published by the National Center for Science Education, which vigorously promotes the teaching of Darwinian evolution in schools. The letter is reproduced below:

> In their article "Of Mousetraps and Men: Behe on Biochemistry" (Reports of the NCSE 20, 25-30, 2000), which has just come to my attention, Shanks and Joplin appear to mistakenly attribute to me the contention that irreducibly complex biochemical systems must have been created ex nihilo. I have never claimed that. I have no reason to think that a designer could not have used suitably modified pre-existent material. My argument in *Darwin's Black Box* is directed merely toward the conclusion of design. How the design was effected is a separate and much more difficult question to address. Although creation ex nihilo is a formal possibility, design might have been produced by some other means which involved no discontinuities in natural law, even if the designer is a supernatural being. One possibility is directed mutations. As noted by Brown University biologist Kenneth Miller in *Finding Darwin's God*, "The indeterminate nature of quantum events would allow a clever and subtle God to influence events in ways that are profound, but scientifically undetectable to us. Those events could include the appearance of mutations...." I have no reason to object to that as a route to irreducibly complex systems. I would just note further that such a process amounts to intelligent design, and that while we may be unable to discern the means by which the design is effected, the resultant design itself may be detected in the structure of the irreducibly complex system.
>
> The core claim of intelligent design theory is quite limited. It says nothing directly about how biological design was produced, who the designer was, common descent, or other such questions. Those can be argued separately. It says only that design can be empirically detected in observable features of physical systems. As an important corollary, it also predicts that mindless processes—such as natural selection or the self-organization scenarios favored by Shanks and Joplin—will not be demonstrated to be able to produce irreducible systems of the complexity found in cells.[42]

None of the possibilities discussed above requires that the picture of the universe developed by modern science be repudiated. The only assertion that intelligent design theory itself properly makes is that some aspects of biology are indeed the product of intelligent design. As discussed earlier in section 1.2.3, this assertion is actually quite consistent with the evidence of biology, although at odds with the claims of Darwinism. The point is that intelligent design theory does not require a person to adhere to any tenet usually associated with the word "creationism". A "creationist" does not have to believe in physically-discernible intelligent design, and an ID proponent does not have to believe in "creationism".

3 What are the gaps and problems with the Darwinian theory of evolution?

3.1 The problem of the origin of new, complex biological features

It is my scientific opinion that the primary problem with Darwin's theory of evolution is the lack of detailed, testable, rigorous explanations for the origin of new, complex, biological features, as explained above in section 1.2.3.1.2. This problem was recognized in the 19th century, shortly after Darwin published *The Origin of Species,* by biologists such as St. George Mivart ("What is to be brought forward [against Darwin's theory] may be summed up as follows: That "Natural Selection" is incompetent to account for the incipient stages of useful structures...")[43], and continues to be a problem today. Although vague stories and speculations are sometimes offered, rarely are such stories testable in a way that could falsify the claim that the complex feature was produced in a Darwinian fashion. For example, as stated above in section 1.2.3.1.2, in the case of the molecular machinery found in cells Franklin M. Harold wrote that: "[W]e must concede that there are presently no detailed Darwinian accounts of the evolution of any biochemical system, only a variety of wishful speculations." And Jerry Coyne wrote "There is no doubt that the pathways described by Behe are dauntingly complex, and their evolution will be hard to unravel.... We may forever be unable to envisage the first proto-pathways." *It is extremely difficult or impossible to test — or even meaningfully critique — "wishful speculations" or un-envisaged proto-pathways.*

It should be strongly emphasized that under this broad category of difficulties lies much of the structure and development of life, including: the existence of the genetic code; transcription of DNA; translation of mRNA; the structure and function of the ribosome; the structure of the cytoskeleton; nucleosome structure; the development of new protein-protein interactions; the existence of the proteosome; the existence of the endoplasmic reticulum; the existence of motility organelles such as the bacterial flagellum and the eukaryotic cilium; the development of the pathways for the construction of the cilium and flagellum; the existence of the defensive apparatus such as the immune system and blood clotting system; and much else. The existence of such unresolved difficulties for Darwinian theory at the molecular level of life makes it reasonable to wonder if a Darwinian framework is the right way to approach such questions. It also makes it reasonable to wonder if Darwinian processes explain major new features of life at higher levels, such as the level of organs and organisms.

3.2 The problem of falsification

There are other major difficulties and problems for Darwin's theory as well. One is the great difficulty in falsifying it. That is, in finding a fact of nature that would be taken by Darwinists as evidence against their theory. For example, for many years in biology textbooks students were shown drawings of vertebrate embryos that looked remarkably similar. The embryos were drawn by the 19th century embryologist Ernst Haeckel, an admirer of Darwin. The striking similarity was thought to strongly support Darwin's theory, that the different classes of vertebrates descended by natural selection from a common ancestor. The rationale for thinking so was given in the widely-used, college-level textbook *Molecular Biology of the Cell*, where president of the National Academy of Sciences Bruce Alberts and other co-authors wrote that:

> Early developmental stages of animals whose adult forms appear radically different are often surprisingly similar... Such observations are not difficult to understand.... The early cells of an embryo are like cards at the bottom of a house of cards—a great deal depends on them, and even small changes in their properties are likely to result in disaster.[44]

In other words, evolution would be expected to conserve the structure of the early embryos, inherited from a common ancestor. Natural selection would not be expected to change such a "locked-in", fundamental structure.

However, in 1997 an international team led by the British embryologist Michael Richardson showed that Haeckel's drawing were very misleading, and that there were significant differences between the embryos. A story entitled "Haeckel's embryos: fraud rediscovered" in the journal *Science* put it this way:

> Not only did Haeckel add or omit features ... but he also fudges the scale to exaggerate similarities among species, even when there were 10-fold differences in size. Haeckel further blurred differences by neglecting to name the species in most cases, as if one representative was accurate for an entire group of animals. In reality ... even closely related embryos such as those of fish vary quite a bit.[45]

Nonetheless, the discovery that the embryos looked very different from what they were pictured in textbooks did not at all cause Bruce Alberts or other scientists to question Darwinian theory. Yet if a theory is equally compatible with one result (nearly identical embryos) and its opposite (variable embryos) than how can it be rigorously tested? If Darwinian theory is compatible with false data, such as the original drawings of Haeckel, then how can we know if the theory is wrong? A story from the *New York Times*, "Biology Text Illustrations More Fiction Than Fact" (which is a sidebar in the longer story "Darwin vs. Design: Evolutionists' New Battle"), concerning the case of Haeckel's embryos is included as Exhibit 6.[46]

3.3 The "crisis" of sex

Another major, longstanding difficulty of Darwinian theory that is little appreciated by the general public is the problem of sexual reproduction. It was recognized as early as the late

19th century that Darwinian theory predicted sexual reproduction should be rare. The reason is that in sexual reproduction a given organism only gets half of its genes reproduced in any particular one of its offspring; its sexual partner contributes the other half. However, if an organism reproduced asexually, then it could contribute all of its genes to each of its offspring. The reason that, contrary to the straightforward expectation of Darwinian theory, sex is common has been debated for over a hundred years. There are so many competing ideas about why sexual reproduction should occur that A. S. Kondrashov, in an article in the *Journal of Heredity* in 1993, found it necessary to try to classify all the hypotheses into groups to keep better track of them!

> After more than a century of debate, the major factors of the evolution of reproduction are still obscure. During the past 25 years, hypotheses have become so numerous and diverse that their classification is a necessity. The time is probably ripe for this: no fundamentally new hypothesis has appeared in the last 5 years, and I would be surprised—and delighted—if some important idea remains unpublished.[47]

The debate continues, with various theories offered by various scientists, but with no accepted resolution of the problem. For example in 1998 the journal *Science* devoted a special section of one issue to the evolution of sex, with articles such as "Why Sex? Putting Theory to the Test" and "Why Sex and Recombination?" One introductory article remarked,

> Yet how sex began and why it thrived remain a mystery. ... Why did sex overtake asexual reproduction some billion or more years ago, and why does it continue to upstage asexuals? ... Biologists have come up with a profusion of theories since first posing these questions a century ago. ... Sex is a paradox in part because if nature puts a premium on genetic fidelity, asexual reproduction should come out ahead. It transmits, intact, a single parental genome that is by definition successful. Sexual reproduction, on the other hand, involves extensive makeovers of the genome. The production of gametes requires recombination, in which the two copies of each chromosome pair up and exchange DNA. Fertilization, in which genes from different parents fuse, creates yet more genetic combinations. All this shuffling is more likely to break up combinations of good genes than to create them—yet nature keeps reshuffling the deck.[48]

(Notice again that the word "theories" is used above to mean competing, tentative ideas — *not* "well-substantiated explanations".) In his 1975 book *Sex and Evolution* the prominent evolutionary biologist George C. Williams wrote:

> This book is written from a conviction that the prevalence of sexual reproduction in higher plants and animals is inconsistent with current evolutionary theory ... there is a kind of crisis at hand in evolutionary biology ...[49]

While discussing Williams remarks on sex, in 2004 Richard Dawkins wrote:

> Maynard Smith and Hamilton said similar things. It is to resolve this crisis that all three Darwinian heroes, along with others of the rising generation, laboured. I shall not attempt an account of their efforts, and certainly I have no rival solution to offer myself.[50]

Yet if Darwinian theory has no good account for sexual reproduction, then the very heart of the theory of evolution — the differential reproduction of organisms — is floating in midair.

A theory of evolution that predicts most species should be asexual is like a theory of gravity

that predicts that most objects will fall up. Either conundrum should make a reasonable person wonder if the proposed theory might be missing some large piece of the puzzle. Furthermore, if Darwinists have tolerated such a large, acknowledged difficulty in the center of their theory for over a hundred years, then one might wonder if they are unreasonably attached to it. Students should be aware of this.

It should be emphasized that the problem with sex is not simply the problem of how the intricate machinery of meiosis, recombination, and other sexual processes could develop in the gradual, undirected manner that Darwinian theory envisions. The theory encounters that difficulty in the explanation of *all* complex biological systems. Rather, the difficulty in explaining sex is the question of why, on Darwinian principles, it should exist at all, even if there were a gradual way to develop it. On straightforward Darwinian principles, sex appears detrimental to the interests of the organism.

4 The origin of life

A major problem for Darwin's theory is the unsolved mystery of the origin of life. Darwin's theory doesn't itself deal with the origin of life; rather, it presupposes that life was present on earth in a form that would be able to undergo evolution by random mutation and natural selection. Even in principle, Darwin's theory cannot account for the origin of life, because the theory concerns the reproduction of already-living organisms. Thus before the beginning of life the earth was missing a prerequisite ingredient for Darwinian evolution to occur.

The problem that the origin of life poses for Darwin's theory is the following. If the beginning of life required something extra, something in addition to the unintelligent operation of natural processes that Darwin's theory invokes, then it would be fair for a curious inquirer to wonder if those other processes ended with the beginning of life, or if they continued to operate throughout the history of life. The acknowledgment of difficulties with the origin of life would likely make it more urgent that Darwinists actually demonstrate that random mutation and natural selection can do what they claim, rather than relying on the presumption that they can.

The importance of the origin of life to Darwin's theory is seen in the fact that high school biology textbooks include a section dealing with the topic. This often leads into discussion of the first cells and then into Darwinian evolution, so that it can appear to the student to be a seamless process. Sometimes a text gives students little warning (or the warning is not emphasized so that students easily overlook it) that the origin of life is an unsolved problem that has remained a mystery despite fifty years of active scientific investigation.

It is easy to find comments by knowledgeable scientists that attest to the lack of progress in the field of origin of life studies. For example, in a recent interview with the PBS science program Nova, the distinguished paleontologist Andrew Knoll, who is the Fisher Professor of Natural History at Harvard University and a leading expert on early life on earth, remarked: "The short answer is we don't really know how life originated on this planet." In response to the interviewer's question, "Will we ever solve the problem [of the origin of

life]?", Knoll responded, "I imagine my grandchildren will still be sitting around saying that it's a great mystery..."[51]

The widely-used, university-level textbook "Biochemistry" by Voet and Voet introduces a section on the origin of life with the following remarks:

> In the remainder of this section, we describe the most widely favored scenario for the origin of life. *Keep in mind, however, that there are valid scientific objections to this scenario as well as to the several others that have been seriously entertained, so that we are far from certain as to how life arose.*[52] [italics in the original]

In other words, like with the problem of sex, there is a profusion of theories, none of which is satisfactory.

In its booklet *Science and Creationism* the National Academy of Sciences called the problem of the origin of life "seemingly intractable." Nonetheless, the National Academy writes:

> For those who are studying the origin of life, the question is no longer whether life could have originated by chemical processes involving nonbiological components. The question instead has become which of many pathways might have been followed to produce the first cells.[53]

This statement subtly shifts the spotlight away from the actual scientific *problem* of the origin of life and onto the subjective *attitudes* of workers in the field. In effect it encourages teachers (to whom the booklet is addressed) to inculcate in their students the presumption that the problem of the origin of life must be addressed in the framework of unintelligent "chemical processes involving nonbiological components". This despite the fact that such a framework has been unsuccessful over the course of half of a century. Students are not encouraged to think, or given any reason to think, that such a framework might possibly be wrong. Students are encouraged to follow in the footsteps of the failures of the past fifty years.

One can also discern in another, quite remarkable passage in *Science and Creationism* the desire to inculcate into students the presumption that "chemical processes involving nonbiological components" simply must be responsible for the origin of life. There the National Academy of Sciences speaks glowingly of a particular *theological* stance, called "theistic evolution", as if the Academy — an organization chartered by the federal government — were expert on religious matters.

> Many religious persons, including many scientists, hold that God created the universe and the various processes driving physical and biological evolution and that these processes then resulted in the creation of galaxies, our solar system, and life on Earth. This belief, which sometimes is termed "theistic evolution," is not in disagreement with scientific explanations of evolution. Indeed, it reflects the remarkable and inspiring character of the physical universe revealed by cosmology, paleontology, molecular biology, and many other scientific disciplines.[54]

A teacher reading that section could easily pick up the Academy's apparent attitude toward religion: theistic evolution, where laws operate continuously — good; religious ideas

requiring interruption of natural laws — bad. A teacher influenced by the Academy's booklet might possibly attempt to influence the religious beliefs of students in the same way.

5 The scientific controversy over intelligent design

My book, *Darwin's Black Box: The Biochemical Challenge to Evolution*, presented the argument that Darwinian processes are unlikely explanations for the biochemical complexity that modern science has found in the cell. Instead, the book argued, a more likely explanation is deliberate intelligent design. Since shortly after the book was published in 1996 scientists who support Darwin's theory of evolution by random mutation and natural selection have offered arguments to try to refute the contention of intelligent design. In turn I have offered counterarguments to show why the Darwinian arguments fail. I think it is safe to say that so far neither side has been persuaded by the other's arguments. Below I will list some of the articles that have been published on both sides.

1) In a symposium published by *Boston Review* in its Feb/March 1997 issue, a dozen academics traded essays arguing the relative merits of intelligent design, Darwinism, and other ideas for explaining the development of life. The essays are available on line.[55] Contributing authors include:[56]

> Michael Behe, professor of biological sciences, Lehigh University
> Phillip E. Johnson, professor of law, University of California, Berkeley,
> David Berlinski, a writer and mathematician,
> Jerry A. Coyne, professor of evolutionary biology, University of Chicago
> Russell F. Doolittle, professor of biochemistry, University of California, San Diego
> Douglas J. Futuyma, professor of evolutionary biology, State University of New York, Stony Brook
> Robert DiSilvestro, professor of nutritional biochemistry, Ohio State University
> Michael Ruse, professor of philosophy, University of Guelph
> James A. Shapiro, professor of biochemistry, University of Chicago
> Daniel Dennett, professor of philosophy, Tufts University
> H. Allen Orr, professor of evolutionary biology, University of Rochester

2) In 1999 Kenneth Miller, a professor of biology at Brown University, published *Finding Darwin's God*[57] (HarperCollins). In the book he defended Darwinian evolution. One of the chapters of the book, Chapter 5 "God the Mechanic", criticizes my argument in *Darwin's Black Box* for intelligent design, and offers scientific arguments against it.

3) In 2001 Robert T. Pennock, professor of philosophy at Michigan State University, edited a book entitled *Intelligent Design Creationism and Its Critics*[58], which was published by MIT Press. The book collected dozens of essays. Each essay by a proponent of intelligent design was subjected to several critical essays by opponents. Proponents usually were not given space to respond to criticisms. Several of the essays concerned the scientific claims of intelligent design.

4) In 1999 Shanks and Joplin published a review of *Darwin's Black Box* in the journal *Philosophy of Science*[59]. They argued that the idea of irreducible complexity (which I discussed in the book) was incorrect, and that complex biochemical systems could develop through a means they called "redundant complexity."

5) In 2000 I replied to Shanks and Joplin's criticisms in an article also published in *Philosophy of Science*[60]. I argued that their criticisms of irreducible complexity were themselves flawed. A copy of the article is included as Exhibit 7.

6) In 2001 I published an article entitled "Reply to my critics: A response to reviews of *Darwin's Black Box: the biochemical challenge to evolution*" in the journal *Biology and Philosophy*[61]. The article responds to many of the criticisms in books and articles listed above, including those of scientists Kenneth Miller, Russell Doolittle, H. Allen Orr, Jerry Coyne. A copy of the article is included as Exhibit 8.

7) In 2000 Thornhill and Ussery published an article in the *Journal of Theoretical Biology* arguing against the concept of irreducible complexity.[62]

8) In 2000 a conference organized by William Dembski was held at Baylor University. It was entitled "the Nature of Nature", and brought together intelligent design proponents and opponents, including many scientists, mathematicians, and philosophers, as well as several Nobel laureates and members of the National Academy of Sciences.[63]

9) In 2003 Lenski et al published an article in the journal *Nature* entitled "The evolutionary origin of complex features".[64] The article concerned the ability of a computer program to develop the ability to perform new functions. It was intended to be a model for how biological features might develop in organisms and possibly get around the difficulty of irreducible complexity.

10) In 2004 Young and Edis edited a volume entitled *Why Intelligent Design Fails: A Scientific Critique of the New Creationism*[65] (Rutgers University Press) which, as its title suggests, offered scientific arguments against intelligent design.

11) In 2004 Dembski and Ruse edited *Debating Design: From Darwin to DNA*[66] (Cambridge University Press), which included contributions from proponents and opponents of intelligent design, as well as contributions from complexity theorists, who disagree with some of the claims of both intelligent design and Darwinian theory, as well as theistic evolutionists. A copy of my chapter in the volume, which responds to criticisms of irreducible complexity and intelligent design, is included as Exhibit 9.

12) In 2004 Behe and Snoke published an article in the journal *Protein Science* entitled "Simulating evolution by gene duplication of protein features that require multiple amino acid residues"[67]. The article attempts to show the difficulty of evolving a new protein feature by random mutation and natural selection when multiple changes are needed for a new function. A copy of the article is included as Exhibit 10.

One point of this compilation is to show that some Darwinian scientists have responded to

intelligent design with scientific arguments that attempt to falsify it — to show ID to be incorrect. Although I think their scientific arguments are incorrect, the fact that scientists offer such arguments demonstrates that intelligent design is amenable to scientific investigation and criticism. It is therefore a scientific claim.

6 The utility of design as a scientific theory

6.1 A scientific theory does not need to be utilitarian

A scientific theory does not have to have an immediately-obvious utilitarian application to be correct. One of the purposes of science is simply to describe nature accurately. If a theory does that, or at least is better than competing theories at describing nature, then it is fulfilling an important purpose of a scientific theory. In the view of proponents of intelligent design theory, ID more accurately describes what we observe in nature than do competing theories. As explained earlier, some scientists admit that Darwinian theory does not have detailed, rigorous explanations for some of the complex systems that have been discovered in the cell. And some scientists such as Richard Dawkins readily admit that aspects of biology strongly appear to have been designed. Thus it is reasonable to conclude, as ID proponents do, that intelligent design is a more accurate description of aspects of nature than other theories.

6.2 Where is the border between design and unintelligent natural processes?

One use of a theory of intelligent design might be to prod scientists to look for limits to the efficacy of the Darwinian processes of random mutation and natural selection, which might lead to describing nature more accurately. If one has reason to believe, as proponents of ID do, that not all of biology can be explained by natural selection, then one can begin to look for the borders of Darwinian processes. A question such as, what are the limits of Darwinian processes in explaining life on earth?, does not easily occur to a Darwinist, who takes it as an assumption that Darwinian processes explain most complex biological systems. A small step toward addressing such questions was recently taken by myself and David Snoke, a professor of physics at the University of Pittsburgh. We recently published an article in the journal *Protein Science* entitled "Simulating evolution by gene duplication of protein features that require multiple amino acid residues". The article attempts to show the difficulty of evolving a new protein feature by random mutation and natural selection when multiple changes are needed for a new function. A copy of the article is included as Exhibit 10.

6.3 Health implications for a limit to Darwinian evolution

Although a scientific theory does not have to have practical implications in order to be correct, a theory of intelligent design nonetheless might be important in understanding such things as the limits of the development of antibiotic resistance, which of course could have great importance for public health. Here is why. It is well known that bacteria and other microorganisms can develop resistance to some antibiotic drugs, and that this is a

formidable public health threat. It is less well known that some bacteria have been unable to develop resistance to some drugs. The reason is that some antibiotic resistance genes do not have the "evolutionary potential" to develop resistance. This is exemplified in some recent articles from the laboratory of Professor Barry G. Hall at the University of Rochester. Although he is not an advocate of intelligent design, Professor Hall nonetheless does not automatically assume that Darwinian processes can do everything. For example, he writes in a paper in the journal *Antimicrobial Agents and Chemotherapy*: "Instead of assuming that metallo-ß-lactamases will evolve rapidly, it would be highly desirable to accurately predict their evolution in response to carbapenem selection." Using a method he developed, he predicts that bacteria will be unable to develop resistance to an antibiotic called imipinem. He writes in the abstract of his article: "The results predict, with >99.9% confidence, that even under intense selection the IMP-1 ß-lactamase will not evolve to confer increased resistance to imipenem." A copy of Hall's article is included as Exhibit 11.

If intelligent design theory is correct, and there are limits to what unaided nature can do, then if we understand in more detail what those limits are, we may be able to design more effective antibiotics, ones to which bacteria will be unable to develop resistance.

I should emphasize that this is just one possible application of intelligent design theory, which may or may not be easily successful. The overarching point, however, is that approaching the study of biology from an intelligent design perspective may afford insights that do not come easily to workers who have a Darwinian perspective.

7 The age-appropriateness of discussing difficulties with Darwin's theory in 9th grade

It is my opinion that discussing difficulties with Darwin's theory is quite appropriate for students in 9th grade, or in whichever grade a high school biology course is given. The reason is that the problems with Darwin's theory are no more difficult to understand than the advantages of Darwin's theory. Indeed, the difficulties are often just the reverse of the advantages. Even molecular difficulties with the theory are appropriate for high school students. In many high school biology texts, students are taught of the underlying chemical, biochemical, genetic, and cellular bases of life. If the students can understand such topics, then they can understand difficulties that arise for Darwinian theory at this level. My opinions on this topic are summarized in a *New York Times* op-ed piece "Teach Evolution—And Ask Hard Questions", which is included as Exhibit 12.

Signed: Michael J Behe Date: 3/24/05

Endnotes

1. Merriam-Webster,I. 1986. *Webster's ninth new collegiate dictionary*. Merriam-Webster: Springfield, Mass., U.S.A.

2. National Academy of Sciences. 1999. *Science and creationism: a view from the National Academy of Sciences*, 2nd ed. ed. National Academy Press: Washington, DC, p. 2.

3. "relativistic mechanics." Encyclopædia Britannica from Encyclopædia Britannica Online. <http://search.eb.com/eb/article?tocId=9110309> [Accessed March 15, 2005].

4. "ether." Encyclopædia Britannica from Encyclopædia Britannica Online. <http://search.eb.com/eb/article?tocId=9033121> [Accessed March 15, 2005].

5. Hulbert,A.J. 2005. On the importance of fatty acid composition of membranes for aging. *J. Theor. Biol.* **234**:277-288.

6. Andrich,D.E., Rickards,D., Landon,D.N., Fowler,C.J., and Mundy,A.R. 2005. Structural assessment of the urethral sphincter in women with urinary retention. *J. Urol.* **173**:1246-1251.

7. Hodgson,M.E., Newman,B., and Millikan,R.C. 2004. Birthweight, parental age, birth order and breast cancer risk in African-American and white women: a population-based case-control study. *Breast Cancer Res.* **6**:R656-R667.

8. Wuethrich,B. 1998. Why sex? Putting theory to the test. *Science* **281**:1980-1982.

9. Kauffman,S.A. 1993. *The origins of order: self-organization and selection in evolution*. Oxford University Press: New York, p. xiii.

10. Mayr,E. 1991. *One long argument: Charles Darwin and the genesis of modern evolutionary thought*. Harvard University Press: Cambridge, Mass., p. 35.

11. Mayr, 1991, pp. 36-37.

12. Mayr, 1991, p. 37.

13. Merriam-Webster,I. 1986. *Webster's ninth new collegiate dictionary*. Merriam-Webster: Springfield, Mass., U.S.A.

14. Behe,M.J. 2001. Reply to my critics: A response to reviews of *Darwin's Black Box: the biochemical challenge to evolution*. *Biology and Philosophy* **16**:685-709.

15. Crick,F. 1988. *What mad pursuit: a personal view of scientific discovery*. Basic Books: New York, pp. 138-139.

16. DeRosier,D.J. 1998. The turn of the screw: the bacterial flagellar motor. *Cell* **93**:17-20.

17. *Cell*, "Review Issue on Macromolecular Machines", February 6, 1998,

18. Dawkins,R. 1986. *The blind watchmaker*. Norton: New York, p. 1.

19. Dawkins, 1986, p. 21.

20. Dawkins, 1986, p. 21.

21. Dawkins, 1986, p. 21.

22. Harold,F.M. 2001. *The Way of the Cell*. Oxford University Press: Oxford, p. 205.

23. Coyne,J.A. 1996. God in the details. *Nature* **383**:227-228.

24. "logic." Britannica Student Encyclopedia from Encyclopædia Britannica Online. <http://search.eb.com/ebi/article?tocId=204014> [Accessed March 18, 2005].

25. "Lemaître, Georges." Encyclopædia Britannica from Encyclopædia Britannica Online. <http://search.eb.com/eb/article?tocId=9047718> [Accessed March 16, 2005].

26. Maddox,J. 1989. Down with the Big Bang. *Nature* **340**:425.

27. Behe,M.J. 2001. Reply to my critics: A response to reviews of *Darwin's Black Box: the biochemical challenge to evolution*. *Biology and Philosophy* **16**:685-709.

28. Thornton, Stephen, "Karl Popper", The Stanford Encyclopedia of Philosophy (Winter 2002 Edition), Edward N. Zalta (ed.), URL = <http://plato.stanford.edu/archives/win2002/entries/popper/>; Ruse M., ed., 1996, "But is it Science?: The Philosophical Question in the Creation/Evolution Controversy," Prometheus Books: Amherst NY.

29. Laudan L., 1996, "Science at the Bar—Causes for Concern," in Ruse, 1996.

30. see my discussion of this in Behe, 2001, pp. 696-697.

31. Shanks,N. and Joplin,K.H. 1999. Redundant complexity: A critical analysis of intelligent design in biochemistry. *Phil. Sci.* **66**:268-282; Doolittle,R.F. A delicate balance. Boston Review [Feb/March], 28-29. 1997; Miller,K.R. 1999. *Finding Darwin's God: a scientist's search for common ground between God and evolution*. Cliff Street Books: New York.

32. Behe,M.J. 2000. Self-organization and irreducibly complex systems: A reply to Shanks and Joplin. *Phil. Sci.* **67**:155-162; Behe,M.J. 2001. Reply to my critics: A response to reviews of *Darwin's Black Box: the biochemical challenge to evolution*.

Biology and Philosophy **16**:685-709; Behe,M.J. 2004. Irreducible Complexity: Obstacle to Darwinian Evolution. In *Debating Design: from Darwin to DNA*. (eds. WA Dembski and M Ruse), pp 352-370. Cambridge University Press: Cambridge.

33. Behe, 2001.

34. Maddox,J. 1989. Down with the Big Bang. *Nature* **340**:425.

35. creationism." TheFreeDictionary.com. <http://www.thefreedictionary.com/creationism> [Accessed March 15, 2005].

36. www.newmanreader.org/biography/ward/volume2/chapter28.html

37. Behe,M.J. 1996. *Darwin's black box: the biochemical challenge to evolution*. The Free Press: New York, Ch. 11.

38. Behe, 2001.

39. Crick,F. and Orgel,L.E. 1973. Directed panspermia. *Icarus* **19**:341-346.

40. www.rael.org

41. "Demiurge." Encyclopædia Britannica from Encyclopædia Britannica Online. <http://search.eb.com/eb/article?tocId=9029894> [Accessed March 16, 2005].

42. Behe,M.J. 2001. Letter to the editor, "Behe replies to Shanks and Joplin",*NCSE Reports* **21**: 4.

43. Mivart,S. 1871. *On the genesis of species*, 2nd ed ed. Macmillan: London, p. 21.

44. Alberts,B. 1994. *Molecular biology of the cell*, 3rd ed. ed. Garland Pub.: New York.

45. Pennisi,E. 1997. Haeckel's embryos: fraud rediscovered. *Science* **277**:1435.

46. Glanz,J. Biology Text Illustrations More Fiction Than Fact. New York Times , A18. 4-8-2001.

47. Kondrashov,A.S. 1993. Classification of hypotheses on the advantage of amphimixis. *J. Hered.* **84**:372-387.

48. Wuethrich,B. 1998. Why sex? Putting theory to the test. *Science* **281**:1980-1982.

49. Quoted in Dawkins,R. 2004. *The ancestor's tale: a pilgrimage to the dawn of evolution*. Houghton Mifflin: Boston, p. 424.

50. Dawkins, 2004, p. 424.

51. www.pbs.org/wgbh/nova/origins/knoll.html

52. Voet,D. and Voet,J.G. 2004. *Biochemistry*, 3rd ed ed. J. Wiley & Sons: New York, p. 31.

53. National Academy of Sciences, 1999, p. 6.

54. National Academy of Sciences 1999, p. 7.

55. www.bostonreview.net/evolution.html

56. The institutional affiliations are those at the time the essays were published. Several authors have since switched jobs.

57. Miller, 1999.

58. Pennock,R.T. 2001. *Intelligent design creationism and its critics: philosophical, theological, and scientific perspectives*. MIT Press: Cambridge, Mass.

59. Shanks,N. and Joplin,K.H. 1999. Redundant complexity: A critical analysis of intelligent design in biochemistry. *Phil. Sci.* **66**:268-282.

60. Behe, 2000.

61. Behe, 2001.

62. Thornhill,R.H. and Ussery,D.W. 2000. A classification of possible routes of Darwinian evolution. *J. Theor. Biol.* **203**:111-116.

63. www.designinference.com/documents/2000.04.nature_of_nature.htm

64. Lenski,R.E., Ofria,C., Pennock,R.T., and Adami,C. 2003. The evolutionary origin of complex features. *Nature* **423**:139-144.

65. Young,M. and Edis,T. 2004. *Why intelligent design fails: a scientific critique of the new creationism*. Rutgers University Press: New Brunswick, N.J.

66. Dembski,W.A. and Ruse,M. 2004. *Debating design: from Darwin to DNA*. Cambridge University Press: Cambridge, U.K.

67. Behe,M.J. and Snoke,D.W. 2004. Simulating evolution by gene duplication of protein features that require multiple amino acid residues. *Protein Sci.* **13**:2651-2664.

Exhibit 1

Abstracts from PubMed which contain the word "theory"

Field: **Title/Abstract**, Limits: **Publication Date from 2000, English**

1: Breast Cancer Res. 2004;6(6):R656-67. Epub 2004 Sep 22.

Birthweight, parental age, birth order and breast cancer risk in African-American and white women: a population-based case-control study.

Hodgson ME, Newman B, Millikan RC.

Department of Epidemiology, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA.
ehodgson@email.unc.edu

INTRODUCTION: Much recent work has focused on hypotheses that very early life exposures influence adult cancer risk. For breast cancer it has been hypothesized that high in utero estrogen exposure may increase risk. METHODS: We used data from the Carolina Breast Cancer Study, a population-based case-control study of incident breast cancer in North Carolina, to examine associations for three possible surrogates of high prenatal estrogen exposure: weight at birth, maternal age, and birth order. We also examined paternal age. Birthweight analyses were conducted for white and African-American women born in North Carolina on or after 1949 (196 cases, 167 controls). Maternal age was analyzed for US born participants younger than 49 years of age (280 cases, 236 controls). RESULTS: There was a weak inverse association between birthweight in the highest tertile and breast cancer overall (odds ratio [OR] 0.7, 95% confidence interval [CI] 0.4-1.2), although associations differed by race (OR 0.5, 95% CI 0.2-1.0, and OR 1.0, 95% CI 0.5-2.1 for African-American and white women, respectively). For maternal age there was an approximately threefold increase in risk in women whose mothers were older than 22 years of age, relative to 19-22 years of age, when the women were born. After adjustment for maternal age, older paternal age increased risk in the oldest and youngest age categories (relative to 23-27 years of age at the woman's birth: OR 1.6, 95% CI 0.8-3.1 for age 15-22 years; OR 1.2, 95% CI 0.7-2.2 for age 28-34 years; and OR 1.5, 95% CI 0.7-3.2 for age 35-56 years). There was no association with older paternal age for white women alone. After adjustment for maternal age (265 cases, 224 controls), a birth order of fifth or higher relative to first had an inverse association with breast cancer for women younger than 49 years old (OR 0.6, 95% CI 0.3-1.3). CONCLUSION: Although the CIs are wide, these results lend support to

the possibility that the prenatal period is important for subsequent breast cancer risk, but they do not support the estrogen hypothesis as a unifying theory for the influence of this period.

PMID: 15535848 [PubMed - in process]

1: J Urol. 2005 Apr;173(4):1246-51.

Structural assessment of the urethral sphincter in women with urinary retention.

Andrich DE, Rickards D, Landon DN, Fowler CJ, Mundy AR.

From the Institute of Urology, the Institute of Neurology (DNL, CJF), and The National Hospital for Neurology and Neurosurgery (CJF), Queen Square, London, United Kingdom.

PURPOSE:: The pathophysiology of urinary retention in women is generally unknown but a subgroup of women with urinary retention have been diagnosed as having so-called primary disorder of sphincter relaxation on the basis of an abnormal urethral sphincter electromyogram. It was suggested this sphincter overactivity could lead to work hypertrophy of the urethral rhabdosphincter and in this study we looked for any evidence of such muscle fiber hypertrophy. MATERIALS AND METHODS:: In 9 women 18 to 45 years old (mean age 31.6) with urinary retention and overactive urethral sphincter electromyogram, light and electron microscopy were used to examine core needle biopsies of the urethral rhabdosphincter taken under transvaginal ultrasound control. Of the 9 patients only 5 biopsies processed for light microscopy and 4 processed for electron microscopy contained striated urethral muscle fibers. The results of these biopsies were compared to the morphology of a control specimen from a postmenopausal woman without a history of urinary retention. RESULTS:: On light microscopy the urethral rhabdosphincter fiber diameter did not differ among patients (mean average 7.6 mum), was less than that reported in the literature (15 to 20), but did not differ from that of the control (mean 9.9). In all patients electron microscopy showed excessive peripheral sarcoplasm with lipid and glycogen deposition, and sarcoplasmic accumulation of normal mitochondria. These ultrastructural abnormalities were not seen in the control. CONCLUSIONS:: To our knowledge this is the first morphological description of the urethral rhabdosphincter in a subgroup of women with urinary retention. Mean rhabdosphincter fiber diameter was approximately the same in patients and controls. This study does not support the previous theory that urethral sphincter overactivity in a subgroup of women with urinary retention leads to work hyperplasia of urethral rhabdosphincter fibers. An alternative hypothesis is suggested.

1: J Theor Biol. 2005 May 21;234(2):277-88. Epub 2005 Jan 24.

On the importance of fatty acid composition of membranes for aging.

Hulbert AJ.

Metabolic Research Centre, University of Wollongong, Wollongong, NSW 2522, Australia; School of Biological Sciences, University of Wollongong, Wollongong, NSW 2522, Australia.

The membrane pacemaker theory of aging is an extension of the oxidative stress theory of aging. It emphasises variation in the fatty acid composition of membranes as an important influence on lipid peroxidation and consequently on the rate of aging and determination of lifespan. The products of lipid peroxidation are reactive molecules and thus potent damagers of other cellular molecules. It is suggested that the feedback effects of these peroxidation products on the oxidative stress experienced by cells is an important part of the aging process. The large variation in the chemical susceptibility of individual fatty acids to peroxidation coupled with the known differences in membrane composition between species can explain the different lifespans of species, especially the difference between mammals and birds as well as the body-size-related variation in lifespan within mammals and birds. Lifespan extension by calorie-restriction can also be explained by changes in membrane fatty acid composition which result in membranes more resistant to peroxidation. It is suggested that lifespan extension by reduced insulin/IGF signalling may also be mediated by changes in membrane fatty acid composition.

PMID: 15757684 [PubMed - in process]

1: J Antimicrob Chemother. 2005 Mar 10; [Epub ahead of print] Related Articles, Links
Full text article at jac.oupjournals.org
LEHIGH SFX LINK

Antitubercular inhaled therapy: opportunities, progress and challenges.

Pandey R, Khuller GK.

Department of Biochemistry, Postgraduate Institute of Medical Education & Research, Chandigarh--160 012, India.

Pulmonary tuberculosis remains the commonest form of this disease and the development of methods for delivering antitubercular drugs directly to the lungs via the respiratory route is a rational therapeutic goal. The obvious advantages of inhaled therapy include direct drug delivery to the diseased organ, targeting to alveolar macrophages harbouring the mycobacteria, reduced risk of systemic toxicity and improved patient compliance. Research efforts have demonstrated the feasibility of various drug delivery systems employing liposomes, polymeric microparticles and nanoparticles to serve as inhalable antitubercular drug carriers. In particular, nanoparticles have emerged as a remarkably useful tool for this purpose. While some researchers have preferred dry powder inhalers, others have emphasized nebulization. Beginning with the respiratory delivery of a single antitubercular drug, it is now possible to deliver multiple drugs simultaneously with a greater therapeutic efficacy. More experience and expertise have been observed with synthetic polymers, nevertheless, the possibility of using natural polymers for inhaled therapy has yet to be explored. Several key issues such as patient education, cost of treatment, stability and large scale production of drug formulations, etc. need to be addressed before antitubercular inhaled therapy finds its way from theory to clinical reality.

PMID: 15761077 [PubMed - as supplied by publisher]

Field: **Title/Abstract,** Limits: **Publication Date from 2000, English**

1: Philos Trans R Soc Lond B Biol Sci. 2001 Jul 29;356(1411):1057-68.

Early effects of climate change: do they include changes in vector-borne disease?

Kovats RS, Campbell-Lendrum DH, McMichael AJ, Woodward A, Cox JS.

Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK.
sari.kovats@lshtm.ac.uk

The world's climate appears now to be changing at an unprecedented rate. Shifts in the distribution and behaviour of insect and bird species indicate that biological systems are already responding to this change. It is well established that climate is an important determinant of the spatial and temporal distribution of vectors and pathogens. In theory, a change in climate would be expected to cause changes in the geographical range, seasonality (intra-annual variability), and in the incidence rate (with or without changes in geographical or seasonal patterns). The detection and then attribution of such changes to climate change is an emerging task for scientists. We discuss the evidence required to attribute changes in disease and vectors to the early effects of anthropogenic climate change. The literature to date indicates that there is a lack of strong evidence of the impact of climate change on vector-borne diseases (i.e. malaria, dengue, leishmaniasis, tick-borne diseases). New approaches to monitoring, such as frequent and long-term sampling along transects to monitor the full latitudinal and altitudinal range of specific vector species, are necessary in order to provide convincing direct evidence of climate change effects. There is a need to reassess the appropriate levels of evidence, including dealing with the uncertainties attached to detecting the health impacts of global change.

Publication Types:
- Review
- Review, Tutorial

PMID: 11516383 [PubMed - indexed for MEDLINE]

1: Expert Rev Vaccines. 2005 Feb;4(1):51-62. Related Articles, Links

DNA vaccines for poultry: the jump from theory to practice.

Haygreen L, Davison F, Kaiser P.

Institute for Animal Health, Compton, Berkshire RG20 7NN, UK.

DNA vaccines could offer a solution to a number of problems faced by the poultry industry; they are relatively easy to manufacture, stable, potentially easy to administer, can overcome neonatal tolerance and the deleterious effects of maternal antibody, and do not cause disease pathology. Combined with this, in ovo vaccination offers the advantage of reduced labor costs, mass administration and the induction of an earlier immune response. Together, this list of advantages is impressive. However, this combined technology is still in its infancy and requires many improvements. The potential of CpG motifs, DNA vaccines and in ovo vaccination, however, can be observed by the increasing number of recent reports investigating their application in challenge experiments. CpG motifs have been demonstrated to be stimulatory both invitro and invivo. In addition, DNA vaccines have been successfully delivered via the in ovo route, albeit not yet through the amniotic fluid. Lastly, a recent report has demonstrated that a DNA vaccine against infectious bronchitis virus administered via in ovo vaccination, followed by live virus boost, can slightly improve on the protective effect induced by the live virus alone. Therefore, DNA vaccination via the in ovo route is promising and offers potential as a poultry vaccine, however, efficacy needs to be improved and the costs of production reduced before it is likely to be beneficial to the poultry industry in the long term.

PMID: 15757473 [PubMed - in process]

Field: **Title/Abstract,** Limits: **Publication Date from 2000, English**

1: J Theor Biol. 2005 May 21;234(2):167-72. Epub 2005 Jan 20.

Daisyworld inhabited with daisies incorporating a seed size/number trade-off: the mechanism of negative feedback on selection from a standpoint of the competition theory.

Seto M, Akagi T.

Faculty of Agriculture, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu-shi, Tokyo 183-8509, Japan.

We reexamined a Daisyworld model from the traditional view of competition theory. Unlike the original model, white and black daisies in our model incorporate a seeding/germination trade-off against bare ground area without assuming the local temperature reward. As a result, the planetary temperature is automatically regulated by two species if the following conditions are met: (i) the species react equally to an environmental condition, but one can alter the environmental condition in the opposite direction to the other. (ii) that one of the two cannot have both a higher maximal growth rate (mu(max)) and lower half-saturation constant (K) than those of the other. In other words, a pair of phenotypes incorporates a trade-off between quality and number of seeds. We found that the homeostatic regulation can also be reconciled with the adaptive evolution of optimal temperature. The results of simulation imply that biotic environmental feedback can also be maintained when the emergence of polymorphisms (black and white daisies) is closely linked to such a trade-off.

PMID: 15757676 [PubMed - in process]

Field: **Title/Abstract,** Limits: **Publication Date from 2000, English**

1: J Comp Physiol A Neuroethol Sens Neural Behav Physiol. 2005 Mar;191(3):201-11. Epub 2004 Oct 12.

Expectations and outcomes: decision-making in the primate brain.

McCoy AN, Platt ML.

Department of Neurobiology, Duke University Medical Center, 325 Bryan Research Building, Box 3209, Durham, NC, 27710, USA.

Success in a constantly changing environment requires that decision-making strategies be updated as reward contingencies change. How this is accomplished by the nervous system has, until recently, remained a profound mystery. New studies coupling economic theory with neurophysiological techniques have revealed the explicit representation of behavioral value. Specifically, when fluid reinforcement is paired with visually-guided eye movements, neurons in parietal cortex, prefrontal cortex, the basal ganglia, and superior colliculus-all nodes in a network linking visual stimulation with the generation of oculomotor behavior-encode the expected value of targets lying within their response fields. Other brain areas have been implicated in the processing of reward-related information in the abstract: midbrain dopaminergic neurons, for instance, signal an error in reward prediction. Still other brain areas link information about reward to the selection and performance of specific actions in order for behavior to adapt to changing environmental exigencies. Neurons in posterior cingulate cortex have been shown to carry signals related to both reward outcomes and oculomotor behavior, suggesting that they participate in updating estimates of orienting value.

PMID: 15759141 [PubMed - in process]

About Entrez

Text Version

Entrez PubMed
Overview
Help | FAQ
Tutorial
New/Noteworthy
E-Utilities

PubMed Services
Journals Database
MeSH Database
Single Citation Matcher
Batch Citation Matcher
Clinical Queries
LinkOut
My NCBI (Cubby)

Related Resources
Order Documents
NLM Catalog
NLM Gateway
TOXNET
Consumer Health
Clinical Alerts
ClinicalTrials.gov
PubMed Central

Field: **Title/Abstract,** Limits: **Publication Date from 2000**

Display Abstract Show: 20 Sort Send to Text

All: 1 Review: 0

1: J Neurol Sci. 2005 Mar 15;229-230(1):109-16. Epub 2004 Dec 8. Related Articles, Links

FULL-TEXT ARTICLE LEHIGH SFX LINK

Effects of arousing emotional scenes on the distribution of visuospatial attention: changes with aging and early subcortical vascular dementia.

Rosler A, Ulrich C, Billino J, Sterzer P, Weidauer S, Bernhardt T, Steinmetz H, Frolich L, Kleinschmidt A.

Department of Neurology, Johann Wolfgang Goethe-University, Frankfurt, Germany.

BACKGROUND: The modulation of attention by emotionally arousing stimuli is highly important for each individual's social function. Disturbances of emotional processing are a supportive feature for the diagnosis of subcortical vascular dementia (SVD). We address here whether these disturbances might be useful as an early disease marker. METHODS: In order to examine the modulation of visual attention by emotionally arousing stimuli of different valence, 12 elderly patients with early SVD, 12 age-comparable healthy adults and 12 young healthy subjects were studied while looking at pairs of pictures from the International Affective Picture Battery that were either neutral-neutral, neutral-positive or neutral-negative in terms of emotional content. Eye movements were recorded with an infrared eye-tracking system. The direction of the first saccade and the dwell time during the 10 s of presentation were measured and compared among groups with parametric tests. RESULTS: All subjects showed a modulation of initial attentional orienting as well as a higher percentage of dwell time towards the pictures containing emotional material. Patients with SVD and old controls did not differ in either experimental measure. Young patients showed a stronger bias towards emotionally negative material than both groups of older individuals. CONCLUSIONS: Modulation of visuospatial attention is preserved in early SVD. This might have implications for therapeutic interventional approaches. A weakened sustained attention towards negative but not positive emotional pictures in the elderly is in accordance with the socioemotional selectivity theory, describing a relative selection of positive stimuli with aging.

PMID: 15760628 [PubMed - in process]

Display Abstract Show: 20 Sort Send to Text

Field: **Title/Abstract,** Limits: **Publication Date from 2000**

Display Abstract Show: 20 Sort Send to Text

All: 1 Review: 0

1: Hum Reprod. 2005 Mar 10; [Epub ahead of print] Related Articles, Links

New debate: natural killer cells and reproductive failure--theory, practice and prejudice.

Rai R, Sacks G, Trew G.

Department of Obstetrics and Gynaecology, Faculty of Medicine, Imperial College London, St Mary's Hospital, London, UK.

The relationship between peripheral blood natural killer (NK) cells and reproductive failure is one of the most controversial areas in reproductive medicine. Amidst much publicity, peripheral blood NK cell testing is being promoted as a useful diagnostic test to guide the initiation of a variety of immunosuppressive therapies amongst patients with either recurrent miscarriage or infertility. We contend (i) that at present there is no scientific basis for the introduction of NK cell testing into routine clinical practice, and (ii) that the use of immunosuppressant agents based on the results of such testing may potentially be harmful.

PMID: 15760961 [PubMed - as supplied by publisher]

Exhibit 2

A drawing of the bacterial flagellum from a biochemistry textbook

The bacterial flagellum

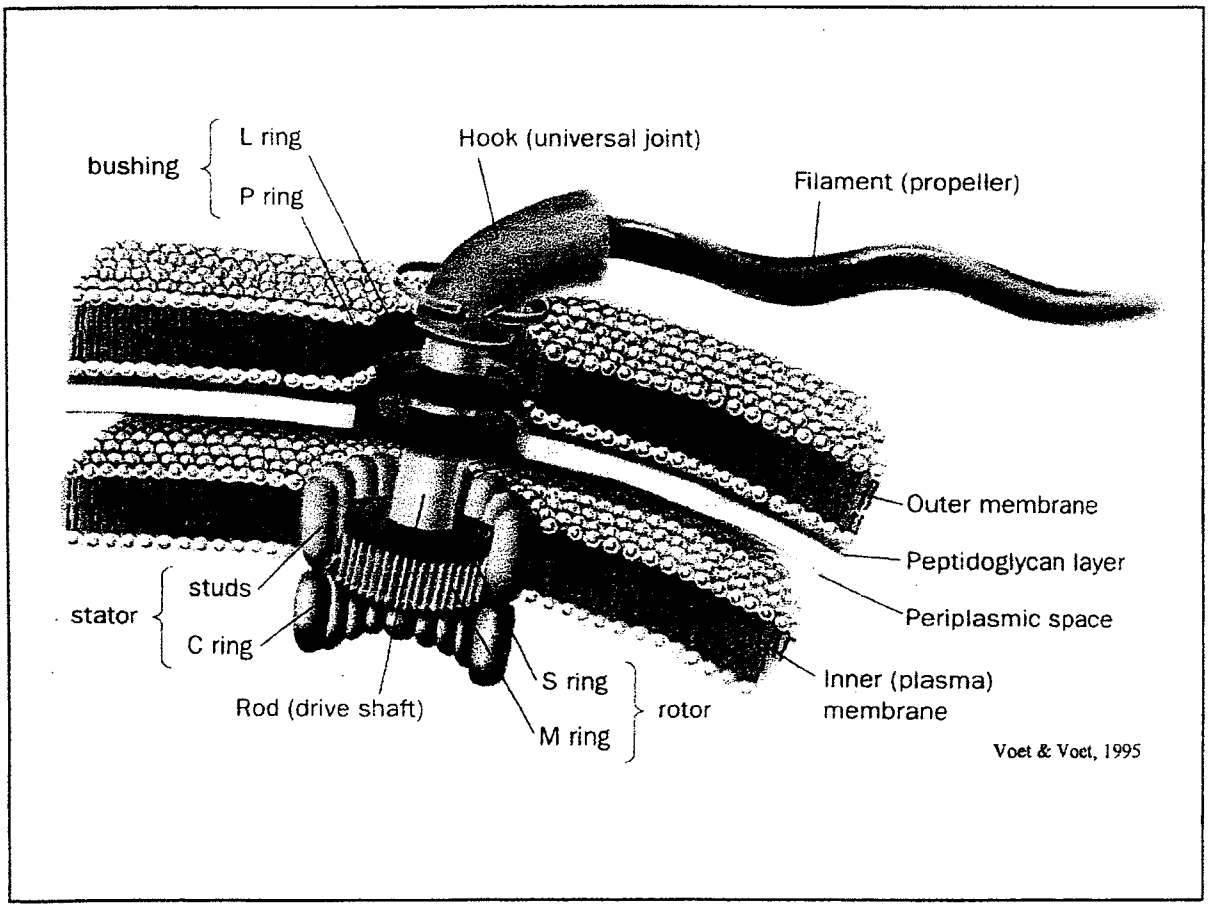


Exhibit 3

Cover and Table of Contents of the February 6, 1998 issue of the journal *Cell*

Review Issue

Macromolecular Machines

Volume 92 Number 3 February 6, 1998

Cell

Reviews on Macromolecular Machines

(continued)

Articles

Like the machines invented by humans to deal efficiently with the macroscopic world, protein assemblies contain highly coordinated moving parts. Reviewed in this issue of *Cell* are the protein machines that control replication, transcription, splicing, nucleocytoplasmic transport, protein synthesis, protein assembly, protein degradation, and protein translocation—the machines that underlie the workings of all living things. The cover, inspired by the idea of protein machines, is a reproduction of the painting "Dilemma of the Helixes" by Rong Li, Ph.D., Department of Cell Biology, Harvard Medical School.

Exhibit 4

New York Times op-ed summarizing the argument for intelligent design

The **New York Times**

February 7, 2005 Monday
Late Edition - Final

SECTION: Section A; Column 1; Editorial Desk; Pg. 21

LENGTH: 965 words

HEADLINE: Design for Living

BYLINE: By Michael J. **Behe**.

Michael J. **Behe,** a professor of biological sciences at Lehigh University and a senior fellow with the Discovery Institute's Center for Science and Culture, is the author of "Darwin's Black Box: The Biochemical Challenge to Evolution."

DATELINE: BETHLEHEM, Pa.

BODY:

IN the wake of the recent lawsuits over the teaching of Darwinian evolution, there has been a rush to debate the merits of the rival theory of intelligent design. As one of the scientists who have proposed design as an explanation for biological systems, I have found widespread confusion about what intelligent design is and what it is not.

First, what it isn't: the theory of intelligent design is not a religiously based idea, even though devout people opposed to the teaching of evolution cite it in their arguments. For example, a critic recently caricatured intelligent design as the belief that if evolution occurred at all it could never be explained by Darwinian natural selection and could only have been directed at every stage by an omniscient creator. That's misleading. Intelligent design proponents do question whether random mutation and natural selection completely explain the deep structure of life. But they do not doubt that evolution occurred. And intelligent design itself says nothing about the religious concept of a creator.

Rather, the contemporary argument for intelligent design is based on physical evidence and a straightforward application of logic. The argument for it consists of four linked claims. The first claim is uncontroversial: we can often recognize the effects of design in nature. For example, unintelligent physical forces like plate tectonics and erosion seem quite sufficient to account for the origin of the Rocky Mountains. Yet they are not enough to explain Mount Rushmore.

Of course, we know who is responsible for Mount Rushmore, but even someone who had never heard of the monument could recognize it as designed. Which leads to the second claim of the intelligent design argument: the physical marks of design are visible in aspects of biology. This is uncontroversial, too. The 18th-century clergyman William Paley likened living things to a watch, arguing that the workings of both point to intelligent design. Modern Darwinists disagree with Paley that the perceived design is real, but they do agree that life overwhelms us with the appearance of design.

For example, Francis Crick, co-discoverer of the structure of DNA, once wrote that biologists must constantly remind themselves that what they see was not designed but evolved. (Imagine a scientist repeating through clenched teeth: "It wasn't really designed. Not really.")

The resemblance of parts of life to engineered mechanisms like a watch is enormously stronger than what Reverend Paley imagined. In the past 50 years modern science has shown that the cell, the very foundation of life, is run by machines made of molecules. There are little molecular trucks in the cell to ferry supplies, little outboard motors to push a cell through liquid.

In 1998 an issue of the journal Cell was devoted to molecular machines, with articles like "The Cell as a Collection of Protein Machines" and "Mechanical Devices of the Spliceosome: Motors, Clocks, Springs and Things." Referring to his student days in the 1960's, Bruce Alberts, president of the National Academy of Sciences, wrote that "the chemistry that makes life possible is much more elaborate and sophisticated than anything we students had ever considered." In fact, Dr. Alberts remarked, the entire cell can be viewed as a factory with an elaborate network of interlocking assembly lines, each of which is composed of a set of large protein machines. He emphasized that the term machine was not some fuzzy analogy; it was meant literally.

The next claim in the argument for design is that we have no good explanation for the foundation of life that doesn't involve intelligence. Here is where thoughtful people part company. Darwinists assert that their theory can explain the appearance of design in life as the result of random mutation and natural selection acting over immense stretches of time. Some scientists, however, think the Darwinists' confidence is unjustified. They note that although natural selection can explain some aspects of biology, there are no research studies indicating that Darwinian processes can make molecular machines of the complexity we find in the cell.

Scientists skeptical of Darwinian claims include many who have no truck with ideas of intelligent design, like those who advocate an idea called complexity theory, which envisions life self-organizing in roughly the same way that a hurricane does, and ones who think organisms in some sense can design themselves.

The fourth claim in the design argument is also controversial: in the absence of any convincing non-design explanation, we are justified in thinking that real intelligent design was involved in life. To evaluate this claim, it's important to keep in mind that it is the profound appearance of design in life that everyone is laboring to explain, not the appearance of natural selection or the appearance of self-organization.

The strong appearance of design allows a disarmingly simple argument: if it looks, walks and quacks like a duck, then, absent compelling evidence to the contrary, we have warrant to conclude it's a duck. Design should not be overlooked simply because it's so obvious.

Still, some critics claim that science by definition can't accept design, while others argue that science should keep looking for another explanation in case one is out there. But we can't settle questions about reality with definitions, nor does it seem useful to search relentlessly for a non-design explanation of Mount Rushmore. Besides, whatever special restrictions scientists adopt for themselves don't bind the public, which polls show, overwhelmingly, and sensibly, thinks that life was designed. And so do many scientists who see roles for both the messiness of evolution and the elegance of design.

URL: http://www.nytimes.com

GRAPHIC: Drawing (Drawing by Leigh Wells)

LOAD-DATE: February 7, 2005

Document 1 of 1.

Exhibit 5

"Directed Panspermia", by
Francis Crick and Leslie Orgel

ICARUS 19, 341–346 (1973)

Directed Panspermia

F. H. C. CRICK

Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge, England

AND

L. E. ORGEL

The Salk Institute for Biological Studies, P.O. Box 1809, San Diego, California 92112

Received June 22, 1972; revised December 20, 1972

It now seems unlikely that extraterrestrial living organisms could have reached the earth either as spores driven by the radiation pressure from another star or as living organisms imbedded in a meteorite. As an alternative to these nineteenth-century mechanisms, we have considered Directed Panspermia, the theory that organisms were deliberately transmitted to the earth by intelligent beings on another planet. We conclude that it is possible that life reached the earth in this way, but that the scientific evidence is inadequate at the present time to say anything about the probability. We draw attention to the kinds of evidence that might throw additional light on the topic.

Introduction

It was not until the middle of the nineteenth century that Pasteur and Tyndall completed the demonstration that spontaneous generation is not occurring on the Earth nowadays. Darwin and a number of other biologists concluded that life must have evolved here long ago when conditions were more favourable. A number of scientists, however, drew a quite different conclusion. They supposed that if life does not evolve from terrestrial nonliving matter nowadays, it may never have done so. Hence, they argued, life reached the earth as an "infection" from another planet (Oparin, 1957).

Arrhenius (1908) proposed that spores had been driven here by the pressure of the light from the central star of another planetary system. His theory is known as Panspermia. Kelvin suggested that the first organisms reached the Earth in a meteorite. Neither of these theories is absurd, but both can be subjected to severe criticism. Sagan (Shklovski and Sagan, 1966; Sagan and Whitehall, 1973) has shown that any known type of radiation-resistant spore would receive so large a dose of radiation during its journey to the Earth from another Solar System that it would be extremely unlikely to remain viable. The probability that sufficiently massive objects escape from a Solar System and arrive on the planet of another one is considered to be so small that it is unlikely that a single meteorite of extrasolar origin has ever reached the surface of the Earth (Sagan, private communication). These arguments may not be conclusive, but they argue against the "infective" theories of the origins of life that were proposed in the nineteenth century.

It has also been argued that "infective" theories of the origins of terrestrial life should be rejected because they do no more than transfer the problem of origins to another planet. This view is mistaken; the historical facts are important in their own right. For all we know there may be other types of planet on which the origin of life *ab initio* is greatly more probable than on our own. For example, such a planet may possess a mineral, or compound, of crucial catalytic importance, which is rare on

Earth. It is thus important to know whether primitive organisms evolved here or whether they arrived here from somewhere else. Here we reexamine this problem in the light of more recent biological and astronomical information.

Our Present Knowledge of the Galaxy

The local galactic system is estimated to be about 13×10^9 yr old (See Metz, 1972). The first generation of stars, because they were formed from light elements, are unlikely to have been accompanied by planets. However, some second generation stars not unlike the Sun must have formed within 2×10^9 yr of the origin of the galaxy (Blaauw and Schmidt, 1965). Thus it is quite probable that planets not unlike the Earth existed as much as 6.5×10^9 yr before the formation of our own Solar System.

We know that not much more than 4×10^9 yr elapsed between the appearance of life on the Earth (wherever it came from) and the development of our own technological society. The time available makes it possible, therefore, that technological societies existed elsewhere in the galaxy *even before the formation of the Earth*. We should, therefore, consider a new "infective" theory, namely that a primitive form of life was deliberately planted on the Earth by a technologically advanced society on another planet.

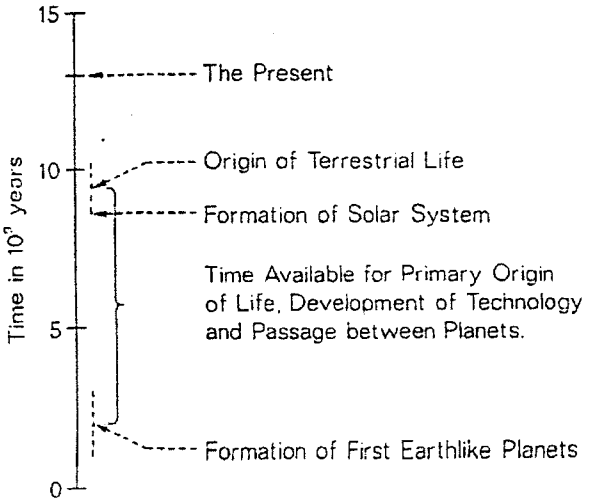


Fig. 1. An approximate time-scale for the events discussed in the paper. To simplify illustration the age of the galaxy has been somewhat arbitrarily taken as 13×10^9 yr.

Are there many planets which could be infected with some chance of success? It is believed, though the evidence is weak and indirect, that in the galaxy many stars, of a size not dissimilar to our Sun, have planets, on a fair fraction of which temperatures are suitable for a form of life based on carbon chemistry and liquid water, as ours is. Experimental studies of the production of organic chemicals under prebiotic conditions make it seem likely that a rich prebiotic soup accumulates on a high proportion of such Earthlike planets. Unfortunately, we know next to nothing about the probability that life evolves within a few billion years in such a soup, either on our own special Earth, or still less on other Earthlike planets.

If the probability that life evolves in a suitable environment is low we may be able to prove that we are likely to be alone in the galaxy (Universe). If it is high the galaxy may be pullulating with life of many different forms. At the moment we have no means at all of knowing which of these alternatives is correct. We are thus free to postulate that there have been (and still are) many places in the galaxy where life could exist but that, in at least a fraction of them, after several billion years the chemical systems had not evolved to the point of self-replication and natural selection. Such planets, if they do exist, would form an excellent breeding ground for external microorganisms. Note that because many if not all such planets would have a reducing atmosphere they would not be very hospitable to the higher forms of life as we know them on Earth.

Our Proposal

The possibility that terrestrial life derives from the deliberate activity of an extraterrestrial society has often been considered in science fiction and more or less light-heartedly in a number of scientific papers. For example, Gold (1960) has suggested that we might have evolved from the microorganisms inadvertently

left behind by some previous visitors from another planet (for example, in their garbage). Here we wish to examine a very specific form of Directed Panspermia. Could life have started on Earth as a result of infection by microorganisms sent here deliberately by a technological society on another planet, by means of a special long-range unmanned spaceship? To show that this is not totally implausible we shall use the theorem of detailed cosmic reversibility; if we are capable of infecting an as yet lifeless extrasolar planet, then, given that the time was available, another technological society might well have infected our planet when it was still lifeless.

The Proposed Spaceship

The spaceship would carry large samples of a number of microorganisms, each having different but simple nutritional requirements, for example blue-green algae, which could grow on CO_2 and water in "sunlight." A payload of 1000 kg might be made up of 10 samples each containing 10^{16} microorganisms, or 100 samples each of 10^{15} microorganisms.

It would not be necessary to accelerate the spaceship to extremely high velocities, since its time of arrival would not be important. The radius of our galaxy is about 10^5 light years, so we could infect most planets in the galaxy within 10^8 yr by means of a spaceship traveling at only one-thousandth of the velocity of light. Several thousand stars are within a hundred light years of the Earth and could be reached within as little as a million years by a spaceship travelling at only 60,000 mph, or within 10,000 yr if a speed of one-hundredth of that of light were possible.

The technology required to carry out such an act of interstellar pollution is not available at the present time. However, it seems likely that the improvements in astronomical techniques will permit the location of extrasolar planets within the next few decades. Similarly, the problem of sending spaceships to other stars, at velocities low compared with that of light, should not prove insoluble once workable nuclear engines are available. This again is likely to be within a few decades. The most difficult problem would be presented by the long flight times; it is not clear how long it will be before we can build components that would survive in space for periods of thousands or millions of years.

Although there are some technological problems associated with the distribution of the microorganisms in viable form after a long journey through space, none of them seems insuperable. Some radiation protection could be provided during the journey. Suitable packaging should guarantee that small samples, including some viable organisms, would be widely distributed. The question of how long microorganisms, and in particular bacterial spores, could survive in a spaceship has been considered in a preliminary way by Sneath (1962). He concludes "that life could probably be preserved for periods of more than a million years if suitably protected and maintained at temperatures close to absolute zero." Sagan (1960) has given a comparable estimate of the effects of radiation damage. We conclude that within the foreseeable future we could, if we wished, infect another planet, and hence that it is not out of the question that our planet was infected.

We can in fact go further than this. It may be possible in the future to send either mice or men or elaborate instruments to the planets of other Solar Systems (as so often described in science fiction) but a rocket carrying microorganisms will always have a much greater effective range and so be advantageous if the sole aim is to spread life. This is true for several reasons. The conditions on many planets are likely to favour microorganisms rather than higher organisms. Because of their extremely small size vast numbers of microorganisms can be carried, so much more wastage can be accepted. The ability of microorganisms to survive, without special equipment, both storage for very long periods at low temperatures and also an abrupt change back to room temperatures is also a great advantage. Whatever the potential range for infection by other organisms, microorganisms can almost

certainly be sent further and probably much further.

It should be noted that most of the earliest "fossils" so far recognized are somewhat similar to our present bacteria or blue-green algae. They occur in cherts of various kinds and are estimated to be up to 3×10^9 yr old. This makes it improbable that the Earth was ever infected merely by higher organisms.

Motivation

Next we must ask what motive we might have for polluting other planets. Since we would not derive any direct advantage from such a programme, presumably it would be carried through either as a demonstration of technological capability or, more probably, through some form of missionary zeal.

It seems unlikely that we would deliberately send terrestrial organisms to planets that we believed might already be inhabited. However, in view of the precarious situation on Earth, we might well be tempted to infect other planets if we became convinced that we were alone in the galaxy (Universe).[1] As we have already explained we cannot at the moment estimate the probability of this. The hypothetical senders on another planet may have been able to prove that they were likely to be alone, and to remain so, or they may have reached this conclusion mistakenly. In either case, if they resembled us psychologically, their motivation for polluting the galaxy would be strong, if they believed that all or even the great majority of inhabitable planets could be given life by Directed Panspermia.

The psychology of extraterrestrial societies is no better understood than terrestrial psychology. It is entirely possible that extraterrestrial societies might infect other planets for quite different reasons than those we have suggested. Alternatively, they might be less tempted than we would be, even if they thought that they were alone. The arguments given above, together with the principle of cosmic reversibility, demonstrate the possibility that we have been infected, but do not enable us to estimate the probability.

[1] In a somewhat different context the seeding of Venus and other solar planets has been suggested by C. Sagan (1961), and T. Gold, private communication.

Possible Biological Evidence

Infective theories of the origins of terrestrial life could be taken more seriously if they explained aspects of biochemistry or biology that are otherwise difficult to understand. We do not have any strong arguments of this kind, but there are two weak facts that could be relevant.

The chemical composition of living organisms must reflect to some extent the composition of the environment in which they evolved. Thus the presence in living organisms of elements that are extremely rare on the Earth might indicate that life is extraterrestrial in origin. Molybdenum is an essential trace element that plays an important role in many enzymatic reactions, while chromium and nickel are relatively unimportant in biochemistry. The abundance of chromium, nickel, and molybdenum on the Earth are 0.20, 3.16, and 0.02%, respectively. We cannot conclude anything from this single example, since molybdenum may be irreplaceable in some essential reaction—nitrogen fixation, for example. However, if it could be shown that the elements represented in terrestrial living organisms corelate closely with those that are abundant in some class of star—molybdenum stars, for example—we might look more sympathetically at "infective" theories.

Our second example is the genetic code. Several orthodox explanations of the universality of the genetic code can be suggested, but none is generally accepted to be completely convincing. It is a little surprising that organisms with somewhat different codes do not coexist. The universality of the code follows naturally from an "infective" theory of the origins of life. Life on Earth would represent a clone derived from a single extraterrestrial organism. Even if many codes were represented at the primary site where life

began, only a single one might have operated in the organisms used to infect the Earth.

Conclusion

In summary, there is adequate time for technological society to have evolved twice in succession. The places in the galaxy where life could start, if seeded, are probably very numerous. We can foresee that we ourselves will be able to construct rockets with sufficient range, delivery ability, and surviving payload if microorganisms are used. Thus the idea of Directed Panspermia cannot at the moment be rejected by any simple argument. It is radically different from the idea that life started here *ab inito* without infection from elsewhere. We have thus two sharply different theories of the origin of life on Earth. Can we choose between them?

At the moment it seems that the experimental evidence is too feeble to make this discrimination. It is difficult to avoid a personal prejudice, one way or the other, but such prejudices find no scientific support of any weight. It is thus important that both theories should be followed up. Work on the supposed terrestrial origin of life is in progress in many laboratories. As far as Directed Panspermia is concerned we can suggest several rather diverse lines of research.

The arguments we have employed here are, of necessity, somewhat sketchy. Thus the detailed design of a long-range spaceship would be worth a careful feasibility study. The spaceship must clearly be able to home on a star, for an object with any appreciable velocity, if dispatched in a random direction, would in almost all cases pass right through the galaxy and out the other side. It must probably have to decelerate as it approached the star, in order to allow the safe delivery of the payload. The packets of microorganisms must be made and dispersed in such a way that they can survive the entry at high velocity into the atmosphere of the planet, and yet be able to dissolve in the oceans. Many uesful feasibility studies could be carried out on the engineering points involved.

On the biological side we lack precise information concerning the life-time of microorganisms held at very low temperatures while traveling through space at relatively high velocities. The rocket would presumably be coasting most of the time so the convenient temperature might approximate to that of space. How serious is radiation damage, given a certain degree of shielding? How many distinct types of organism should be sent and which should they be? Should they collectively be capable of nitrogen fixation, oxidative phosphorylation and photosynthesis? Although many "soups" have been produced artifically in the laboratory, following the pioneer experiments of Miller, as far as we know no careful study has been made to determine which present-day organisms would grow well in them under primitive Earth conditions.

At the same time present-day organisms should be carefully scrutinized to see if they still bear any vestigial traces of extraterrestrial origin. We have already mentioned the uniformity of the genetic code and the anomalous abundance of molybdenum. These facts amount to very little by themselves but as already stated there may be other as yet unsuspected features which, taken together, might point to a special type of planet as the home of our ancestors.

These enquiries are not trivial, for if successful they could lead to others which would touch us more closely. Are the senders or their descendants still alive? Or have the hazards of 4 billion years been too much for them? Has their star inexorably warmed up and frizzled them, or were they able to colonise a different Solar System with a short-range spaceship? Have they perhaps destroyed themselves, either by too much aggression or too little? The difficulties of placing any form of life on another planetary system are so great that we are unlikely to be their sole descendants. Presumably they would have made many attempts to infect the galaxy. If the range of their rockets were small this might suggest that we have cousins on planets which are not too distant. Perhaps the galaxy is lifeless except for a local village, of which we are one member.

One further point deserves emphasis. We feel strongly that under no circumstances should we risk infecting other planets at the present time. It would be wise to wait until we know far more about the probability of the development of life on extrasolar planets before causing terrestrial organisms to escape from the solar system.

ACKNOWLEDGMENTS

We are indebted to the organisers of a meeting on Communication with Extraterrestrial Intelligence, held at Byurakan Observatory in Soviet Armenia in September 1971, which crystallized our ideas about Panspermia. We thank Drs. Freeman Dyson, Tommy Gold, and Carl Sagan for discussion and important comments on our argument.

REFERENCES

ARRHENIUS, S. (1908). "Worlds in the Making." Harper and Row, New York.

BLAAUW, A., AND SCHMIDT, M. (1965). "Galactic Structure." University of Chicago Press, Chicago.

GOLD, T. (May 1960). "Cosmic Garbage" "Air Force and Space Digest", p. 65.

METZ, W. D. (1972). Reporting a speech by Allan Sandage. *Science* **178**, 600.

OPARIN, A. I. (1957). "The Origins of Life on Earth." Academic Press, New York, New York (gives a general discussion on Panspermia).

SAGAN, C. (1960). Biological contamination of the Moon. *Proc. Nat. Acad. Sci.* **46**, 396.

SAGAN, C. (1961). The planet Venus. *Science* **133**, 849.

SAGAN, C., AND WHITEHALL, L. (1973). To be submitted to *Icarus*.

SHKLOVSKII, I. S., AND SAGAN, C. (1966 and 1967). "Intelligent Life in the Universe," p. 207. Holden-Day, San Francisco and Dell Publishing Co., New York.

SNEATH, P. H. A. (1962). Longevity of microorganisms. *Nature* (*London*) **195**, 643.

Exhibit 6

"Biology Text illustrations More Fiction Than Fact", New York Times, 4/8/01 (sidebar)

The New York Times

SUNDAY, APRIL 8, 2001 VOL. CL . . . . No. 51,717

Darwin vs. Design: Evolutionists' New Battle

By JAMES GLANZ

When Kansas school officials restored the theory of evolution to statewide education standards a few weeks ago, biologists might have been inclined to declare victory over creationism.

Instead, some evolutionists say, the latter stages of the battle in Kansas, along with new efforts in Michigan and Pennsylvania as well as in a number of universities and even in Washington, suggest that the issue is far from settled.

This time, though, the evolutionists find themselves arrayed not against traditional creationism, with its roots in biblical literalism, but against a more sophisticated idea: the intelligent design theory.

Proponents of this theory, led by a group of academics and intellectuals and including some biblical creationists, accept that the earth is billions of years old, not the thousands of years suggested by a literal reading of the Bible.

But they dispute the idea that natural selection, the force Darwin suggested drove evolution, is enough to explain the complexity of the earth's plants and animals. That complexity, they say, must be the work of an intelligent designer.

This designer may be much like the biblical God, proponents say, but they are open to other explanations, such as the proposition that life was seeded by a meteorite from elsewhere in the cosmos, possibly involving extraterrestrial intelligence, or the new age philosophy that the universe is suffused with a mysterious but inanimate life force.

In recent months, the proponents of intelligent design have advanced their case on several fronts.

¶In Kansas, after the backlash against the traditional biblical creationism, proponents of the design theory have become the dominant anti-evolution force, though they lost an effort to have theories like intelligent design considered on an equal basis with evolution in school curriculums.

Michigan, nine legislators in the House of Representatives have introduced legislation to amend state education standards to put intelligent design on an equal basis with evolution.

Salvatore C. DiMarco Jr. for The New York Times

An originator of a theory on life that challenges Darwin's theory of evolution, Dr. Michael J. Behe argues that various biochemical structures in cells could not have been built in a stepwise Darwinian fashion.

¶In Pennsylvania, where biblical creationists and design theorists have operated in concert, state officials are close to adopting educational standards that would allow the teaching of theories on the origin and development of life other than evolution.

¶Backers of intelligent design organized university-sanctioned conferences at Yale and Baylor last year, and the movement has spawned at least one university student organization — called Intelligent Design and Evolution Awareness, or the IDEA club — at the University of California in San Diego.

¶The Discovery Institute, a research institute in Seattle that promotes conservative causes, organized a briefing on intelligent design last year on Capitol Hill for prominent members of Congress.

"They are skilled in analyzing evidence and ideas," said Representative Tom Petri, a Wisconsin Republican and one of several members of Congress who was a host at the session in a Congressional hearing room. "They are making a determined effort to attempt to present the intelligent design theory, and ask that it be judged by normal scientific criteria."

Polls show that the percentage of Americans who say they believe in creationism is about 45 percent. George W. Bush took the position in the presidential campaign that children should be exposed to both creationism and evolution in school.

Supporters of Darwin see intelligent design as more insidious than creationism, especially given that many of its advocates have mainstream scientific credentials, which creationists often lack.

"The most striking thing about the intelligent design folks is their potential to really make anti-evolutionism intellectually respectable," said Dr. Eugenie Scott, executive director of the National Center for Science Education in Oakland, Calif., which promotes the teaching of evolution.

Dr. Adrian Melott, a professor of physics and astronomy at the University of Kansas in Lawrence and a member of Kansas Citizens for Science, a group that helped win the restoration of evolution to the state education standards, said the design theory was finding adherents among doctors, engineers and people with degrees in the humanities.

Intelligent design is "the language that the creationists among the student body tend to use now," Dr. Melott said.

One of the first arguments for the

design theory was set out in "Darwin's Black Box: The Biochemical Challenge to Evolution" (Simon & Schuster, 1996), by Dr. Michael J. Behe, a professor of biological sciences at Lehigh University in Pennsylvania. Dr. Behe argued that various biochemical structures in cells could not have been built in a stepwise Darwinian fashion.

Since then, the movement has gained support among a few scientists in other disciplines, most of them conservative Christians.

"I'm very impressed with the level of scientific work and the level of scientific dialogue among the leaders of the design movement," said Dr. Guillermo Gonzalez, an astronomer at the University of Washington in Seattle. The theory "warrants further research," Dr. Gonzalez said.

Leaders of the design movement also look for flaws in evolutionist thinking and its presentation, and have scored heavily by publicizing embarrassing mistakes in prominent biology textbooks.

"There is a legitimate intellectual project here," said Dr. William Dembski, a leading proponent of intelligent design who has a doctorate in mathematics from the University of Chicago and who is on the faculty at Baylor, which receives a small part of its financing from the Texas Baptist Convention. "It is not creationism. There's not a commitment to Genesis literalism."

Dr. Dembski conceded that his interest in alternatives to Darwinian theory was partly brought on by the fact that he is an evangelical Christian, but he said intelligent design could withstand strict scientific scrutiny.

"The religious conviction played a role," he said. But he added, "As far as making me compromise in my work, that's the last thing I want to do."

Evolutionary biologists maintain that the arguments of intelligent design do not survive scrutiny, but they concede that a specialist's knowledge of particular mathematical or biological disciplines is often needed to clinch the point.

"I would use the words 'devilishly clever,'" said Dr. Jerry Coyne, a professor of ecology and evolution at the University of Chicago, speaking of the way the theory is constructed. "It has an appeal to intellectuals who don't know anything about evolutionary biology, first of all because the proponents have Ph.D.'s and second of all because it's not written in the sort of populist, folksy, anti-intellectual style. It's written in the argot of academia."

Despite that gloss, Dr. Leonard Krishtalka, a biologist and director of the University of Kansas Natural History Museum and Biodiversity Research Center, said recently, "Intelligent design is nothing more than creationism dressed in a cheap tuxedo."

Dr. Dembski said his rather vague doubts about Darwinism did not take scientific shape until he attended an academic conference in 1988, just after finishing his doctoral thesis. The conference explored the difficulty of preparing perfectly random strings of numbers, which are important in cryptography, in computer science and in statistics.

One problem is that seemingly random strings often contain patterns discernible only with mathematical tests. Dr. Dembski wondered whether he could devise a way to find evidence of related patterns in the randomness of nature.

Dr. Dembski eventually developed what he called a mathematical "explanatory filter" that he asserted can distinguish randomness from complexity designed by an intelligent agent. He explained this idea in "The Design Inference" (Cambridge University Press, 1998).

Dr. Dembski has applied his explanatory filter to the biochemical structures in cells — and concluded that blind natural selection could not have created them.

But in a detailed critique of Dr. Dembski's filter theory, published in the current issue of the magazine The Skeptical Inquirer, Dr. Taner Edis, a physicist at Truman State University in Kirksville, Mo., said that while Dr. Dembski's mathematics were impressive, his analysis was probably detecting only the complexity that evolution itself would normally produce.

> The intelligent design theory offers ideas about the origin of life.

"They have come up with something genuinely interesting in the information-theory arguments," Dr. Edis said of intelligent design theorists. "At least they make an effort to get rid of some of the blatantly fundamentalist elements of creationism."

Dr. Behe, whose book provided the biochemical basis for Dr. Dembski's work, said he believed that certain intricate structures in cells, involving the cooperative action of many protein molecules, were "irreducibly complex," because removing just one of the proteins could leave those structures unable to function. If the structure serves no function without all of its parts, Dr. Behe asks, then how could evolution have built it up step by step over the ages?

"I don't think something like that could have happened by simple natural laws," he said.

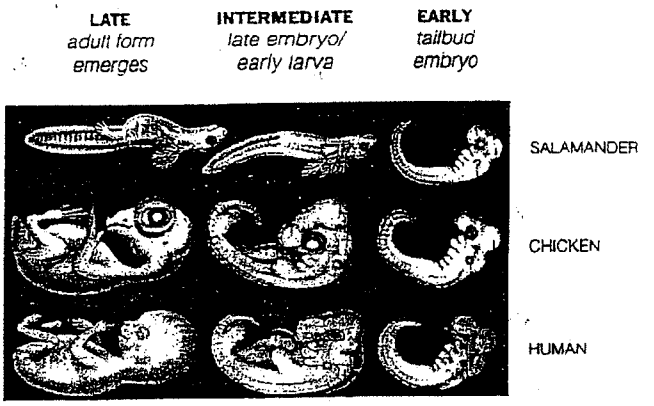
Most biologists disagree.

"It's flat wrong," said Dr. H. Allen Orr, an evolutionary geneticist and professor at the University of Rochester. Dr. Orr said that cell structures might have been put together in all sorts of unpredictable ways over the course of evolution and that a protein added might not have been indispensable at first, but only later, when many more proteins were woven around it.

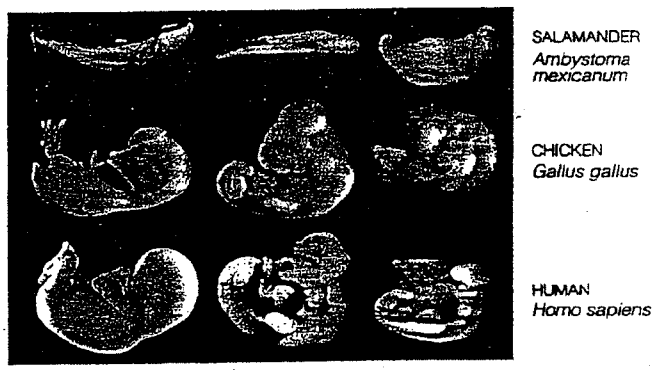
"The fact that that system is irreducibly complex doesn't mean you can't get there by Darwinian evolu-

Variations on an Embryo

A cornerstone college textbook, "Molecular Biology of the Cell," reprints Ernst Haeckel's doctored 1874 illustration suggesting that early embryonic stages of many animals, including humans, were virtually identical. But photographs of the embryos show great variation in very early stages of development.



Ernst Haeckel's 1874 illustration



Light microscope photographs

Source: James Hanken, Harvard University; Michael K. Richardson, Leiden University, Netherlands; Ernst Haeckel, Anthropogenie oder Entwickelungsgeschichte des Menschen

Biology Text Illustrations More Fiction Than Fact

The anti-evolution movement called intelligent design has helped its cause by publicizing some embarrassing mistakes in leading biology textbooks.

Biologists attribute them to inattention, but design proponents say the errors show that Darwinists are more than willing to accept shoddy evidence if it supports evolution.

In particular, design proponents cite the 19th-century drawings of the German biologist Ernst Haeckel, who asserted that the early embryonic stages of many animals, including humans, were virtually identical and diverged only later. He said that the resemblance proved that all animals had a common ancestor.

The drawings were reproduced in textbook after textbook for more than a century.

Several years ago, though, biologists discovered that many of the drawings were fraudulent and that the true resemblances were not nearly so striking. Nevertheless, some textbooks still contain them.

One of the texts that includes the faulty drawings is the third edition of "Molecular Biology of the Cell," the bedrock text of the field. Its authors include Dr. Bruce Alberts, a biochemist who is president of the National Academy of Sciences, and Dr. James D. Watson, the geneticist who shared a Nobel Prize for unraveling the structure of DNA.

In an interview, Dr. Alberts said he believed Haeckel's drawings were "overinterpreted," or highly idealized, rather than outright fakes. But he said they would be removed from the fourth edition of the textbook, to appear at the end of this year.

Biologists say the findings do not shake their confidence in the theory of evolution.

JAMES GLANZ

tion," Dr. Orr said.

Exactly how a designer might have assembled cell structures, say, is a question seldom addressed by design theorists. But they point out that Darwinists cannot necessarily offer detailed, step-by-step sequences of events for them either.

Dr. Behe, Dr. Dembski and Phillip E. Johnson, a professor emeritus of the law school at the University of California at Berkeley, are regarded as the intellectual fathers of the design theory movement. Mr. Johnson's book "Darwin on Trial" (InterVarsity Press, 1991) has become its manifesto. The book focuses on what Mr. Johnson says are the difficulties Darwinian theory has in explaining the fossil record.

Until last fall, Dr. Dembski was the director of a center at Baylor that was dedicated to the study of intelligent design theory. After complaints from other Baylor faculty members, the center's focus and leadership were changed, and it now includes design theory as well as other philosophical, theological and scientific topics.

Dr. Dembski and Dr. Behe are fellows of the Discovery Institute, the Seattle research institute that promotes intelligent design in its Center for the Renewal of Science and Culture.

The center's $1.1 million annual budget is supplied largely by Christian foundations that broadly endorse the implications of the intelligent design theory, said Bruce Chapman, Discovery's president. Mr. Johnson is an adviser to the institute, he said.

The center, which reaches people through books, articles, lectures and local activism, "is going to be of interest to academics," Mr. Chapman said. "But it's also going to be of interest to people in a more grassroots situation because they're teaching science or because they're on a school board somewhere."

Exhibit 7

Philosophy of Science journal article

Self-Organization and Irreducibly Complex Systems: A Reply to Shanks and Joplin*

Michael J. Behe†
Department of Biological Sciences, Lehigh University

Some biochemical systems require multiple, well-matched parts in order to function, and the removal of any of the parts eliminates the function. I have previously labeled such systems "irreducibly complex," and argued that they are stumbling blocks for Darwinian theory. Instead I proposed that they are best explained as the result of deliberate intelligent design. In a recent article Shanks and Joplin analyze and find wanting the use of irreducible complexity as a marker for intelligent design. Their primary counterexample is the Belousov-Zhabotinsky reaction, a self-organizing system in which competing reaction pathways result in a chemical oscillator. In place of irreducible complexity they offer the idea of "redundant complexity," meaning that biochemical pathways overlap so that a loss of one or even several components can be accommodated without complete loss of function. Here I note that complexity is a quantitative property, so that conclusions we draw will be affected by how well-matched the components of a system are. I also show that not all biochemical systems are redundant. The origin of non-redundant systems requires a different explanation than redundant ones.

1. Introduction. In the past half-century biology has made astonishing progress in understanding the molecular and cellular basis of life. In light of this progress it is fair to ask whether Darwin's mechanism of natural selection acting on random variation appears to be a good explanation for the origin of all, or just some, of the molecular systems science has discovered. In *Darwin's Black Box: The Biochemical Challenge to Evolution* (Behe 1996) I argued that some biochemical systems, such as the blood clotting cascade or bacterial flagellum, are resistant to Darwinian expla-

*Received August 1999; revised November 1999.

†Send requests for reprints to the author, Department of Biological Sciences, Lehigh University,111 Research Drive, Bethlehem, PA 18015.

Philosophy of Science, 67 (March 2000) pp. 155–162. 0031-8248/2000/6701-0008$2.00

nation because they are irreducibly complex. I defined irreducible complexity as

> a single system which is composed of several well-matched, interacting parts that contribute to the basic function, and where the removal of any one of the parts causes the system to effectively cease functioning. (1996, 39)

The difficulty for Darwinian theory is that

> An irreducibly complex system cannot be produced directly (that is, by continuously improving the initial function, which continues to work by the same mechanism) by slight, successive modifications of a precursor system, because any precursor to an irreducibly complex system that is missing a part is by definition nonfunctional. (1996, 39)

To illustrate the concept with a familiar example for a general readership, I pointed to a simple mechanical mousetrap, composed of several parts such as the base, hammer, spring, and so on, and noted that the absence of any of the parts destroys the mouse-catching ability of the trap. Darwin's vision of natural selection gradually improving function in "numerous, successive, slight modifications" (Darwin 1859) appears not to fit well with such systems. I went on to argue that, since intelligent agents are the only entities known to be able to construct irreducibly complex systems, the biochemical systems are better explained as the result of deliberate intelligent design.

But are gradual Darwinian natural selection and intelligent design the only potential explanations? Shanks and Joplin (1999) direct our attention to complexity theory, which concerns the ability of systems to self-organize abruptly, sometimes in surprising ways. They suggest that irreducibly complex biochemical systems might in principle be explained by self-organization, eliminating the need to invoke intelligence. They then go on to argue that biochemical systems are "redundantly complex"—that is, contain components that can be removed without entirely eliminating function.

After briefly describing the Belousov-Zhabotinsky reaction—Shanks and Joplin's main counterexample—I will first argue that the reaction does not meet the definition of irreducibly complex, because the interacting components are not "well-matched." I will then agree that redundant complexity exists, but show that not all of biochemistry is redundant.

2. A Closer Look At Chemical Self-Organization. The dissipation of energy in nature can organize matter and produce reaction pathways. A simple example is the clumping of matter into stars under the influence of gravity. More complex examples are tornados and the stellar nuclear pathways

that lead to the production of the heavy elements. These examples, however, have no direct relevance to the origin of biochemical systems. Shanks and Joplin (1999) offer what they think is a more pertinent example—the Belousov-Zhabotinsky reaction, a self-organizing chemical system discovered in the 1950s by B. P. Belousov in an attempt to model the Krebs cycle. The term "BZ reaction" is applied to a group of chemical reactions in which an organic substrate is oxidized by bromate ions in the presence of a transition metal ion and acid. Instead of proceeding monotonically to equilibrium, the reaction oscillates between two pathways because of a competition between bromide ion and bromous acid for reaction with bromate ion. Bromate oxidizes the metal ions, which in turn are re-reduced by reaction with organic substrate. When the reaction is well-stirred, the visible result is a solution that switches from one color to another at constant time intervals until the reaction materials are consumed. When the same reaction is set up as a thin, unstirred layer, waves of color change propagate through the layer. For details of the reaction pathways, see Gray and Scott 1994 and references therein.

Shanks and Joplin write that the BZ reaction "satisfies Behe's criteria for irreducible chemical complexity" because if any of the chemical components is removed "the characteristic behavior of the system is disrupted." Thus "Irreducible complexity in a self-organizing system" can be generated "without the aid of a designing deus ex machina" (1999, 272–273).

I disagree that the BZ reaction "satisfies Behe's criteria" for an irreducibly complex system. Although it does have interacting parts that are required for the reaction, the system lacks a crucial feature—the components are not "well-matched." The appearance of the modifier "well-matched" in the definition I constructed (above) reflects the fact that complexity is a quantitative property. A system can be more or less complex, so the likelihood of coming up with any particular interactive system by chance can be more or less probable. As an illustration, contrast the greater complexity of a mechanical mousetrap (mentioned above) with the much lesser complexity of a lever and fulcrum. Together a lever and fulcrum form an interactive system which can be used to move weights. Nonetheless, the parts of the system can have a wide variety of shapes and sizes and still function. Because the system is not well-matched, it could easily be formed by chance.

Systems requiring several parts to function that need not be well-matched, we can call "simple interactive" systems (designated 'SI'). Ones that require well-matched components are irreducibly complex ('IC'). The line dividing SI and IC systems is not sharp, because assignment to one or the other category is based on probabilistic factors which often are hard to calculate and generally have to be intuitively estimated based on always-incomplete background knowledge. Moreover, no law of physics auto-

matically rules out the chance origin of even the most intricate IC system. As complexity increases, however, the odds become so abysmally low that we reject chance as an explanation (Dembski 1998).

Just as I think that a gradual origin by natural selection is a good explanation for some things, I agree that a discontinuous origin by self-organization explains some things too. Nonetheless, I do not think either explains irreducible complexity. I argue that Shanks and Joplin's counterexample—the BZ reaction—is not IC; it is SI, because the components are not well-matched. To justify my position, let me first illustrate a well-matched system using the blood clotting cascade (Stubbs and Bode 1994). The active form of one protein of the cascade is called thrombin, which cleaves the soluble protein fibrinogen to produce fibrin, the insoluble meshwork of a blood clot. The chemistry catalyzed by thrombin is simply the hydrolysis of a certain fibrinogen peptide bond. However, all proteins are made of amino acid residues joined by peptide bonds. A typical protein contains several hundred peptide bonds. There is nothing remarkable about the bond in fibrinogen that is cleaved by thrombin. Yet thrombin selects that particular bond for cleavage out of literally hundreds of thousands of peptide bonds in its environment and ignores almost all others. It can do this because the shape of thrombin is well-matched to the shape of fibrinogen around the bond it cleaves. It "recognizes" not only the bond it cuts, but also a number of other features of its target. The other proteins of the clotting cascade (Stuart factor, proaccelerin, tissue factor, and so on) have similar powers of discrimination. So do virtually all of the components of the molecular machines I discussed in *Darwin's Black Box*.

Let us contrast this biochemical specificity with a comparable chemical reaction lacking such specificity. The peptide bonds of proteins can also be cleaved by simple chemicals. A typical procedure calls for incubating the protein in 6*N* hydrochloric acid at 110°C for twenty four hours. If fibrinogen were incubated under those conditions, the peptide bond that thrombin cleaves would be broken, but so would every other peptide bond in the protein. It would be completely reduced to amino acids. If thrombin were in the mix, it too would be completely destroyed. If the other proteins of the clotting cascade were there, no clotting would take place, even though the peptide bonds that are cleaved in the cascade would be cleaved, because all other peptide bonds would be hydrolyzed too. There is virtually no specificity to the chemical hydrolysis beyond the type of bond that is cleaved.

Similarly, the reactants of the BZ reaction are small organic or inorganic chemicals that show little specificity for each other. One ingredient, sodium bromate, is a general purpose oxidizing reagent and is capable of degrading a very large spectrum of chemicals besides the ones used in BZ reactions (thus its transport aboard airlines is forbidden). Another re-

quirement of the reaction is simply for a transition metal that can change its oxidation state, and a number of such metals are known, including iron, cerium, and manganese ions (Field 1972). A third requirement is for an organic molecule that can be oxidized. Many candidates could fulfill this role (ones that have been used include malonic, citric, maleic, and malic acids), and organic molecules can be oxidized by many reagents other than bromate. The last ingredient is simply a high concentration of sulfuric acid. As Field (1972, 308) noted, setting up BZ reactions "is an exceedingly easy task as they will occur over a wide range of concentrations and conditions."

The BZ class of self-organizing reactions—chemical oscillations—is surprising and interesting. Nonetheless, its complexity can be likened to other self-organizing systems found outside of biology, such as, say, tornados, which, although they command our attention, do not approach the specificity of well-matched, irreducibly complex biochemical systems.

3. Biochemical Self-Organization: Behavior vs. Origin. The dynamical behavior of the BZ reaction has been modeled by a set of two ordinary differential equations (Tyson 1994, 577). Because some biological systems can be modeled by similar mathematics, Shanks and Joplin (1999) conclude that self-organization can explain the behavior of the biological systems. There are several reasons to question the relevance of their point. First, they also note that "the substrates and products in these systems are very different from those in the BZ reaction" (1999, 273). In other words, we have traveled far from cerium, sodium bromate, and the other constituents of the chemical system. Second, and more importantly, the behavior of a system must be distinguished from its origin. As an illustration, consider highway traffic flow. A number of mathematical models have been used to describe traffic flow, some drawing on theories of self-organization (Schreckenberg and Wolf 1998). The mathematics, however, have not called the automobiles into being. The mathematics simply try to describe the typical behavior of traffic when a certain density is reached under conditions of restricted movement on a highway.

Examples of biological processes that show BZ dynamical behavior include glycolysis and aggregation of dispersed cells of the slime mold *Dictyostelium discoideum* into a slug. But consider the sophisticated components of the aggregation-signaling system of *D. discoideum*, which include: a cyclic AMP membrane receptor protein that can exist in an active and inactive form; an adenylate cyclase that binds to the active form of the receptor and itself becomes activated; a protein to export cyclic AMP into the extracellular medium; and more (Goldbeter 1996, Part I). All of that complicated machinery is ignored in BZ models—treated as a black box. Oscillations in the cellular concentration of glycolytic intermediates

are due in large part to the multi-talented phosphofructokinase (PFK), a tetrameric enzyme that can exist in two conformational states (an active form and a less-active one) and which has binding sites for a dozen different activators and inhibitors (Goldbeter 1996, Part III). Mathematical models of BZ behavior do not explain the origin of the impressive abilities of PFK any more than models of traffic flow explain the origin of brakes or gas pedals. Thus, even if a biological system displays self-organizing behavior, the question of its origin remains.

4. Not All Biochemical Systems Are Redundant. In contrast to claims about irreducible complexity, Shanks and Joplin write that "Real biochemical systems, we argue, manifest *redundant complexity*—a characteristic result of evolutionary processes" (1999, 268). By this they mean that biochemical pathways overlap and are interconnected, so that removal of one or even several components does not completely destroy the function. In support of their position they cite a diverse array of biochemical examples: the synthesis of an alternate pine tree lignin with increased content of dihydroconiferyl alcohol; viable mice in which the gene for the tumor suppressor *p53* was knocked out; and more. Their initial illustration is the metabolic pathways for the synthesis of glucose-6-phosphate. They point out that the molecule can be made by "several different isoforms or variants of hexokinase, and all are present, as a result of gene duplication, in varying proportions in different tissues." What's more, "Knock out one enzyme isoform and the other isoforms in the tissue can take over its function" (277).

True enough. The observation that some biochemical systems are redundant, however, does not entail that all are. And, in fact, some are not redundant. Consider the following examples of nonredundant metabolic pathways. Primates, including humans, cannot synthesize ascorbic acid (vitamin C) because they lack a functional gene for L-gulono-gamma-lactone oxidase, although a pseudogene is present (Nishikimi and Yagi 1991). Vitamin C is made by no other pathway. Hexosaminidase A is required to catabolize ganglioside G_{M2}; its loss results in Tay-Sachs disease (Kolter and Sandhoff 1998). These enzymes are parts of "real biochemical systems," but they do not "manifest redundant complexity." (For many, many additional examples, see Scriver 1995 or other texts on inborn errors of metabolism.) Therefore, arguments developed about the origin of redundant systems do not necessarily apply to all biochemical systems.

Shanks and Joplin's argument for redundant complexity has the same strengths and weaknesses when the subject moves from metabolic pathways to other biochemical systems. That is, they are right to notice that some systems or components are redundant, but wrong to extrapolate the conclusion to all systems. For example, they point to mice in which the

gene for the protein *p53* has been knocked out. *p53* is "involved in a number of fundamental cell processes, such as affecting gene transcription, acting as control points in the cell cycle, initiating programmed cell death," and more. Shanks and Joplin write that "Looking at this case from the standpoint of a 'genetic mousetrap model', one would naturally predict that the removal of this gene . . . would lead to catastrophic collapse of the developmental process. . . . . Such is not the case" (1999, 279). Yet contrast this case with that of mice in which the gene for either fibrinogen (Bugge et al. 1996a), tissue factor (Bugge et al. 1996b), or prothrombin (Sun et al. 1998) has been knocked out. Those proteins are all components of the blood clotting cascade, which I discussed prominently in *Darwin's Black Box* (1996, Ch. 4), claiming it is irreducibly complex. The loss of any one of those proteins prevents clot formation—the clotting cascade is broken. Thus Shanks and Joplin's concept of redundant complexity does not apply to all biochemical systems.

REFERENCES

Behe, Michael J. (1996), *Darwin's Black Box: The Biochemical Challenge to Evolution*. New York: The Free Press.

Bugge, Thomas H., Keith W. Kombrinck, Matthew J. Flick, Cynthia C. Daugherty, Mary Jo S. Danton, and Jay L. Degen (1996a), "Loss of Fibrinogen Rescues Mice from the Pleiotropic Effects of Plasminogen Deficiency", *Cell* 87: 709–719.

Bugge, Thomas H., Qing Xiao, Keith W. Kombrinck, Matthew J. Flick, Kenn Holmbäck, Mary Jo S. Danton, Melissa C. Colbert, David P. Witte, Kazuo Fujikawa, Earl W. Davie, and Jay L. Degen (1996b), "Fatal Embryonic Bleeding Events in Mice Lacking Tissue Factor, the Cell-Associated Initiator of Blood Coagulation", *Proceedings of the National Academy of Sciences of the United States of America* 93, 6258–6263.

Darwin, Charles. (1859), *The Origin of Species*. New York: Bantam Books.

Dembski, William A. (1998), *The Design Inference: Eliminating Chance Through Small Probabilities*. Cambridge: Cambridge University Press.

Field, Richard J. (1972), "A Reaction Periodic in Time and Space", *Journal of Chemical Education* 49: 308–311.

Goldbeter, Albert. (1996), *Biochemical Oscillations and Cellular Rhythms: The Molecular Bases of Periodic and Chaotic Behaviour*. Cambridge: Cambridge University Press.

Gray, Peter and Stephen K. Scott (1994), *Chemical Oscillations and Instabilities: Non-Linear Chemical Kinetics*. Oxford: Clarendon Press.

Kolter, Thomas and Konrad Sandhoff (1998), "Glycosphingolipid Degradation and Animal Models of G_{M2}-Gangliosidoses", *Journal of Inherited Metabolic Disease* 21: 548–563.

Nishikimi, Morimitsu and Kunio Yagi (1991), "Molecular Basis for the Deficiency in Humans of Gulonolactone Oxidase, a Key Enzyme for Ascorbic Acid Biosynthesis", *American Journal of Clinical Nutrition* 54: 1203S–1208S.

Schreckenberg, Michael and Dietrich E. Wolf (1998), *Traffic and Granular Flow*. Singapore: Springer.

Scriver, Charles R. (1995), *The Metabolic and Molecular Bases of Inherited Disease*. New York: McGraw-Hill, Health Professions Division.

Shanks, Niall and Karl H. Joplin (1999), "Redundant Complexity: A Critical Analysis of Intelligent Design in Biochemistry", *Philosophy of Science* 66: 268–282.

Stubbs, Milton T. and Wolfram Bode (1994), "Coagulation Factors and Their Inhibitors", *Current Opinion in Structural Biology* 4: 823–832.

Sun, William Y., David P. Witte, Jay L. Degen, Melissa C. Colbert, Melissa C. Burkart, Kenn Holmbäck, Qing Xiao, Thomas H. Bugge, and Sandra J. F. Degen (1998), "Prothrombin Deficiency Results in Embryonic and Neonatal Lethality in Mice", *Proceedings of the National Academy of Sciences of the United States of America* 95: 7597–7602.

Tyson, John J. (1994), "What Everyone Should Know About the Belousov-Zhabotinsky Reaction", in Simon A. Levin (ed.), *Frontiers in Mathematical Biology*. Berlin: Springer-Verlag, 569–587.

Exhibit 8

Biology and Philosophy journal article

Biology and Philosophy **16:** 685–709, 2001.

Reply to My Critics: A Response to Reviews of *Darwin's Black Box: The Biochemical Challenge to Evolution*

MICHAEL J. BEHE
Department of Biological Sciences
Lehigh University
111 Research Drive
Bethlehem, PA 18015
U.S.A.
E-mail: michael.behe@lehigh.edu

Abstract. In *Darwin's Black Box: The Biochemical Challenge to Evolution* I argued that purposeful intelligent design, rather than Darwinian natural selection, better explains some aspects of the complexity that modern science has discovered at the molecular foundation of life. In the five years since its publication the book has been widely discussed and has received considerable criticism. Here I respond to what I deem to be the most fundamental objections. In the first part of the article I address empirical criticisms based on experimental studies alleging either that biochemical systems I discussed are not irreducibly complex or that similar systems have been demonstrated to be able to evolve by Darwinian processes. In the remainder of the article I address methodological concerns, including whether a claim of intelligent design is falsifiable and whether intelligent design is a permissible scientific conclusion.

Key words: Darwinism, evolution, falsifiability, intelligent design, irreducible complexity, natural selection

1. Empirical objections

1.1. Is the question open?

In *Darwin's Black Box* (Behe 1996) I argued there are good reasons, based on the physical structures and functional properties of some biochemical systems, to think that they had been deliberately designed. (The focus of the book was exclusively on the mechanism of evolution. I agreed that descent-with-modification is well-supported.) The necessary starting point of the book was to show that the question is open – that, contrary to common assumption, the origins of many intricate cellular systems have not yet been

adequately explained in Darwinian terms. This point has met with general agreement. While most reviewers disagreed (often emphatically) with my proposal of intelligent design, most did admit to a current lack of Darwinian explanations. Here is a sampling of comments on the particular question of whether successful Darwinian accounts have yet been offered for complex biochemical systems:

> Microbiologist James Shapiro of the University of Chicago wrote in *National Review* that "There are no detailed Darwinian accounts for the evolution of any fundamental biochemical or cellular system, only a variety of wishful speculations" (Shapiro 1996). In *Nature* University of Chicago evolutionary biologist Jerry Coyne stated, "There is no doubt that the pathways described by Behe are dauntingly complex, and their evolution will be hard to unravel. ... [W]e may forever be unable to envisage the first proto-pathways" (Coyne 1996). In *Trends in Ecology and Evolution* Tom Cavalier-Smith, an evolutionary biologist at the University of British Columbia, commented, "For none of the cases mentioned by Behe is there yet a comprehensive and detailed explanation of the probable steps in the evolution of the observed complexity. The problems have indeed been sorely neglected – though Behe repeatedly exaggerates this neglect with such hyperboles as 'an eerie and complete silence'" (Cavalier-Smith 1997). University College, London, evolutionary biologist Andrew Pomiankowski agreed in *New Scientist*, "Pick up any biochemistry textbook, and you will find perhaps two or three references to evolution. Turn to one of these and you will be lucky to find anything better than 'evolution selects the fittest molecules for their biological function'" (Pomiankowski 1996). In *American Scientist* Yale molecular biologist Robert Dorit averred, "In a narrow sense, Behe is correct when he argues that we do not yet fully understand the evolution of the flagellar motor or the blood clotting cascade" (Dorit 1997).

Several scientists, on the other hand, have maintained that experimental evidence is actually already in hand showing either that the systems I described are not irreducibly complex ("irreducibly complex" means roughly that if one removes a component from a system, function is lost; much more about this later) or that similar systems can be produced by natural selection. In the next two sections I will address several such assertions. As I will briefly demonstrate, the arguments rest on mistaken readings or faulty interpretations of the data.

1.2. Is an "irreducibly complex" biochemical system actually reducible?

In its Feb/March 1997 issue, the Massachusetts Institute of Technology publication *Boston Review* featured a symposium discussing *Darwin's Black Box* and Richard Dawkins' *Climbing Mount Improbable*. Among the dozen essays was one by Russell Doolittle, an eminent biochemist at the University of California, San Diego, and member of the National Academy of Sciences. Doolittle took direct issue with my claims regarding the blood clotting system. I had devoted a chapter of *Darwin's Black Box* to blood clotting, asserting that it is an irreducibly complex system, does not fit well within a Darwinian framework, and that "*no one on earth has the vaguest idea how the coagulation cascade came to be*" (Behe 1996: 97; emphasis in the original). Doolittle, an expert on blood clotting, disagreed.

Professor Doolittle cited a paper by Bugge et al. (1996a), entitled "Loss of Fibrinogen Rescues Mice from the Pleiotropic Effects of Plasminogen Deficiency." Of the paper he wrote:

> Recently the gene for plaminogen [*sic*] was knocked out of mice, and, predictably, those mice had thrombotic complications because fibrin clots could not be cleared away. Not long after that, the same workers knocked out the gene for fibrinogen in another line of mice. Again, predictably, these mice were ailing, although in this case hemorrhage was the problem. And what do you think happened when these two lines of mice were crossed? For all practical purposes, the mice lacking both genes were normal! Contrary to claims about irreducible complexity, the entire ensemble of proteins is not needed. Music and harmony can arise from a smaller orchestra (Doolittle 1997).

A closer look at Bugge et al (1996a) shows Doolittle to have misread the paper.[1] Briefly, plasminogen is the precursor of plasmin, a protein that degrades blood clots (Clots eventually have to be cleared away). Fibrinogen, on the other hand, is the precursor of fibrin, the clot material which entraps blood cells and blocks bleeding. The point of Bugge et al. (1996a) was that if one crosses the two knock-out strains, producing plasminogen-plus-fibrinogen deficiency in individual mice, the mice do not suffer the same problems that afflict mice lacking plasminogen alone[2] (Bugge et al. 1995). Since the title emphasized that mice are "rescued" from some ill-effects, one might be misled into thinking that the double-knockout mice were normal. They are not. Bugge et al. (1996a) state in their abstract, "Mice deficient in plasminogen and fibrinogen are phenotypically indistinguishable from fibrinogen-deficient mice." In other words, the double-knockouts have all the problems that mice lacking only fibrinogen were previously shown to have: they do not form clots, they hemorrhage, and the females die if they become

Table 1. Symptoms of gene knock-out mice

Lacking plasminogen[1]	*Lacking fibrinogen*[2]	*Lacking both*[3]
thrombosis	failure to clot	failure to clot
ulcers	hemorrhage	hemorrhage
high mortality	death during pregnancy	death during pregnancy

[1]Bugge et al. 1995;
[2]Suh et al. 1995;
[3]Bugge et al. 1996a.

pregnant (Suh et al. 1995) [Table 1]. They are definitely not "normal." More to the point, they have no functioning clotting system and clearly are not viable candidates for evolutionary intermediates.

Although the knock-out mice in this study are not successful models for Darwinian evolutionary development of the blood clotting system, I believe the study is nonetheless quite relevant to the question of the possible step-by-step Darwinian origin of the clotting system, for two reasons. First, because it highlights the importance of the *regulation* of biochemical systems. As Halkier (1992: 104) observed concerning the coagulation cascade: "A system of this kind cannot just be allowed to free-wheel. ... Too little or too much activity would be equally damaging for the organism. Regulation is a central issue in blood coagulation." In discussing the blood clotting system in *Darwin's Black Box*, even though I briefly noted its function of removing clots, and though I highlighted the importance of regulation elsewhere in the book, I did not count plasminogen as part of the irreducibly complex clotting cascade, because it is not involved in the actual formation of the clot (Behe 1996: 86). Nonetheless, the careful experimental work of Bugge et al (1996a) reinforces Halkier's point of the serious consequences of failure to regulate a system such as the clotting cascade. Thus the study shows that from its inception the clotting system would have to be tightly regulated. Any Darwinian scheme purporting to account for clotting, therefore, would have to demonstrate how an incipient cascade would be regulated. To the extent this creates yet another impediment to a Darwinian explanation, it leaves the field open to other possible explanations.

The second reason Bugge et al.'s results are relevant is that they confirm my claim that the system is irreducibly complex. As expected, when fibrinogen is deleted, the blood clotting cascade no longer functions. Further work by the same authors in which other components of the clotting cascade – prothrombin (Sun et al. 1998) and tissue factor (Bugge et al. 1996b) – were knocked out shows that those components are also necessary for a functioning cascade.

1.3. Has the development of irreducible complexity by Darwinian means already been experimentally demonstrated?

In his book *Finding Darwin's God* Brown University cell biologist Kenneth Miller claims that "a true acid test" of the ability of Darwinism to deal with irreducible complexity would be to "[use] the tools of molecular genetics to wipe out an existing multipart system and then see if evolution can come to the rescue with a system to replace it" (Miller 1999: 145). I agree – a decisive blow against the argument of *Darwin's Black Box* would be to experimentally demonstrate the production of an irreducibly complex biochemical system under selective pressure in a model organism. Miller then claims it has already been done. With that I completely disagree. I will show that the experimental results Miller cites do not at all justify his claims.

In a section entitled "Parts is Parts," in which he discusses the careful work over the past quarter-century of Barry Hall of the University of Rochester on the experimental evolution of a lactose-utilizing system in *E. coli*, Miller excitedly remarks:

> Think for a moment – if we were to happen upon the interlocking biochemical complexity of the reevolved lactose system, wouldn't we be impressed by the intelligence of its design? Lactose triggers a regulatory sequence that switches on the synthesis of an enzyme that then metabolizes lactose itself. The products of that successful lactose metabolism then activate the gene for the lac permease, which ensures a steady supply of lactose entering the cell. Irreducible complexity. What good would the permease be without the galactosidase? ... No good, of course. By the very same logic applied by Michael Behe to other systems, therefore, we could conclude that the system had been designed. Except we *know* that it was *not* designed. We know it evolved because we watched it happen right in the laboratory (Miller 1999: 146; Miller's emphasis)!

When one examines Hall's publications directly, however, without the intervening lens of Miller's enthusiasm, one sees that the work is entirely consistent with my claim that irreducibly complex biochemical systems require intelligent design. Indeed, I could have used it as an example in *Darwin's Black Box*. I stress three points which I will discuss only briefly here. A more complete treatment can be found elsewhere (Behe et al. 2000).

1) *Only one part of the pre-existing, multi-part, lactose-utilizing system Hall studied was knocked out.* Hall eliminated the gene for just the galactosidase and clearly emphasized that:

> All of the other functions for lactose metabolism, including lactose permease and the pathways for metabolism of glucose and galactose, the products of lactose hydrolysis, remain intact, thus re-acquisition of lactose utilization requires only the evolution of a new ß-galactosidase function (Hall 1999).

Thus, contrary to Miller's own criterion for "a true acid test," a multipart system was not "wiped out" – only one component was deleted. Replacing one component of a pre-existing system does not show that a system lacking multiple components could be repaired by Darwinian means.

2) *The changes required to recover activity are quite small and can be classified as microevolution.* The function of the deleted enzyme was eventually taken over by a previously unnoticed cellular enzyme – a homologous galactosidase with an active site that was already nearly identical to that of the deleted enzyme (Hall 1999). The unmutated replacement enzyme already possessed a slight ability to hydrolyze lactose; selection of mutants simply improved the pre-existing hydrolytic activity. Importantly, by phylogenetic analysis Hall concluded that the mutations in the homologous protein he studied are the *only* ones in *E. coli* that confer the ability to hydrolyze lactose.

> The phylogenetic evidence indicates that either Asp-92 and Cys/Trp-977 are the only acceptable amino acids at those positions, or that all of the single base substitutions that might be on the pathway to other amino acid replacements at those sites are so deleterious that they constitute a deep selective valley that has not been traversed in the 2 billion years since those proteins diverged from a common ancestor (Hall 1999).

Such results hardly support extravagant claims for the creativeness of Darwinian processes. They are microevolutionary changes on the order of the development of antibiotic resistance in bacteria, and far from the development of a new irreducibly complex system that Miller claimed.

3) *The re-development of the system required intelligent intervention.* Although only one component of the multi-part system was knocked out, and only small changes were needed to restore it, yet the system still had to be artificially supported by intelligent intervention through phases when the bacteria would otherwise have been unable to utilize lactose. The intervention was in the form of the addition of isopropylthiogalactoside (IPTG), a chemical which induces the gene coding for a pre-existing permease that allows lactose to enter the cell. As Barry Hall forthrightly notes:

> At this point it is important to discuss the use of IPTG in these studies. Unless otherwise indicated, IPTG is *always* included in media

> containing lactose or other ß-galactoside sugars. The sole function of the IPTG is to induce synthesis of the lactose permease, and thus to deliver lactose to the inside of the cell. Neither the constitutive nor the inducible evolved strains grew on lactose in the absence of IPTG (Hall 1982; Hall's emphasis).

This aspect of Hall's results can be likened to an origin-of-life researcher who, at a critical point in an experiment, buys a necessary chemical from a laboratory supply house and adds it to her reaction. Whatever the results of the experiment, interesting though they may be, they crucially reflect the intelligence of the experimenter rather than the course of unaided natural processes.

Miller's writing notwithstanding, it certainly seems to me that Barry Hall's experiments actually count in favor of intelligent design, and against Darwinian evolution, because: 1) despite extensive work over the course of decades Darwinian processes were seen to produce only minor changes; and 2) intelligent intervention was necessary to keep the bacterial cells growing when the galactosidase was deleted. Such results are exactly what an intelligent design proponent would expect, given the complexity of the system.

In closing this section I would like to point out that two noted scientists, Doolittle and Miller, who were intent on showcasing the power of Darwinian processes specifically to rebut my argument for design, both cited work which on closer inspection is at best unsupportive of their position, and at worst antagonistic to it. I think this strongly confirms the view of the majority of scientific reviewers of my book who agreed that the origins of many intricate cellular systems have not yet been explained in Darwinian terms. Thus I conclude that, at the least, the question of whether Darwinian processes can account for irreducibly complex systems remains open.

1.4. Defects in the definition of irreducible complexity

The concept of irreducible complexity is central to my argument against the sufficiency of Darwinian processes. Thus it requires careful scrutiny. In *Darwin's Black Box* I defined the term in the following way:

> By irreducibly complex I mean a single system which is composed of several well-matched, interacting parts that contribute to the basic function, and where the removal of any one of the parts causes the system to effectively cease functioning (Behe 1996: 39).

As an illustration of the concept I showed a mousetrap built of a number of pieces (spring, hammer, platform, and so on), all of which were necessary to its function. It is now clear that, although the mousetrap paradigm remains

a good one, there is some ambiguity in the written definition, as discussed below. Nonetheless, I think the definition can be repaired.

In his review of *Darwin's Black Box* in *Boston Review* University of Rochester evolutionary biologist H. Allen Orr agrees that many biological systems are "irreducibly complex," but argues that Darwinian evolution can, at least in theory, directly account for them. As I will show, in critical respects he has not followed my definition of irreducible complexity, which causes confusion. Because others have followed Orr's reasoning, I will go into detail about where it goes wrong. Nonetheless, Orr has also helpfully put his finger on an ambiguity in the definition, which I will discuss subsequently. Elimination of the ambiguity will aid in focusing attention on the difficulty for Darwinian gradualism.

Attributing the following scenario to the early 20th-century geneticist H. J. Muller, Orr writes:

> An irreducibly complex system can be built gradually by adding parts that, while initially just advantageous, become – because of later changes – essential. The logic is very simple. Some part (A) initially does some job (and not very well, perhaps). Another part (B) later gets added because it helps A. This new part isn't essential, it merely improves things. But later on, A (or something else) may change in such a way that B now becomes indispensable. This process continues as further parts get folded into the system. And at the end of the day, many parts may all be required (Orr 1996–7).

Orr later gives a biological example of what he has in mind.

> The transformation of air bladders into lungs that allowed animals to breathe atmospheric oxygen was initially just advantageous: such beasts could explore open niches – like dry land – that were unavailable to their lung-less peers. But as evolution built on this adaptation (modifying limbs for walking, for instance), we grew thoroughly terrestrial and lungs, consequently, are no longer luxuries – they are essential. The punch-line is, I think, obvious: although this process is thoroughly Darwinian, we are often left with a system that is irreducibly complex (Orr 1996–7).

In his example Orr has not adhered to the concept of irreducible complexity as I defined it. First, my definition requires that one consider "a single system." Whole organs, such as lungs or swim bladders, are not "single systems." Indeed, lung tissue contains many of the separate, irreducibly complex systems I described in *Darwin's Black Box*: cilia; intracellular transport systems; blood clotting proteins; and so on. If the origins of those molecular systems are currently unexplained, then systems built on them (such as cells

or organs) are unexplained as well. In my book I strongly emphasized that one has to examine biological systems at the molecular level to determine if they were likely produced by Darwinian processes or not. The reason is that whole cells and organs contain so many active, unknown components – a typical cell contains thousands of specific, separate macromolecules, most acting, both separately and together, in unknown ways – that one is dealing with a "black box" whose capacities are substantially obscure.[3]

A second confusion in Orr's example is with regard to the specification of the system and function under consideration. I consider the function to be the activity that the *system itself* performs: triggered snapping for a mousetrap; rotary propulsion for a flagellum; controlled formation of a barrier for the blood clotting system; and so on. My definition is intended to mean that removal of a part causes the system itself – the discrete system currently under consideration – to cease functioning. Yet it is not at all clear from Orr's example what is the system he is considering, whether lungs, swim bladders, or even the whole animal. If he is considering that the system is, say, the lungs, then what are the parts of the lungs without which they will not work? Orr does not list them, so perhaps that is not what he had in mind. The only other part, besides swim bladders and lungs, that he mentions is "limbs for walking." But vertebrate limbs are certainly not part of an irreducibly complex system that includes the lungs, at least not in my sense of the term. For example, if one removes the spring from a mousetrap, or the drive shaft from a bacterial flagellum, the systems themselves immediately cease working. But if a limb is amputated from a terrestrial vertebrate, the lungs can easily continue to function.

It seems likely that Orr had in mind that the "system" was the entire animal, and that he was thinking of an alternate conception of irreducible complexity that can perhaps be paraphrased as, "if you remove this part, the organism will eventually die," or "it will not compete successfully with other organisms." And the "function" he seems to have in mind is to help the whole animal or species prosper. So without lungs a terrestrial vertebrate would die after a minute or so, and without limbs the animal couldn't work well on land. However, that is not the same concept as I discussed. In my thinking, if one removes a part of a clearly defined, irreducibly complex system, the system itself immediately and necessarily ceases to function.

Although I think Orr's lung example is off the mark, and although the scenario doesn't help the problem of how Darwinian processes could have put together molecular systems such as I discussed, Orr's "part A, part B" scenario does identify an ambiguity in my definition of irreducible complexity. As I constructed it the definition is equivocal; it doesn't distinguish between systems that necessarily must have several parts because of the

mechanism by which they perform the task at hand, and systems which use a number of parts to do a task which could in theory be done with one. I had intended the definition to cover only the former group.

Some systems necessarily require several parts to function as they do. A simple mechanical example is a lever and fulcrum. A molecular example is the bacterial flagellum, of which I wrote: "The bacterial flagellum uses a paddling mechanism. Therefore it must meet the same requirements as other such swimming systems. Because the bacterial flagellum is necessarily composed of at least three parts – a paddle, a rotor, and a motor – it is irreducibly complex" (Behe 1996: 72). And of the intracellular transport system I wrote "Because gated transport requires a minimum of three separate components to function, it is irreducibly complex." The components were: 1) an identification mark; 2) a component to recognize the mark; and 3) a gate that is activated when the mark is recognized. Thus systems such as the flagellum and intracellular transport must have several components to do their jobs the way they do them. They do not fit Orr's scenario – there is no single part A that does the job, even poorly, so that a part B can come along later to help it.

However, one can indeed imagine a different type of molecular system where a task can be performed by one part. Perhaps some protein has a weak activity by itself, and another protein comes along to act as Orr's part B, perhaps by binding to the first protein and stabilizing the active conformation.[4] If that were to happen further mutations might change the system such that, although it functioned when both components were present, in the absence of part B, part A wouldn't have even a weak activity. Nonetheless, as can be seen from the fact that the system started out with just one component (Orr's part A), there is nothing about the mechanism of the task that makes it impossible for it to be carried out by a single component. Thus we have two conceptually distinguishable categories: 1) one in which a given task can theoretically be done by a given mechanism using a single component, but in fact several components are used by the cell; and 2) one in which more than one component is necessarily required to carry out a given task by a given mechanism.

Although I only intended to include the second category, my definition of irreducible complexity currently does not distinguish between the two. Fortunately, however, the confusion does not affect the science and the defect can be repaired easily enough by inserting a word to define irreducible complexity as: a single system which is *necessarily* composed of several well-matched, interacting parts that contribute to the basic function, and where the removal of any one of the parts causes the system to effectively cease functioning. I think that, with the discussion above in mind, such a definition would

include those systems I discussed (the flagellum, mousetrap, and others) while excluding even molecular instances of the "part A, part B" situation Orr had in mind.

After defining the term in *Darwin's Black Box*, I went on to argue that irreducibly complex systems are obstacles for Darwinian explanations.

> An irreducibly complex system cannot be produced directly (that is, by continuously improving the initial function, which continues to work by the same mechanism) by slight, successive modifications of a precursor system, because any precursor to an irreducibly complex system that is missing a part is by definition nonfunctional (Behe 1996: 39).

However, commentary by Robert Pennock and others has made me realize that there is a weakness in that view of irreducible complexity. The current definition puts the focus on removing a part from an already-functioning system. Thus, seeking a counterexample to irreducible complexity, in *Tower of Babel* Pennock writes about a part in a sophisticated chronometer, whose origin is simply assumed, which breaks to give a system that he posits can nonetheless work in a simpler watch in a less demanding environment.[5] The difficult task facing Darwinian evolution, however, would not be to *remove* parts from sophisticated pre-existing systems; it would be to *bring together* components to make a new system in the first place. Thus there is an asymmetry between my current definition of irreducible complexity and the task facing natural selection. I hope to repair this defect in future work.

2. Methodological objections

2.1. Is intelligent design falsifiable?

In addition to empirical questions about the results of particular experiments and their interpretation, concerns have been expressed about whether intelligent design is a scientific theory at all. In particular, it has been claimed that intelligent design is not falsifiable or that it is tantamount to invoking a miracle, which is no explanation. Either of these features, the argument goes, disqualifies design as a scientific hypothesis. I will discuss these objections in the following two sections.[6]

Some reviewers of *Darwin's Black Box* have objected that intelligent design is not falsifiable. I will argue that it is. However, to decide whether, or by what evidence, it is falsifiable, one first has to be sure what is meant by "intelligent design." By that phrase someone might mean that the laws of nature themselves are designed to produce life and the complex systems that undergird it. In fact, something like that position has been taken by the

physicist Paul Davies and the geneticist Michael Denton in their recent books, respectively, *The Fifth Miracle: The Search for the Origin and Meaning of Life* (Davies 1999) and *Nature's Destiny: How the Laws of Biology Reveal Purpose in the Universe* (Denton 1998). That stance, although not exactly endorsed, seems at least to be acceptable to the National Academy of Sciences:

> Many religious persons, including many scientists, hold that God created the universe and the various processes driving physical and biological evolution and that these processes then resulted in the creation of galaxies, our solar system, and life on Earth. This belief, which sometimes is termed "theistic evolution," is not in disagreement with scientific explanations of evolution (National Academy of Sciences 1999: 7).

In such a view even if we observe new complex systems being produced by selection pressure in the wild or in the laboratory, design would not be falsified because it is considered to be built into natural laws. Without commenting on the merits of the position, let me just say that that is not the meaning I assign to the phrase. By "intelligent design" (ID) I mean to imply design beyond the simple laws of nature. That is, taking the laws of nature as given, are there other reasons for concluding that life and its component systems have been intentionally arranged, just as there are reasons beyond the laws of nature for concluding a mousetrap was designed? In my book, and in this article, whenever I refer to ID I mean this stronger sense of design-beyond-laws. Virtually all academic critics of my book have taken the phrase in the strong sense I meant it.

In the strong sense ID is no longer condoned by the National Academy, for a specific reason: "[I]ntelligent design ... [is] not science because [it is] not testable by the methods of science" (National Academy of Sciences 1999: 25). In his review of *Darwin's Black Box* for *Nature*, University of Chicago evolutionary biologist Jerry Coyne explains in some detail why he also thinks intelligent design is unfalsifiable.

> If one accepts Behe's idea that both evolution and creation can operate together, and that the Designer's goals are unfathomable, then one confronts an airtight theory that can't be proved wrong. I can imagine evidence that would falsify evolution (a hominid fossil in the Precambrian would do nicely), but none that could falsify Behe's composite theory. Even if, after immense effort, we are able to understand the evolution of a complex biochemical pathway, Behe could simply claim that evidence for design resides in the other unexplained pathways. Because we will never explain everything, there will always be evidence for design. This regressive ad hoc creationism may seem clever, but it is certainly not science (Coyne 1996).

Coyne's and the National Academy's conclusion that design is unfalsifiable, however, seems to be at odds with the arguments of other reviewers of my book. Clearly, Russell Doolittle, Kenneth Miller, and others have advanced scientific arguments aimed at falsifying ID. Now, one can't have it both ways. One can't say both that ID is unfalsifiable (or untestable) and that there is evidence against it. Either it is unfalsifiable and floats serenely beyond experimental reproach, or it can be criticized on the basis of our observations and is therefore testable. The fact that critical reviewers advance scientific arguments against ID (whether successfully or not) shows that intelligent design is indeed falsifiable.

In fact, *intelligent design is open to direct experimental rebuttal.* Here is a thought experiment that makes the point clear. In *Darwin's Black Box* I claimed that the bacterial flagellum was irreducibly complex and so required deliberate intelligent design. The flip side of this claim is that the flagellum can't be produced by natural selection acting on random mutation, or any other unintelligent process. To falsify such a claim, a scientist could go into the laboratory, place a bacterial species lacking a flagellum under some selective pressure (for mobility, say), grow it for ten thousand generations, and see if a flagellum – or *any* equally complex system – was produced. If that happened, my claims would be neatly disproven.

What about Professor Coyne's concern that, if one system were shown to be the result of natural selection, proponents of ID could just claim that some other system was designed? I think the objection has little force. If natural selection were shown to be capable of producing a system of a certain degree of complexity, then the presumption would be that it could produce any other system of an equal or lesser degree of complexity. If Coyne demonstrated that the flagellum (which requires approximately forty gene products) could be produced by selection, I would be rather foolish to then assert that the blood clotting system (which consists of about twenty proteins) required intelligent design.

Let's turn the tables and ask, how could one falsify a claim that a particular biochemical system was produced by Darwinian processes? (Coyne's remarks about a Precambrian fossil hominid are beside the point since I dispute the mechanism of natural selection, not common descent. I would no more expect to find a fossil hominid out of sequence than he would.) Kenneth Miller announced an "acid test" for the ability of natural selection to produce irreducible complexity. He then decided that the test had been passed, and unhesitatingly proclaimed intelligent design to be falsified ("Behe is wrong"; Miller 1999: 147). But if, as it certainly seems to me, *E. coli* actually fails the lactose-system "acid test," would Miller consider Darwinism to be falsified? Almost certainly not. He would surely say that the

experiment started with the wrong bacterial species, used the wrong selective pressure, and so on. Leave aside the question of whether that is a legitimate response or not. The point here is that ID could potentially be falsified by the results of a single series of rather straightforward experiments, such as Barry Hall conducted (Hall 1982, 1999). Darwinian evolution can't.

Does the falsifiability of the argument to design change if the possibility is left open that the designer is God or some other supernatural agent? No – not if one understands the phrase "intelligent design" in the sense which I used. Because I argue that unintelligent means cannot produce irreducibly complex systems such as I described, an experimental demonstration of the ability of natural selection to do just that would show my argument to be false, no matter who one thought the designer might otherwise have been. The only way around such a result would be to say that the designer was acting furtively during the experiment itself – and during any subsequent repetition of the experiment. In my opinion that is a defensible position if one thinks the experimenters were consciously or unconsciously biasing the results. However, I would not think it defensible to claim that supernatural agents were controlling the experiment. Although someone somewhere might conceivably be found who would take that position, I certainly would not. I would understand the results as a definitive refutation of my position.

I think Professor Coyne and the National Academy of Sciences have it exactly backwards. A strong point of intelligent design is its vulnerability to falsification.[7] A weak point of Darwinian theory is its resistance to falsification. What experimental evidence could possibly be found that would falsify the contention that complex molecular machines evolved by a Darwinian mechanism? I can think of none.

2.2. *Is intelligent design equivalent to invoking a miracle?*

Intelligent design has been criticized as tantamount to invoking a miracle (For example, see Futuyma 1997; Ruse 1997). Simply declaring "God did it", the argument goes, is not a scientific explanation. Furthermore, understanding is not advanced by postulating a designer that is surely more complex than the designed system one is trying to account for. Therefore intelligent design is no explanation of any kind.

I will reply to such criticisms in this section. Although I will not give a complete answer, I will strive to show that such objections do not currently have enough force to constitute an obstacle to a theory of intelligent design. Questions which I will address are the following: Is it possible that the designer of terrestrial life is a natural entity? Is it plausible that the designer is a natural entity? If the designer is a supernatural entity, is intelligent design an "explanation"? Would progress in science be stymied by a theory

of intelligent design? Must a designer be more complex than the systems it designs?

Is it possible that the designer is a natural entity? If the designer were a natural entity then questions about God, the supernatural, and miracles would be moot. Thus we can begin by asking whether it is even possible for the designer to be a natural entity. This can be divided into two questions: 1) is it logically possible? and, 2) is it physically possible? The key consideration is that of irreducible complexity. If irreducible complexity requires intelligent design, and if any natural designer must contain irreducibly complex systems, and if the universe is not infinitely old allowing infinite regress, then eventually one runs into the problem of "who designed the designer?" Nonetheless, it is at least logically possible to have a natural designer who does not contain irreducibly complex systems, because there is nothing in the concept of intelligent designer that entails irreducible complexity. "Not irreducibly complex" and "natural intelligent designer" are not contradictory terms, so the conjunction of the terms violates no logical tenet.

Logical possibility is perhaps the least demanding criterion. A more interesting question is whether a non-irreducibly-complex natural designer is physically possible. By "physically possible" I mean only that something is not contradicted by known physical laws – not that we have any evidence of it. In other words, by the phrase physically possible I intend to leave the door open to all speculative phenomena for which we do not have strong positive evidence that they are directly contrary to well-established physical principles. In this sense I think a natural intelligent designer is physically possible. As I wrote in *Darwin's Black Box*, perhaps the designer could be made of gas particles or self-organizing electromagnetic fields or something else which would strike us as fantastic, but is physically possible and does not involve irreducible complexity. It is noteworthy that serious scientists have proposed that life could exist in such places as the atmosphere of Jupiter (Shapiro1999) and that it could at least begin as clay crystals (Cairns-Smith 1985).

We may not need to resort to utterly alien life forms, however. A non-irreducibly complex entity could conceivably be based on carbon chemistry not very much unlike our own. After all, although some terrestrial biochemical systems are irreducibly complex, others aren't (such as some metabolic pathways, cell membranes, oxygen transport, etc.). Furthermore, functions that are performed by irreducibly complex terrestrial systems may be accomplished by simpler systems elsewhere. For example, the function of a mechanical mousetrap can be performed by a glue trap, which is not irreducibly complex. In speculating on the origin of life and the first cells scientists often invoke relatively simple systems that can accomplish a known

cellular function at least to a degree. Perhaps functions that are performed by irreducibly complex biochemical systems in humans can be performed by simpler systems, or somehow gotten around, while sticking with familiar chemical principles. I must admit that I am not prepared to spell out how that might be done. My purpose here is only to argue that we can't currently rigorously rule it out.

Is it plausible that the designer is a natural entity? While such designers as discussed above may be possible in the sense that there is not positive proof they cannot exist, we can ask further whether it is plausible that they actually do or did exist. Plausibility, of course, is to a large extent in the eye of the beholder. For example, while some scientists consider it highly likely that the universe is teeming with intelligent life (Shapiro 1999), others think it very probable ours is the only planet with intelligent life (Ward and Brownlee 2000). And these convictions are held despite the absence of an accepted theory for how life may originate or of much understanding of what constitutes intelligence.

The problem is the following. Currently we have knowledge of only one type of natural intelligent designer even remotely capable of conceiving such structures as are found in the cell, and that is a human. Our intelligence depends critically on physical structures in the brain which are irreducibly complex. Extrapolating from this sample of one, it may be that all possible natural designers require irreducibly complex structures which themselves were designed. If so, then at some point a supernatural designer must get into the picture.

I myself find this line of reasoning persuasive. In my estimation, although possible in a broadly permissive sense, it is not plausible that the original intelligent agent is a natural entity. The chemistry and physics that we do know weigh heavily against it. If natural intelligence depends on physical organization, then the organization seems likely to have to be enormously complex and stable over reasonable periods of time. While simpler systems may perform the tasks that irreducibly complex systems perform in terrestrial life, they would likely perform them more slowly and less efficiently, so that the complexity required for intelligence would not ultimately be achieved. Thus in my judgment it is implausible that the designer is a natural entity.

I should add that there is nothing in the previous reasoning to rule out the hypothesis that we terrestrials were designed by a natural designer which was itself designed by a supernatural designer, or that there was a series of designers between the supernatural one and us, or some variation of this. It simply means that at the beginning of the chain, input from beyond nature was required.

If the designer is a supernatural entity, is intelligent design an "explanation"? Is it a "miracle"? Let me first acknowledge that, as there could have been a series of natural designers, there could also have been a series of supernatural ones, with one designing another which eventually designed an intelligent natural being. Or several supernatural entities could have collaborated to design natural beings, or some variation of this. I am not proposing these scenarios seriously, but just to show that a bare hypothesis of intelligent design leaves open many possibilities. For simplicity, in the following discussion I will speak of just one supernatural designer which, as Thomas Aquinas might say, we will call God. In addressing the above questions I will first assume that God exists. To justify the assumption I simply note that, in addition to the majority of humankind, a number of prominent philosophers and scientists profess to believe in God's existence. Later I will consider the situation where God's existence is in dispute.

Assuming that God does exist, then, is the hypothesis of intelligent design an "explanation"? To answer this question we must first pay some attention to the limited nature of a claim of intelligent design for any designer. An assertion that some device or system was intelligently designed is *not an explanation of the mechanism* by which it was assembled. It is simply the claim that intelligent input was involved at some point in its assembly. To illustrate, a rag doll in a store might carry a tag saying "hand-sewn", but its unscrupulous manufacturers actually used automated machinery to produce it. Looking at the doll in the store we may not know how it was manufactured, but we easily apprehend that it was purposely designed, whether directly by hand, indirectly by machine, or some other means. Or consider a science fiction example. Suppose we are shown two identical-appearing hand guns. One was manufactured in a gun factory. The other is a duplicate of the first where the original gun was put in a black box, a beam shone upon it, a few dials twirled and lights blinked, and out of the box came an exact replica. Examining the two guns we may not be able to tell which was which, or we might be able to tell only after long and strenuous investigation. Yet we have no trouble at all deciding they were both intelligently designed. Furthermore, in the case of the duplicator, we may never be able to figure out the mechanism for how the second gun was made, yet the conclusion of design remains solid.

With the above examples in mind, it seems to me that a conclusion of intelligent design in cases where the designer is likely to be God is as much an explanation as a conclusion of intelligent design for cases where the designer is a natural being. One is simply asserting that design is part of the causal history of the system and that without design the system would not exist. But one is not specifying the mechanism by which it was produced. The question

of the mechanism by which the system was designed is separate from the question of whether it was designed, and may be much more difficult to answer. If the designer was in fact God, then there is good reason to suppose that the mechanism of design will forever remain beyond us. Yet, whether progress on the mechanism of design is possible or not, the conclusion of design does not absolutely require it.

Perhaps intelligent design in biochemistry is some sort of an explanation, but is it a "scientific" explanation if the designer is likely to be God? I contend that it is. Without getting into the difficult problem of trying to define science, I will just say that I think any explanation which rests wholly on empirical evidence and basic logic deserves the appellation "scientific".[8] The conclusion of intelligent design in biochemistry rests exclusively on empirical evidence – the structures and functions of the biochemical systems – plus principles of logic (for example, see Dembski's (1998) *The Design Inference* and Ratzsch's (2001) *Nature, Design, and Science*). No particular tenet of faith is involved.[9] Therefore, I consider design to be a scientific explanation (whether ultimately correct or not).

Well, if one thinks that the most plausible designer of life is God, then is the hypothesis of intelligent design tantamount to invoking a miracle?[10] I think there are actually two questions here: 1) does the hypothesis imply a miracle probably happened? and 2) if so, does the hypothesis concern the miracle itself? Yes to the first, no to the second. Although a hypothesis of the intelligent design of aspects of life may reasonably be taken to imply the involvement of supernatural agency – a "miracle" – at some (perhaps quite remote) point, it does not concern it directly. Rather, it reaches its conclusion based on tangible, empirical features of a system and proceeds from there. That, I argue, is not unprecedented in science. To illustrate the point, consider the Big Bang hypothesis. In the middle part of the 20th century the observation of galactic red shifts led to the proposal of an expanding universe and to the hypothesis that the universe had a beginning in the distant past – the Big Bang. A number of scientists thought that the Big Bang hypothesis had theistic implications.[11] After all, what might cause the beginning of nature if not something outside of nature? The actual coming-into-being of the universe may have been a supernatural event, some thought – a miracle. Nonetheless, the Big Bang hypothesis justified itself with empirical evidence, and scientific investigations started with the fact of the Big Bang itself and proceeded to examine physical consequences which flowed from it. In other words, the question of the (possibly miraculous) mechanism producing the Big Bang was bracketed; the beginning of the universe was treated as a boundary condition.

The beginning of nature may indeed have been a miracle, but the Big Bang hypothesis is a scientific one which does not concern miraculous matters.[12] I place the intelligent design hypothesis in the same category. Although the process by which some structures of life were designed may have involved supernatural agency, we can bracket that question, treat the designed structures as akin to boundary conditions, and proceed from there.

Is science stymied if we hypothesize that aspects of life were designed? Another objection to intelligent design theory is that it is a "science stopper", that it places some questions off-limits to scientific investigation and thus discourages progress. I think much of that objection arises from a failure to recognize that all scientific theories do the same. To the extent a theory proposes to explain an aspect of nature, it simultaneously claims that other explanations are incorrect, predicts that experiments that aren't designed in conformity with the theory will fail, and in that way discourages them. To illustrate, among other things Einstein's theory of relativity holds that no object with mass can travel faster than light. Certainly any person working within the framework of the theory will be discouraged from conducting extensive experiments to show that a massive object can travel faster than light (except perhaps to confirm the expected failure to do so). Some questions are just ill-formed. From the point of view of Einstein's theory, avoidance of the question of supraluminal travel is not a limitation. Rather, the theory is simply helping an investigator avoid an ill-formed question, which is a positive feature of the theory. Similarly, for intelligent design theory a question such as "How did random mutation and natural selection construct the bacterial flagellum?" is ill-formed because the theory holds that in fact the flagellum could not arise that way, and spending a lot of resources to investigate it is likely to be a waste. Again, helping investigators avoid ill-formed questions is a positive contribution which all theories seek to make. Nonetheless, a number of other empirical questions (such as those listed by Ratzsch 2001: 143) can open up to design theorists, which would not be asked in the framework of other theories.

Those skeptical of ID, who worry that science would be stuck in a blind alley if design became the majority view in biology, should relax. As a practical matter, no theory, including intelligent design, can stop scientists from investigating alternatives. If a theory could do that, then theories would never be overturned. Within the broad community of scientists there are always some who question the dominant view. Even if intelligent design theory were some day to be the majority position in biology, there is every reason to think experiments would continue to be conducted by skeptical investigators to challenge its postulates.

Must a designer be more complex than the systems it designs? The evolutionary biologist Richard Dawkins (Dawkins 1986: 141) has argued briefly that we should not entertain the notion of a supernatural designer, because such a designer would have to be more complex than the system being explained. An appeal to the more complex to explain the less complex, the argument goes, is no explanation at all. Thus we should prefer a hypothesis of Darwinian evolution to intelligent design.

It seems to me that Professor Dawkins' argument is open to question and his conclusion is not obvious. First, is it true that an appeal to the more complex cannot explain the less complex? It cannot be true in general since we do it all the time. A smooth formation of igneous rock might be explained as a lava flow from an ancient volcano, yet it is quite arguable that the volcano is more complex than the rock formation. I might explain tracks across my lawn as the result of my daughter's erratic driving. By most measures, however, the car is probably more complex than the tracks explained by reference to it. The presence of a wood-and-mud dam at a particular river might bring the conclusion that it was likely built by a beaver. All of these effects are less complex than their causes, yet most of us would be perfectly satisfied with the explanations.

Perhaps Dawkins meant in particular that an ultimate explanation of life or nature must be simple, where the chain of causation begins. Again, that is not obvious to me – perhaps the beginning of the chain of causation is infinitely complex in some sense. For purposes of argument, however, let us assume he's right in this narrower sense. Yet is it indeed true that a designer must be more complex than the systems it designs? It is not self-evidently true even for natural designers. Consider computers. Is it possible for a human to build a computer that is more complex than a human? It may be. In fact some people in the field are quite confident that one day it will happen. Even if today's computers are not as complex as human beings, given time there is no logical reason to suppose a computer can't be built that exceeded the complexity of humans by some measure.

If a natural designer can in principle be less complex than systems she designs, then why should we think a supernatural designer must be more complex than systems it designs? I can think of no reason. Like Dawkins, I am no theologian and I am ill equipped to argue about the nature and attributes of God. Nonetheless, one attribute assigned to God by theologians such as Aquinas is simplicity. If those theologians are right, perhaps there is no basis in fact for Dawkins' objection. In any event, unless and until his argument is much more fully developed I do not think it presents a barrier to a conclusion of intelligent design in biochemistry.

What if the existence of God is in dispute or is denied? So far I have assumed the existence of God. But what if the existence of God is denied at the outset, or is in dispute? Is the plausibility of the argument to design affected? As a matter of my own experience the answer is clearly yes, the argument is less plausible to those for whom God's existence is in question, and is much less plausible for those who deny God's existence. People I speak with who already believe in God generally agree with the idea of design in biology (although there are certainly exceptions), those who are in doubt are interested in the argument but often are skeptical, and as a rule those who actively deny God's existence are either very skeptical or wholly disbelieving (Apparently, the idea of a natural intelligent designer of terrestrial life is not entertained by a large percentage of people).

Without becoming entangled in philosophical or religious arguments about the existence of God, what are we to make of these various reactions? I think that one can simply view them as arising from different estimations of the strengths of competing hypotheses. As an analogy, imagine that a shipwreck marooned you on a desert island. If you see a few rocks strewn about, you might think nothing of it. But if you see some rocks on the island in a circle, then if you also think some other person may have escaped the wreck and ended up on the island, you may surmise the rocks were arranged by that other survivor, even if you have not yet encountered her face-to-face. On the other hand, if no one else was on the sunken boat and you have no reason to think another person is on the island, then you might shrug off the circle of stones as a peculiar coincidence. However, as the example becomes more and more insistent (say, instead of a circle of stones one sees a stone chimney, or stones spelling out the message "hi" vs "hello" vs "welcome, survivor") then the hypothesis of intelligent design becomes more and more plausible. It might even reach the point where, if a natural designer were nowhere to be seen, a person would judge it more likely that God was arranging the stones than that it was a coincidence.[13]

To many theists such as myself, the state of the biological evidence is such that the hypothesis of design is more compelling than that unintelligent processes produced the irreducibly complex systems seen in the cell, like the shipwreck survivor who thought someone else might be on the island. However, like the survivor of the shipwreck who thinks no one else is on the island, many (although not all) agnostics and nontheists draw the line differently, and reach the opposite conclusion. This is unsurprising; it is frequently the case in science that when new theories are proposed people judge the evidence differently. The only unusual (but not unprecedented) feature here is that a person's judgment on the existence of God enters into the balance.

Nonetheless, the more common – and most important – feature is that people's judgments can be affected by the further development of the evidence, just as in the shipwreck example an opinion might change if the evidence became more compelling.[14] If future work shows natural selection capable of doing more than skeptics of Darwinism had thought, or if another mechanism is discovered which can generate irreducible complexity without intelligent input, then fewer people will deem intelligent design to be a sound hypothesis. On the other hand, if further investigations exacerbate problems for Darwinism and no plausible alternative explanation appears, or especially if design is seen to have real empirical payoffs, then it is likely that more people will be attracted to the idea of intelligent design as I have developed it.

Notes

[1] Doolittle's point has been echoed by other critics of intelligent design. For example, in *Free Inquiry* Michael Ruse, a prominent Darwinian scholar, wrote:

> Behe is a real scientist, but this case for the impossibility of a small-step natural origin of biological complexity has been trampled upon contemptuously by the scientists working in the field. They think his grasp of the pertinent science is weak and his knowledge of the literature curiously (although conveniently) outdated.
>
> For example, far from the evolution of clotting being a mystery, the past three decades of work by Russell Doolittle and others has thrown significant light on the ways in which clotting came into being. More than this, it can be shown that the clotting mechanism does not have to be a one-step phenomenon with everything already in place and functioning. One step in the cascade involves fibrinogen, required for clotting, and another, plaminogen [*sic*], required for clearing clots away (Ruse 1998).

And Ruse went on to quote Doolittle's passage from *Boston Review* cited above.

[2] The intent of Bugge et al. (1996a) was to determine if plasminogen had any other function in the organism besides its role in blood clotting. The fact that knocking out fibrinogen relieved all the symptoms of plasminogen deficiency led them to conclude that it did not.

[3] As an aside, ordinary mechanical contraptions such as a mousetrap or a clock don't have to be examined at the molecular level because the parts are not themselves complex assemblages of active components, as are cells. However, more sophisticated artificial devices, such as computers, may indeed have to be examined at the molecular – or at least microscopic – level to determine if they are irreducibly complex.

[4] One example of this could be myoglobin/hemoglobin. The single-chain myoglobin binds oxygen by itself, but several myoglobin-like chains in tetrameric hemoglobin bind oxygen with greater flexibility. I discussed the potential evolution of hemoglobin from a myoglobin-like precursor in a section entitled "Making Distinctions" (Behe 1996: 205–208).

[5] In *Tower of Babel: The Evidence Against the New Creationism* philosopher Robert Pennock protests that I have tried to get an empirical conclusion from a conceptual argument (Pennock 1999: 267–268) mostly, it seems, because of my unfortunate use of the phrase "by definition" in describing the problem irreducible complexity poses to Darwinism. However, Pennock seriously misunderstands my argument, which is a scientific/empirical one, not a philosophical/a

priori one. The confusion may be partly my fault because, as a scientist, I was not sensitive to how a professional philosopher might construe my words, that the phrase "by definition" would be a red flag and become the unintended focus of scrutiny to the exclusion of the real empirical difficulties of constructing irreducibly complex biochemical systems. In fact I was not attempting to rule out Darwinian explanations a priori (nor did I say that I was) or to "prove" in a logical sense that Darwinian processes could not possibly have produced irreducibly complex systems. Such a heavy burden of logical proof, completely ruling out alternative explanations, is rarely if ever borne in science, even by time-tested theories, and I do not feel the need to take it on either. For his part, Pennock focuses on the phrase "by definition" to the point of overlooking important qualifications I made and examples I gave, and he ignores or dismisses without engagement the empirical problems I concentrated on. Pennock's assertion notwithstanding, I did not claim that "there could never be any functional intermediates that natural selection could have selected for on the way to *any* irreducibly complex system" (Pennock 1999: 267–268). On the contrary, I forthrightly wrote that "Even if a system is irreducibly complex (and thus cannot have been produced directly), however, one cannot definitively rule out the possibility of an indirect, circuitous route," continuing that "As the complexity of an interacting system increases, though, the likelihood of such an indirect route drops precipitously" (Behe 1996: 40). Thus the essence of my argument is probabilistic rather than definitional.

[6] These questions have recently been addressed at length by Ratzsch (2001) who concludes that design can indeed be a legitimate scientific conclusion. For example, he writes: "However, if one takes the second option – science as an attempt to discover as much as possible concerning the structure, operation, and history of actual reality, whatever that reality may be or include – then the situation is very different. In particular, prohibition on the supernatural does not even superficially appear to emerge out of the definition or primary aim of science. In fact, under this second conception, science – aimed now at the truth, whatever the truth turns out to be – might be required to think about the possibilities of supernatural causation and phenomena within even the empirical realm" (p. 95).

[7] Indeed, some of my religious critics dislike intelligent design theory precisely because they worry that it will be falsified, and thus religion will appear to suffer another blow from science. See, for example, Flietstra 1998 and Oakes 2001.

[8] On the other hand, if an explanation depends critically on specific tenets of a particular faith, such as the Trinity or Incarnation, or on sacred texts, then that of course is not a scientific explanation.

[9] I do not regard the existence of God as a tenet of faith, since it is a subject of philosophical argument from first principles. Of course, different persons arrive at different conclusions about God's existence, but that only means the arguments persuade some people and not others. It does not mean that the affirmation of God's existence need be dogmatic.

[10] I am using the term miracle here to mean "the involvement at some point of supernatural agency". One should be careful to note, however, that the "some point" doesn't have to be during the course of the universe's history, but could be at its inception. Thus, even if one does think the designer is God, subscribing to a theory of intelligent design does not necessarily commit one to miracles in the sense of "intervention" or the contravening of the laws of nature – no more than thinking that the laws of nature were designed by God (a view, as we've seen, condoned by the National Academy of Sciences (National Academy of Sciences 1999)). In either case one could hold that the information for the subsequent unfolding of life was present at the very start of the universe. In one case, the information is present just in general laws. In the other case, in addition to general laws, much more information is present in other factors too, such as initial conditions. The difference might boil down simply to the

question of whether there was more or less explicit design information present at the beginning – hardly a point of principle.

[11] The question of the theistic implications of the Big Bang hypothesis has been treated explicitly in, for example, Craig and Smith (1993). Furthermore, that scientists recognized the Big Bang had theistic implications can sometimes be seen in statements by those who didn't welcome them. For example, in an article entitled "Down with the Big Bang," whose subtitle calls the theory "philosophically unacceptable," former *Nature* editor John Maddox wrote "Creationists and those of similar persuasions seeking support for their opinions have ample justification in the doctrine of the Big Bang. That, they might say, is when (and how) the Universe was created" (Maddox 1989).

[12] Of course, some scientists are now trying to explain the Big Bang itself (For example, see Guth 1997). Nonetheless, the point remains that for decades there was no attempt at a scientific explanation for the Big Bang. Thus for a time science quietly accepted a possibly miraculous beginning to the universe.

[13] Of course the preceding example is not directly parallel to biological examples because there are claims that the apparently-designed biological systems can be explained by natural processes, and are not sheer coincidence. However, if one examines those claims and becomes convinced they are incorrect, then biological examples take on the same force as nonbiological ones.

[14] To be sure, people might not respond linearly to the evidence.

References

Behe, M.J.: 1996, *Darwin's Black Box: The Biochemical Challenge to Evolution*, Free Press, New York.

Behe, M.J., Dembski, W.A. and Meyer, S.: 2000, *Science and Evidence for Design in the Universe*, Ignatius Press, San Francisco.

Bugge, T.H., Flick, M.J., Daugherty, C.C. and Degen, J.L.: 1995, 'Plasminogen Deficiency Causes Severe Thrombosis but Is Compatible With Development and Reproduction', *Genes and Development* **9**, 794–807.

Bugge, T.H., Kombrinck, K.W., Flick, M.J., Daugherty, C.C., Danton, M.J. and Degen, J.L.: 1996a, 'Loss of Fibrinogen Rescues Mice from the Pleiotropic Effects of Plasminogen Deficiency', *Cell* **87**, 709–715.

Bugge, T.H., Xiao, Q., Kombrinck, K.W., Flick, M.J., Holmback, K., Danton, M.J., Colbert, M.C., Witte, D.P., Fujikawa, K., Davie, E.W. and Degen, J.L.: 1996b, 'Fatal Embryonic Bleeding Events in Mice Lacking Tissue Factor, the Cell- Associated Initiator of Blood Coagulation', *Proceedings of the National Academy of Sciences U.S.A.* **93**, 6258–6263.

Cairns-Smith, A.G.: 1985, *Seven Clues to the Origin of Life: a Scientific Detective Story*, Cambridge University Press, Cambridge.

Cavalier-Smith, T.: 1997, 'The Blind Biochemist', *Trends in Ecology and Evolution* **12**, 162–163.

Coyne, J.A.: 1996, 'God in the Details', *Nature* **383**, 227–228.

Craig, W.L. and Smith, Q.: 1993, *Theism, Atheism, and Big Bang Cosmology*, Clarendon Press, Oxford.

Dawkins, R.: 1986, *The Blind Watchmaker*, Norton, New York.

Davies, P.C.W.: 1999, *The Fifth Miracle: The Search for the Origin and Meaning of Life*, Simon and Schuster, New York.

Dembski, W.A.: 1998, *The Design Inference: Eliminating Chance Through Small Probabilities*, Cambridge University Press, Cambridge.

Denton, M.J.: 1998, *Nature's Destiny: How the Laws of Biology Reveal Purpose in the Universe*, Free Press, New York.

Doolittle, R.: 1997, 'A Delicate Balance' *Boston Review* Feb/March, 28–29.

Dorit, R.: 1997, 'Molecular Evolution and Scientific Inquiry, Misperceived', *American Scientist* **85**, 474–475.

Flietstra, R.: 1998, 'A Response to Michael Behe', *Books & Culture* Sept/Oct, 37.

Futuyma, D.J.: 1997, 'Miracles and Molecules', *Boston Review* Feb/March, 29–30.

Guth, A.H.: 1997, *The Inflationary Universe: the Quest for a New Theory of Cosmic Origins*, Addison-Wesley Publishing, Reading, Mass.

Halkier, T.: 1992, *Mechanisms in Blood Coagulation Fibrinolysis and the Complement System*, Cambridge University Press, Cambridge.

Hall, B.G.: 1982, 'Evolution on a Petri Dish: The Evolved ß-galactosidase System as a Model for Studying Acquisitive Evolution in the Laboratory', in M.K. Hecht, B. Wallace and G.T. Prance (eds), *Evolutionary Biology*, Plenum Press, New York, pp. 85–150.

Hall, B.G.: 1999, 'Experimental Evolution of Ebg Enzyme Provides Clues about the Evolution of Catalysis and to Evolutionary Potential', *FEMS Microbiology Letters* **174**, 1–8.

Maddox, J.: 1989, 'Down With the Big Bang', *Nature* **340**, 425.

Miller, K.R.: 1999, *Finding Darwin's God: a Scientist's Search for Common Ground Between God and Evolution*, Cliff Street Books, New York.

National Academy of Sciences: 1999, *Science and Creationism: a View from the National Academy of Sciences*, 2nd ed, National Academy Press, Washington, DC.

Oakes, E.T.: 2001, 'Edward T. Oakes and His critics: An Exchange', *First Things* April, 5–13.

Orr, H.A.: 1996–1997, 'Darwin V. Intelligent Design (Again)', *Boston Review* Dec/Jan, 28–31.

Pennock, R.: 1999, *Tower of Babel: The Evidence Against the New Creationism*, MIT Press, Cambridge, MA.

Pomiankowski, A.: 1996, 'The God of the Tiny Gaps', *New Scientist* Sept. **14**, 44–45.

Quill, H.: 1966, *John Harrison. The Man Who Found Longitude*, John Baker Publishers, London.

Ratzsch, D.: 2001, 'Nature, Design, and Science: The Status of Design in Natural Science', in D.E. Shaner (ed.), *SUNY Series in Philosophy and Biology*, State University of New York Press, Albany, New York.

Ruse, M.: 1997, 'Enough Speculation', *Boston Review* Feb/March, 31.

Ruse, M.: 1998, 'Answering the creationists: Where they go wrong and what they're afraid of', *Free Inquiry* **18**, 2.

Shapiro, J.A.: 1996, 'In the Details ... What?', *National Review* Sept. **16**, 62–65.

Shapiro, R.: 1999, *Planetary Dreams: the Quest to Discover Life Beyond Earth*, J. Wiley, New York.

Suh, T.T., Holmback, K., Jensen, N.J., Daugherty, C.C., Small, K., Simon, D.I., Potter, S. and Degen, J.L.: 1995, 'Resolution of Spontaneous Bleeding Events but Failure of Pregnancy in Fibrinogen-deficient Mice', *Genes and Development* **9**, 2020–2033.

Sun, W.Y., Witte, D.P., Degen, J.L., Colbert, M.C., Burkart, M.C., Holmback, K., Xiao, Q., Bugge, T.H. and Degen, S.J.: 1998, 'Prothrombin Deficiency Results in Embryonic and Neonatal Lethality in Mice', *Proceedings of the National Academy of Sciences U.S.A.* **95**, 7597–7602.

Ward, P.D. and Brownlee, D.: 2000, *Rare Earth: Why Complex Life Is Uncommon in the Universe*, Copernicus, New York.

Exhibit 9

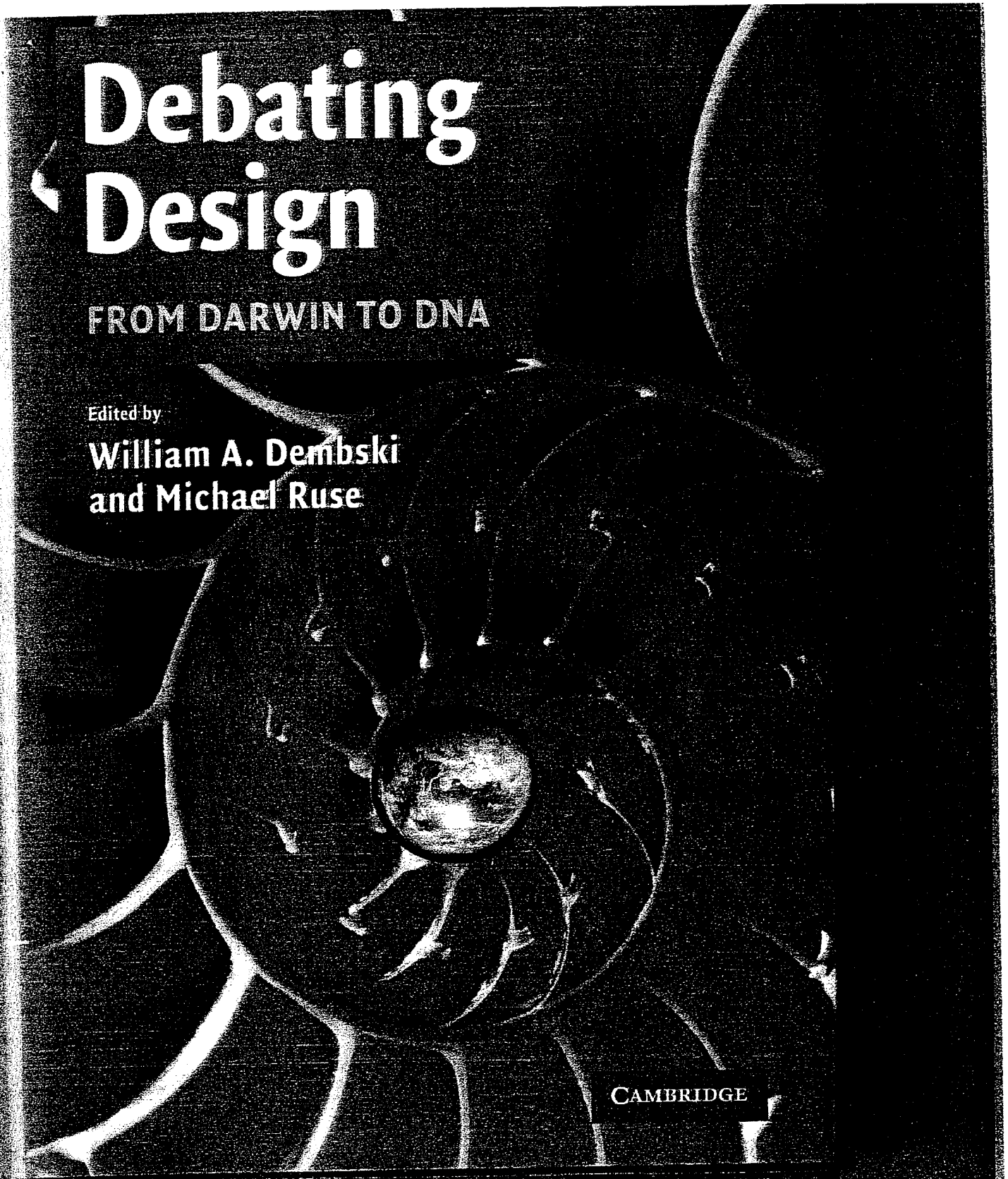
My chapter from *Debating Design: From Darwin to DNA*

Debating Design

FROM DARWIN TO DNA

Edited by

William A. Dembski
and Michael Ruse



CAMBRIDGE

19

Irreducible Complexity

Obstacle to Darwinian Evolution

Michael J. Behe

A SKETCH OF THE INTELLIGENT DESIGN HYPOTHESIS

In his seminal work *On the Origin of Species*, Darwin hoped to explain what no one had been able to explain before – how the variety and complexity of the living world might have been produced by simple natural laws. His idea for doing so was, of course, the theory of evolution by natural selection. In a nutshell, Darwin saw that there was variety in all species. For example, some members of a species are bigger than others, some faster, some brighter in color. He knew that not all organisms that are born will survive to reproduce, simply because there is not enough food to sustain them all. So Darwin reasoned that the ones whose chance variation gives them an edge in the struggle for life would tend to survive and leave offspring. If the variation could be inherited, then over time the characteristics of the species would change, and over great periods of time, perhaps great changes could occur.

It was an elegant idea, and many scientists of the time quickly saw that it could explain many things about biology. However, there remained an important reason for reserving judgment about whether it could actually account for all of biology: the basis of life was as yet unknown. In Darwin's day, atoms and molecules were still theoretical constructs – no one was sure if such things actually existed. Many scientists of Darwin's era took the cell to be a simple glob of protoplasm, something like a microscopic piece of Jell-O. Thus the intricate molecular basis of life was utterly unknown to Darwin and his contemporaries.

In the past hundred years, science has learned much more about the cell and, especially in the past fifty years, much about the molecular basis of life. The discoveries of the double helical structure of DNA, the genetic code, the complicated, irregular structure of proteins, and much else have given us a greater appreciation for the elaborate structures that are necessary to sustain Indeed, we have seen that the cell is run by machines – literally,

machines made of molecules. There are molecular machines that enable the cell to move, machines that empower it to transport nutrients, machines that allow it to defend itself.

In light of the enormous progress made by science since Darwin first proposed his theory, it is reasonable to ask if the theory still seems to be a good explanation for life. In *Darwin's Black Box: The Biochemical Challenge to Evolution* (Behe 1996), I argued that it is not. The main difficulty for Darwinian mechanisms is that many systems in the cell are what I termed "irreducibly complex." I defined an irreducibly complex system as: a single system that is necessarily composed of several well-matched, interacting parts that contribute to the basic function, and where the removal of any one of the parts causes the system to effectively cease functioning (Behe 2001). As an example from everyday life of an irreducibly complex system, I pointed to a mechanical mousetrap such as one finds in a hardware store. Typically, such traps have a number of parts: a spring, a wooden platform, a hammer, and other pieces. If one removes a piece from the trap, it can't catch mice: Without the spring, or hammer, or any of the other pieces, one doesn't have a trap that works half as well as it used to, or a quarter as well; one has a broken mousetrap, which doesn't work at all.

Irreducibly complex systems seem very difficult to fit into a Darwinian framework, for a reason insisted upon by Darwin himself. In the *Origin*, Darwin wrote that "[i]f 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. But I can find out no such case" (Darwin 1859, 158). Here Darwin was emphasizing that his was a gradual theory. Natural selection had to improve systems by tiny steps, over a long period of time, because if things improved too rapidly, or in large steps, then it would begin to look as if something other than natural selection were driving the process. However, it is hard to see how something like a mousetrap could arise gradually by something akin to a Darwinian process. For example, a spring by itself, or a platform by itself, would not catch mice, and adding a piece to the first nonfunctioning piece wouldn't make a trap either. So it appears that irreducibly complex biological systems would present a considerable obstacle to Darwinian evolution.

The question then becomes, are there any irreducibly complex systems in the cell? Are there any irreducibly complex molecular machines? Yes, there are many. In *Darwin's Black Box*, I discussed several biochemical systems as examples of irreducible complexity: the eukaryotic cilium, the intracellular transport system, and more. Here I will just briefly describe the bacterial flagellum (DeRosier 1998; Shapiro 1995), since its structure makes the difficulty for Darwinian evolution easy to see (Figure 19.1). The flagellum can be thought of as an outboard motor that bacteria use to swim. It was the first truly rotary structure discovered in nature. It consists of a lon mentous

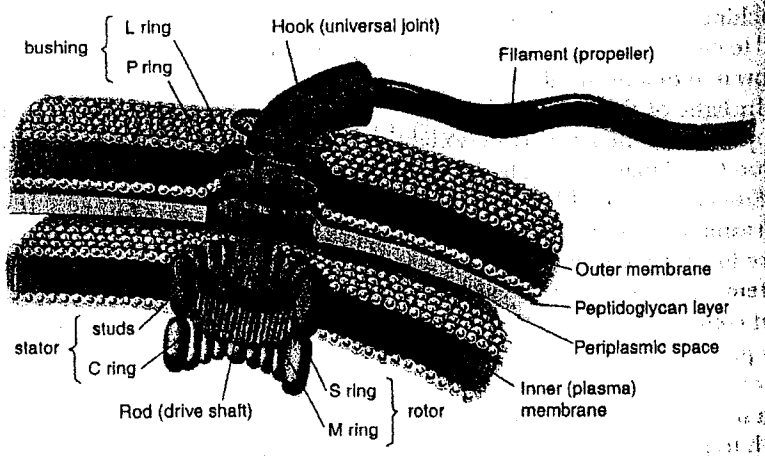


FIGURE 19.1. The bacterial flagellum. Reproduced from D. Voet and J. G. Voet, *Biochemistry*, 2nd ed. (New York: Wiley, 1995), Figure 34–84, with permission of John Wiley Publishers and Donald Voet, who wished to emphasize that "this is an artist-drawn representation of the flagellum rather than a photo or drawing of an actual flagellum."

tail that acts as a propeller; when it is spun, it pushes against the liquid medium and can propel the bacterium forward. The propeller is attached to the drive shaft indirectly through something called the hook region, which acts as a universal joint. The drive shaft is attached to the motor, which uses a flow of acid or sodium ions from the outside to the inside of the cell to power rotation. Just as an outboard motor has to be kept stationary on a motorboat while the propeller turns, there are proteins that act as a stator structure to keep the flagellum in place. Other proteins act as bushings to permit the drive shaft to pass through the bacterial membrane. Studies have shown that thirty to forty proteins are required to produce a functioning flagellum in the cell. About half of the proteins are components of the finished structure, while the others are necessary for the construction of the flagellum. In the absence of almost any of the proteins – in the absence of the parts that act as the propeller, drive shaft, hook, and so forth – no functioning flagellum is built.

As with the mousetrap, it is quite difficult to see how Darwin's gradualistic process of natural selection sifting random mutations could produce the bacterial flagellum, since many pieces are required before its function appears. A hook by itself, or a driveshaft by itself, will not act as a propulsive device. But the situation is actually much worse than it appears from this cursory description, for several reasons. First, there is associated with the functioning of the flagellum an intricate control system, which tells the flagellum ı to rotate, when to stop, and sometimes when to reverse itself

and rotate in the opposite direction. This allows the bacterium to swim toward or away from an appropriate signal, rather than in a random direction that could much more easily take it the wrong way. Thus the problem of accounting for the origin of the flagellum is not limited to the flagellum itself but extends to associated control systems as well.

Second, a more subtle problem is how the parts assemble themselves into a whole. The analogy to an outboard motor fails in one respect: an outboard motor is generally assembled under the direction of a human – an intelligent agent who can specify which parts are attached to which other parts. The information for assembling a bacterial flagellum, however (or, indeed, for assembling any biomolecular machine), resides in the component proteins of the structure itself. Recent work shows that the assembly process for a flagellum is exceedingly elegant and intricate (Yonekura et al. 2000). If that assembly information is absent from the proteins, then no flagellum is produced. Thus, even if we had a hypothetical cell in which proteins homologous to all of the parts of the flagellum were present (perhaps performing jobs other than propulsion) but were missing the information on how to assemble themselves into a flagellum, we would still not get the structure. The problem of irreducibility would remain.

Because of such considerations, I have concluded that Darwinian processes are not promising explanations for many biochemical systems in the cell. Instead, I have noted that, if one looks at the interactions of the components of the flagellum, or cilium, or other irreducibly complex cellular system, they look like they were designed – purposely designed by an intelligent agent. The features of the systems that indicate design are the same ones that stymie Darwinian explanations: the specific interaction of multiple components to accomplish a function that is beyond the individual components. The logical structure of the argument to design is a simple inductive one: whenever we see such highly specific interactions in our everyday world, whether in a mousetrap or elsewhere, we unfailingly find that the systems were intentionally arranged – that they were designed. Now we find systems of similar complexity in the cell. Since no other explanation has successfully addressed them, I argue that we should extend the induction to subsume molecular machines, and hypothesize that they were purposely designed.

MISCONCEPTIONS ABOUT WHAT A HYPOTHESIS OF DESIGN ENTAILS

The hypothesis of Intelligent Design (ID) is quite controversial, mostly because of its philosophical and theological overtones, and in the years since *Darwin's Black Box* was published a number of scientists and philosophers have tried to refute its main argument. I have found these rebuttals to be unpersuasive, at best. Quite the opposite, I think that e putative

counterexamples to design are unintentionally instructive. Not only do they fail to make their case for the sufficiency of natural selection, they show clearly the obstacle that irreducible complexity poses to Darwinism. They also show that Darwinists have great trouble recognizing problems with their own theory. I will examine two of those counterexamples in detail a little later in this chapter. Before I do, however, I will first address a few common misconceptions that surround the biochemical design argument.

First of all, it is important to understand that a hypothesis of Intelligent Design has no quarrel with evolution per se – that is, evolution understood simply as descent with modification, but leaving the mechanism open. After all, a designer may have chosen to work that way. Rather than common descent, the focus of ID is on the *mechanism* of evolution – how did all this happen, by natural selection or by purposeful Intelligent Design?

A second point that is often overlooked but should be emphasized is that Intelligent Design can happily coexist with even a large degree of natural selection. Antibiotic and pesticide resistance, antifreeze proteins in fish and plants, and more may indeed be explained by a Darwinian mechanism. The critical claim of ID is not that natural selection doesn't explain *anything*, but that it doesn't explain *everything*.

My book, *Darwin's Black Box*, in which I flesh out the design argument, has been widely discussed in many publications. Although many issues have been raised, I think the general reaction of scientists to the design argument is well and succinctly summarized in the recent book *The Way of the Cell*, published by Oxford University Press and authored by the Colorado State University biochemist Franklin Harold. Citing my book, Harold writes, "We should reject, as a matter of principle, the substitution of intelligent design for the dialogue of chance and necessity (Behe 1996); but we must concede that there are presently no detailed Darwinian accounts of the evolution of any biochemical system, only a variety of wishful speculations" (Harold 2001, 205).

Let me emphasize, in reverse order, Harold's two points. First, as other reviewers of my book have done,[1] Harold acknowledges that Darwinists have no real explanation for the enormous complexity of the cell, only hand-waving speculations, more colloquially known as "just-so stories." I had claimed essentially the same thing six years earlier in *Darwin's Black Box* and encountered fierce resistance – mostly from internet fans of Darwinism who claimed that, why, there were hundreds or thousands of research papers describing the Darwinian evolution of irreducibly complex biochemical systems, and who set up web sites to document them.[2]

As a sufficient response to such claims, I will simply rely on Harold's statement quoted here, as well as the other reviewers who agree that there is a dearth of Darwinian explanations. After all, if prominent scientists who are no fans of Intelligent Design agree that the systems remain unexplained, then that should settle the matter. Let me pause, however, to note that I find

this an astonishing admission for a theory that has dominated biology for so long. That Darwinian theory has borne so little fruit in explaining the molecular basis of life – despite its long reign as the fundamental theory of biology – strongly suggests that it is not the right framework for understanding the origin of the complexity of life.

Harold's second point is that there is some principle that forbids us from investigating Intelligent Design, even though design is an obvious idea that quickly pops into your mind when you see a drawing of the flagellum (Figure 19.1) or other complex biochemical system. What principle is that? He never spells it out, but I think the principle probably boils down to this: design appears to point strongly beyond nature. It has philosophical and theological implications, and that makes many people uncomfortable. Because they think that science should avoid a theory that points so strongly beyond nature, they want to rule out intelligent design from the start.

I completely disagree with that view and find it fainthearted. I think science should follow the evidence wherever it seems to lead. That is the only way to make progress. Furthermore, not only Intelligent Design, but *any* theory that purports to explain how life occurred will have philosophical and theological implications. For example, the Oxford biologist Richard Dawkins has famously said that "Darwin made it possible to be an intellectually-fulfilled atheist" (Dawkins 1986, 6). A little less famously, Kenneth Miller has written that "[God] used evolution as the tool to set us free" (Miller 1999, 253). Stuart Kauffman, a leading complexity theorist, thinks Darwinism cannot explain all of biology: "Darwinism is not enough. . . . [N]atural selection cannot be the sole source of order we see in the world" (Kauffman 1995, viii). But Kauffman thinks that his theory will somehow show that we are "at home in the universe." The point, then, is that all theories of origins carry philosophical and theological implications. There is no way to avoid them in an explanation of life.

Another source of difficulty for some people concerns the question, how could biochemical systems have been designed? A common misconception is that designed systems would have to be created from scratch in a puff of smoke. But that isn't necessarily so. The design process may have been much more subtle. In fact, it may have contravened no natural laws at all. Let's consider just one possibility. Suppose the designer is indeed God, as most people would suspect. Well, then, as Kenneth Miller points out in his book, *Finding Darwin's God*:

> The indeterminate nature of quantum events would allow a clever and subtle God to influence events in ways that are profound, but scientifically undetectable to us. Those events could include the appearance of mutations . . . and even the survival of individual cells and organisms affected by the chance processes of radioactive decay. (Miller 1999, 241)

Miller doesn't think that guidance is necessary in evolution, but if it were (as I believe), then a route would be open for a subtle God to design life without overriding natural law. If quantum events such as radioactive decay are not governed by causal laws, then it breaks no law of nature to influence such events. As a theist like Miller, that seems perfectly possible to me. I would add, however, that such a process would amount to Intelligent Design, not Darwinian evolution. Further, while we might not be able to detect quantum manipulations, we may nevertheless be able to conclude confidently that the final structure was designed.

MISCONCEPTIONS CONCERNING SUPPOSED WAYS AROUND THE IRREDUCIBILITY OF BIOCHEMICAL SYSTEMS

Consider a hypothetical example where proteins homologous to all of the parts of an irreducibly complex molecular machine first had other individual functions in the cell. Might the irreducible system then have been put together from individual components that originally worked on their own, as some Darwinists have proposed? Unfortunately, this picture greatly oversimplifies the difficulty, as I discussed in *Darwin's Black Box* (Behe 1996, 53). Here analogies to mousetraps break down somewhat, because the parts of a molecular system have to find each other automatically in the cell. They can't be arranged by an intelligent agent, as a mousetrap is. In order to find each other in the cell, interacting parts have to have their surfaces shaped so that they are very closely matched to each other, as pictured in Figure 19.2. Originally, however, the individually acting components would not have had complementary surfaces. So all of the interacting surfaces of

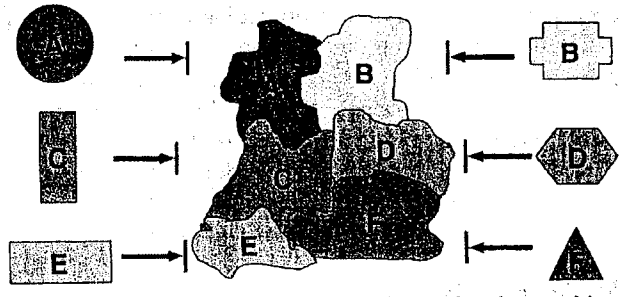


FIGURE 19.2. The parts of an irreducibly complex molecular machine must have surfaces that are closely matched to each other to allow specific binding. This drawing emphasizes that even if individually acting proteins homologous to parts of a complex originally had separate functions, their surfaces would not be complementary to each other. Thus the problem of irreducibility remains even if the separate parts originally had individual functions. (The blocked arrows indicate that the original protein s are not suitable to bind other proteins in the molecular machine.)

all of the components would first have to be adjusted before they could function together. And only then would the new function of the composite system appear. Thus, I emphasize strongly, *the problem of irreducibility remains, even if individual proteins homologous to system components separately and originally had their own functions.*

Another area where one has to be careful is in noticing that some systems that have extra or redundant components may have an irreducibly complex *core.* For example, a car with four spark plugs might get by with three or two, but it certainly can't get by with none. Rat traps often have two springs, to give them extra strength. The trap can still work if one spring is removed, but it can't work if both springs are removed. Thus in trying to imagine the origin of a rat trap by Darwinian means, we still have all the problems we had with a mousetrap. A cellular example of redundancy is the hugely complex eukaryotic cilium, which contains about 250 distinct protein parts (Dutcher 1995). The cilium has multiple copies of a number of components, including multiple microtubules and dynein arms. Yet a working cilium needs at least one copy of each in order to work, as I pictured in my book (Behe 1996, 60). Thus, like the rat trap's, its gradual Darwinian production remains quite difficult to envision. Kenneth Miller has pointed to the redundancy of the cilium as a counterexample to my claim of its irreducibility (Miller 1999, 140–3). But redundancy only delays irreducibility; it does not eliminate it.

Finally, rather than showing how their theory could handle the obstacle, some Darwinists are hoping to get around irreducible complexity by verbal tap dancing. At a debate between proponents and opponents of Intelligent Design sponsored by the American Museum of Natural History in April 2002, Kenneth Miller actually claimed (the transcript is available at the web site of the National Center for Science Education) that a mousetrap isn't irreducibly complex because subsets of a mousetrap, and even each individual part, could still "function" on their own. The holding bar of a mousetrap, Miller observed, could be used as *a toothpick,* so it still has a "function" outside the mousetrap. Any of the parts of the trap could be used as a paperweight, he continued, so they all have "functions." And since any object that has mass can be a paperweight, then any part of anything has a function of its own. *Presto,* there is no such thing as irreducible complexity! Thus the acute problem for gradualism that any child can see in systems like the mousetrap is smoothly explained away.

Of course, the facile explanation rests on a transparent fallacy, a brazen equivocation. Miller uses the word "function" in two different senses. Recall that the definition of irreducible complexity notes that removal of a part "causes the *system* to effectively cease functioning." Without saying so, in his exposition Miller shifts the focus from the separate function of the intact *system* itself to the question of whether we can find a different use (or "function") for some of the *parts.* However, if one removes a part from the mousetrap that I have pictured, it can no longer catch mice. The *system* has

indeed effectively ceased functioning, so the *system* is irreducibly complex, just as I have written. What's more, the functions that Miller glibly assigns to the parts – paperweight, toothpick, key chain, and so forth – have little or nothing to do with the function of the system – catching mice (unlike the mousetrap series proposed by John McDonald, to be discussed later) – so they give us no clue as to how the system's function could arise gradually. Miller has explained precisely nothing.

With the problem of the mousetrap behind him, Miller then moved on to the bacterial flagellum – and again resorted to the same fallacy. If nothing else, one has to admire the breathtaking audacity of verbally trying to turn another severe problem for Darwinism into an advantage. In recent years, it has been shown that the bacterial flagellum is an even more sophisticated system than had been thought. Not only does it act as a rotary propulsion device, it also contains within itself an elegant mechanism used to transport the proteins that make up the outer portion of the machine from the inside of the cell to the outside (Aizawa 1996). Without blinking, Miller asserted that the flagellum is not irreducibly complex because some proteins of the flagellum could be missing and the remainder could still transport proteins, perhaps independently. (Proteins similar – but not identical – to some found in the flagellum occur in the type III secretory system of some bacteria. See Hueck 1998). Again, he was equivocating, switching the focus from the function of the system, acting as a rotary propulsion machine, to the ability of a subset of the system to transport proteins across a membrane. However, taking away the parts of the flagellum certainly destroys the ability of the system to act as a rotary propulsion machine, as I have argued. Thus, contra Miller, the flagellum is indeed irreducibly complex. What's more, the function of transporting proteins has as little directly to do with the function of rotary propulsion as a toothpick has to do with a mousetrap. So discovering the supportive function of transporting proteins tells us precisely nothing about how Darwinian processes might have put together a rotary propulsion machine.

THE BLOOD CLOTTING CASCADE

Having dealt with some common misconceptions about intelligent design, in the next two sections I will examine two systems that were proposed as serious counterexamples to my claim of irreducible complexity. I will show not only that they fail, but also how they highlight the seriousness of the obstacle of irreducible complexity.

In *Darwin's Black Box*, I argued that the blood clotting cascade is an example of an irreducibly complex system (Behe 1996, 74–97). At first glance, clotting seems to be a simple process. A small cut or scrape will bleed for a while and then slow down and stop as the visible blood congeals. However, stud over the past fifty years have shown that the visible simplicity is

undergirded by a system of remarkable complexity (Halkier 1992). In all, there are over a score of separate protein parts involved in the vertebrate clotting system. The concerted action of the components results in the formation of a weblike structure at the site of the cut, which traps red blood cells and stops the bleeding. Most of the components of the clotting cascade are involved not in the structure of the clot itself, but in the control of the timing and placement of the clot. After all, it would not do to have clots forming at inappropriate times and places. A clot that formed in the wrong place, such as in the heart or brain, could lead to a heart attack or stroke. Yet a clot that formed even in the right place, but too slowly, would do little good.

The insoluble weblike fibers of the clot material itself are formed of a protein called fibrin. However, an insoluble web would gum up blood flow before a cut or scrape happened, so fibrin exists in the bloodstream initially in a soluble, inactive form called fibrinogen. When the closed circulatory system is breached, fibrinogen is activated by having a piece cut off from one end of two of the three proteins that comprise it. This exposes sticky sites on the protein, which allows them to aggregate. Because of the shape of the fibrin, the molecules aggregate into long fibers that form the meshwork of the clot. Eventually, when healing is completed, the clot is removed by an enzyme called plasmin.

The enzyme that converts fibrinogen to fibrin is called thrombin. Yet the action of thrombin itself has to be carefully regulated. If it were not, then thrombin would quickly convert fibrinogen to fribrin, causing massive blood clots and rapid death. It turns out that thrombin exists in an inactive form called prothrombin, which has to be activated by another component called Stuart factor. But by the same reasoning, the activity of Stuart factor has to be controlled, too, and it is activated by yet another component. Ultimately, the component that usually begins the cascade is tissue factor, which occurs on cells that normally do not come in contact with the circulatory system. However, when a cut occurs, blood is exposed to tissue factor, which initiates the clotting cascade.

Thus in the clotting cascade, one component acts on another, which acts on the next, and so forth. I argued that the cascade is irreducibly complex because, if a component is removed, the pathway is either immediately turned on or permanently turned off. It would not do, I wrote, to postulate that the pathway started from one end, fibrinogen, and then added components, since fibrinogen itself does no good. Nor is it plausible even to start with something like fibrinogen and a nonspecific enzyme that might cleave it, since the clotting would not be regulated and would be much more likely to do harm than good.

So said I. But Russell Doolittle – an eminent protein biochemist, a professor of biochemistry at the University of California–San Diego, a member of the National Academy of Sciences, and a lifelong student ıe blood

clotting system – disagreed. As part of a symposium discussing my book and Richard Dawkins' *Climbing Mount Improbable* in the *Boston Review*, which is published by the Massachusetts Institute of Technology, Doolittle wrote an essay discussing the phenomenon of gene duplication – the process by which a cell may be provided with an extra copy of a functioning gene. He then conjectured that the components of the blood clotting pathway, many of which have structures that are similar to each other, arose by gene duplication and gradual divergence. This is the common view among Darwinists. Professor Doolittle went on to describe a then-recent experiment that, he thought, showed that the cascade is not irreducible after all. Professor Doolittle cited a paper by Bugge and colleagues (1996a) entitled "Loss of Fibrinogen Rescues Mice from the Pleiotropic Effects of Plasminogen Deficiency." Of that paper, he wrote:

> Recently the gene for plaminogen [sic] was knocked out of mice, and, predictably, those mice had thrombotic complications because fibrin clots could not be cleared away. Not long after that, the same workers knocked out the gene for fibrinogen in another line of mice. Again, predictably, these mice were ailing, although in this case hemorrhage was the problem. And what do you think happened when these two lines of mice were crossed? For all practical purposes, the mice lacking both genes were normal! Contrary to claims about irreducible complexity, the entire ensemble of proteins is not needed. Music and harmony can arise from a smaller orchestra. (Doolittle 1997)

(Again, fibrinogen is the precursor of the clot material itself. Plasminogen is the precursor of plasmin, which removes clots once their purpose is accomplished.) So if one knocks out either one of those genes of the clotting pathway, trouble results; but, Doolittle asserted, if one knocks out both, then the system is apparently functional again. That would be a very interesting result, but it turns out to be incorrect. Doolittle misread the paper.

The abstract of the paper states that "[m]ice deficient in plasminogen and fibrinogen are phenotypically indistinguishable from fibrinogen-deficient mice." In other words, the double mutants have all the problems that the mice lacking just fibrinogen have. Those problems include inability to clot, hemorrhaging, and death of females during pregnancy. Plasminogen deficiency leads to a different suite of symptoms – thrombosis, ulcers, and high mortality. Mice missing both genes were "rescued" from the ill effects of plasminogen deficiency only to suffer the problems associated with fibrinogen deficiency.[3] The reason for this is easy to see. Plasminogen is needed to remove clots that, left in place, interfere with normal functions. However, if the gene for fibrinogen is also knocked out, then clots can't form in the first place, and their removal is not an issue. Yet if clots can't form, then there is no functioning clotting system, and the mice suffer the predictable conseq es.

TABLE 19.1. *Effects of knocking out genes for blood clotting components*

Missing Protein	Symptoms	Reference
Plasminogen	Thrombosis, high mortality	Bugge et al. 1995
Fibrinogen	Hemorrhage, death in pregnancy	Suh et al. 1995
Plasminogen/fibrinogen	Hemorrhage, death in pregnancy	Bugge et al. 1996a
Prothrombin	Hemorrhage, death in pregnancy	Sun et al. 1998
Tissue factor	Hemorrhage, death in pregnancy	Bugge et al. 1996b

Clearly, the double-knockout mice are not "normal." They are not promising evolutionary intermediates.

The same group that produced the mice missing plasminogen and fibrinogen has also produced mice individually missing other components of the clotting cascade – prothrombin and tissue factor. In each case, the mice are severely compromised, which is *exactly* what one would expect if the cascade is irreducibly complex (Table 19.1).

What lessons can we draw from this incident? The point is certainly not that Russell Doolittle misread a paper, which anyone might do. (Scientists, as a rule, are not known for their ability to write clearly, and Bugge and colleagues were no exception.) Rather, the main lesson is that irreducible complexity seems to be a much more severe problem than Darwinists recognize, since the experiment Doolittle himself chose to demonstrate that "music and harmony can arise from a smaller orchestra" showed exactly the opposite. A second lesson is that gene duplication is not the panacea that it is often made out to be. Professor Doolittle knows as much about the structures of the clotting proteins and their genes as anyone on Earth, and he is convinced that many of them arose by gene duplication and exon shuffling. Yet that knowledge did not prevent him from proposing utterly nonviable mutants as possible examples of evolutionary intermediates. A third lesson is that, as I had claimed in *Darwin's Black Box*, there are no papers in the scientific literature detailing how the clotting pathway could have arisen by Darwinian means. If there were, Doolittle would simply have cited them.

Another significant lesson that we can draw is that, while the majority of academic biologists and philosophers place their confidence in Darwinism, that confidence rests on no firmer grounds than Professor Doolittle's. As an illustration, consider the words of the philosopher Michael Ruse:

> For example, Behe is a real scientist, but this case for the impossibility of a small-step natural origin of biological complexity has been trampled upon contemptuously by the scientists working in the field. They think his grasp of the pertinent science is weak and his knowledge of the literature curiously (although conveniently) outdated.

> For example, far from the evolution of clotting being a mystery, the past three decades of work by Russell Doolittle and others has thrown significant light on the ways in which clotting came into being. More than this, it can be shown that the clotting mechanism does not have to be a one-step phenomenon with everything already in place and functioning. One step in the cascade involves fibrinogen, required for clotting, and another, plaminogen [*sic*], required for clearing clots away. (Ruse 1998)

And Ruse goes on to quote Doolittle's passage from the *Boston Review* that I quoted earlier. Now, Ruse is a prominent Darwinist and has written many books on various aspects of Darwiniana. Yet, as his approving quotation of Doolittle's mistaken reasoning shows (complete with his copying of Doolittle's typo-misspelling of "plaminogen"), Ruse has no independent knowledge of how natural selection could have put together complex biochemical systems. As far as the scientific dispute is concerned, Ruse has nothing to add.

Another such example is seen in a recent essay in *The Scientist*, "Not-So-Intelligent Design," by Neil S. Greenspan, a professor of pathology at Case Western Reserve University, who writes (Greenspan 2002), "The Design advocates also ignore the accumulating examples of the reducibility of biological systems. As Russell Doolittle has noted in commenting on the writings of one ID advocate . . ." Greenspan goes on to cite approvingly Doolittle's argument in the *Boston Review*. He concludes, with unwitting irony, that "[t]hese results cast doubt on the claim by proponents of ID that they know which systems exhibit irreducible complexity and which do not." But since the results are precisely the opposite of what Greenspan supposed, the shoe is now on the other foot. This incident casts grave doubt on the claim by Darwinists – both biologists and philosophers – that they know that complex cellular systems are explainable in Darwinian terms. It demonstrates that Darwinists either cannot or will not recognize difficulties for their theory.

THE MOUSETRAP

The second counterargument to irreducibility I will discuss here concerns not a biological example but a conceptual one. In *Darwin's Black Box*, I pointed to a common mechanical mousetrap as an example of irreducible complexity. Almost immediately after the book's publication, some Darwinists began proposing ways in which the mousetrap could be built step by step. One proposal that has gotten wide attention, and that has been endorsed by some prominent scientists, was put forward by John McDonald, a professor of biology at the University of Delaware, and can be seen on his web site.[4] His series of traps is shown in Figure 19.3. McDonald's main point was that the trap that I pictured in my book consisted of five parts, yet he could b a trap with fewer parts.

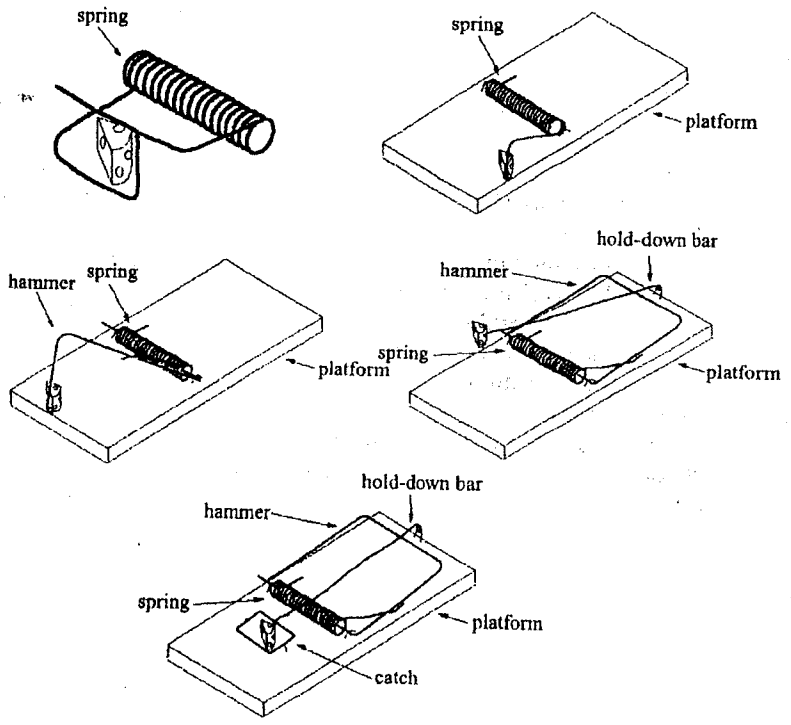


FIGURE 19.3. A series of mousetraps with an increasing number of parts, as proposed by John McDonald <http://udel.edu/~mcdonald/oldmousetrap.html> and reproduced here with his permission. Yet intelligence is still required to construct one trap from another, as described in the text.

I agree. In fact, I said exactly the same thing in my book. I wrote:

> We need to distinguish between a *physical* precursor and a *conceptual* precursor. The trap described above is not the only system that can immobilize a mouse. On other occasions my family has used a glue trap. In theory at least, one can use a box propped open with a stick that could be tripped. Or one can simply shoot the mouse with a BB gun. However, these are not physical precursors to the standard mousetrap since they cannot be transformed, step-by-Darwinian-step, into a trap with a base, hammer, spring, catch, and holding bar. (Behe 1996, 43)

Thus the point is not that mousetraps can be built in different ways, with different numbers of pieces. (My children have a game at home called "Mousetrap," which has many, many pieces and looks altogether different from the common mechanical one.) Of course they can. The only questi s whether a particular trap can be built by "numerous, successive, slight n ications"

to a simple starting point – without the intervention of intelligence – as Darwin insisted that his theory required.

The McDonald traps cannot. Shown at the top of Figure 19.3 are his one-piece trap and his two-piece trap. The structure of the second trap, however, is not a single, small, random step away from the first. First notice that the one-piece trap is not a simple spring – it is shaped in a very special way. In fact, the shape was deliberately chosen by an intelligent agent, John McDonald, to act as a trap. Well, one has to start somewhere. But if the mousetrap series is to have any relevance at all to Darwinian evolution, then intelligence can't be involved at any further point.

Yet intelligence saturates the whole series. Consider what would be necessary to convert the one-piece trap to the "two-piece" trap. One can't just place the first trap on a simple piece of wood and have it work as the second trap does. Rather, as shown in Figure 19.3, the two protruding ends of the spring first have to be reoriented. What's more, two staples (barely visible in Figure 19.3) are added to hold the spring onto the platform so that it can be under tension in the two-piece trap. So we have gone not from a one-piece to a two-piece trap, but from a one-piece to a four-piece trap. Notice also that the placement of the staples in relation to the edge of the platform is critical. If the staples were moved a quarter-inch from where they are, the trap wouldn't work. Finally, consider that, in order to have a serious analogy to the robotic processes of the cell, we can't have an intelligent human setting the mousetrap – the first trap would have to be set by some unconscious charging mechanism. So, when the pieces are rearranged, the charging mechanism too would have to change for the second trap.

It's easy for us intelligent agents to overlook our role in directing the construction of a system, but nature cannot overlook any step at all, so the McDonald mousetrap series completely fails as an analogy to Darwinian evolution. In fact, the second trap is best viewed not as some Darwinian descendant of the first but as a completely different trap, designed by an intelligent agent, perhaps using a refashioned part or two from the first trap.

Each of the subsequent steps in the series suffers from analogous problems, which I have discussed elsewhere.[5]

In his endorsement of the McDonald mousetrap series, Kenneth Miller wrote: "If simpler versions of this mechanical device [the mousetrap] can be shown to work, then simpler versions of biochemical machines could work as well . . . and this means that complex biochemical machines could indeed have had functional precursors."[6] But that is exactly what it doesn't show – if by "precursor" Miller means "Darwinian precursor." On the contrary, McDonald's mousetrap series shows that even if one does find a simpler system to perform some function, that gives one no reason to think that a more complex system performing the same function could be produced by a Darwinian process starting with the simpler system. Rather, the difficulty in doing so for a simple mousetrap gives us compelling reason to think it cannot be done for complex molecular machines.

FUTURE PROSPECTS OF THE INTELLIGENT DESIGN HYPOTHESIS

The misconceived arguments by Darwinists that I have recounted here offer strong encouragement to me that the hypothesis of Intelligent Design is on the right track. After all, if well-informed opponents of an idea attack it by citing data that, when considered objectively, actually demonstrate its force, then one is entitled to be confident that the idea is worth investigating.

Yet it is not primarily the inadequacy of Darwinist responses that bodes well for the design hypothesis. Rather, the strength of design derives mainly from the work-a-day progress of science. In order to appreciate this fact, it is important to realize that the idea of Intelligent Design arose not from the work of any individual but from the collective work of biology, particularly in the last fifty years. Fifty years ago, the cell seemed much simpler, and in our innocence it was easier then to think that Darwinian processes might have accounted for it. But as biology progressed and the imagined simplicity vanished, the idea of design became more and more compelling. That trend is continuing inexorably. The cell is not getting any simpler; it is getting much more complex. I will conclude this chapter by citing just one example, from the relatively new area of proteomics.

With the successful sequencing of the entire genomes of dozens of microorganisms and one vertebrate (us), the impetus has turned toward analyzing the cellular interactions of the proteins that the genomes code for, taken as a whole. Remarkable progress has already been made. Early in 2002, an exhaustive study of the proteins comprising the yeast proteome was reported. Among other questions, the investigators asked what proportion of yeast proteins work as groups. They discovered that nearly fifty percent of proteins work as complexes of a half-dozen or more, and many as complexes of ten or more (Gavin et al. 2002).

This is not at all what Darwinists had expected. As Bruce Alberts wrote earlier in the article "The Cell as a Collection of Protein Machines":

> We have always underestimated cells. Undoubtedly we still do today. But at least we are no longer as naive as we were when I was a graduate student in the 1960s. Then most of us viewed cells as containing a giant set of second-order reactions. . . .
>
> But, as it turns out, we can walk and we can talk because the chemistry that makes life possible is much more elaborate and sophisticated than anything we students had ever considered. Proteins make up most of the dry mass of a cell. But instead of a cell dominated by randomly colliding individual protein molecules, we now know that nearly every major process in a cell is carried out by assemblies of 10 or more protein molecules. And, as it carries out its biological functions, each of these protein assemblies interacts with several other large complexes of proteins.

Indeed, the entire cell can be viewed as a factory that contains an elaborate network of interlocking assembly lines, each of which is composed of a set of large protein machines. (Alberts 1998)

The important point here for a theory of Intelligent Design is that molecular machines are not confined to the few examples that I discussed in *Darwin's Black Box*. Rather, most proteins are found as components of complicated molecular machines. Thus design might extend to a large fraction of the features of the cell, and perhaps beyond that into higher levels of biology.

Progress in twentieth-century science has led us to the design hypothesis. I expect progress in the twenty-first century to confirm and extend it.

Notes

1. For example, the microbiologist James Shapiro of the University of Chicago declared in *National Review* that "[t]here are no detailed Darwinian accounts for the evolution of any fundamental biochemical or cellular system, only a variety of wishful speculations" (Shapiro 1996, 65). In *Nature*, the University of Chicago evolutionary biologist Jerry Coyne stated, "There is no doubt that the pathways described by Behe are dauntingly complex, and their evolution will be hard to unravel.... [W]e may forever be unable to envisage the first proto-pathways" (Coyne 1996, 227). In a particularly scathing review in *Trends in Ecology and Evolution*, Tom Cavalier-Smith, an evolutionary biologist at the University of British Columbia, nonetheless wrote, "For none of the cases mentioned by Behe is there yet a comprehensive and detailed explanation of the probable steps in the evolution of the observed complexity. The problems have indeed been sorely neglected – though Behe repeatedly exaggerates this neglect with such hyperboles as 'an eerie and complete silence' " (Cavalier-Smith 1997, 162). The Evolutionary biologist Andrew Pomiankowski, writing in *New Scientist*, agreed: "Pick up any biochemistry textbook, and you will find perhaps two or three references to evolution. Turn to one of these and you will be lucky to find anything better than 'evolution selects the fittest molecules for their biological function' " (Pomiankowski 1996, 44). In *American Scientist*, the Yale molecular biologist Robert Dorit averred, "In a narrow sense, Behe is correct when he argues that we do not yet fully understand the evolution of the flagellar motor or the blood clotting cascade" (Dorit 1997, 474).
2. A good example is found on the "World of Richard Dawkins" web site, maintained by a Dawkins fan named John Catalano at <www.world-of-dawkins.com/Catalano/box/published.htm>. It is to this site that the Oxford University physical chemist Peter Atkins was referring when he wrote in a review of *Darwin's Black Box* for the "Infidels" web site: "Dr. Behe claims that science is largely silent on the details of molecular evolution, the emergence of complex biochemical pathways and processes that underlie the more traditional manifestations of evolution at the level of organisms. Tosh! There are hundreds, possibly thousands, of scientific papers that deal with this very subject. For an entry into this important and flourishing field, and an idea of the intense scientific effort that it repr 's (see the first link above) [*sic*]" (Atkins 1998).

3. Bugge and colleagues (1996a) were interested in the question of whether plasminogen had any role in metabolism other than its role in clotting, as had been postulated. The fact that the direct effects of plasminogen deficiency were ameliorated by fibrinogen deficiency showed that plasminogen probably had no other role.
4. <http://udel.edu/~mcdonald/oldmousetrap.html>. Professor McDonald has recently designed a new series of traps that can be seen at <http://udel.edu/~mcdonald/mousetrap.html>. I have examined them and have concluded that they involve his directing intelligence to the same degree.
5. M. J. Behe, "A Mousetrap Defended: Response to Critics." <www.crsc.org>
6. <http://biocrs.biomed.brown.edu/Darwin/DI/Mousetrap.html>

References

Aizawa, S. I. 1996. Flagellar assembly in Salmonella typhimurium. *Molecular Microbiology* 19: 1–5.

Alberts, B. 1998. The cell as a collection of protein machines: Preparing the next generation of molecular biologists. *Cell* 92: 291–4.

Atkins, P. W. 1998. Review of Michael Behe's *Darwin's Black Box*. <*www.infidels.org/library/modern/peter_atkins/behe.html*>.

Behe, M. J. 1996. *Darwin's Black Box: The Biochemical Challenge to Evolution*. New York: The Free Press.

2001. Reply to my critics: A response to reviews of *Darwin's Black Box: The Biochemical Challenge to Evolution*. *Biology and Philosophy* 16: 685–709.

Bugge, T. H., M. J. Flick, C. C. Daugherty, and J. L. Degen. 1995. Plasminogen deficiency causes severe thrombosis but is compatible with development and reproduction. *Genes and Development* 9: 794–807.

Bugge, T. H., K. W. Kombrinck, M. J. Flick, C. C. Daugherty, M. J. Danton, and J. L. Degen. 1996a. Loss of fibrinogen rescues mice from the pleiotropic effects of plasminogen deficiency. *Cell* 87: 709–19.

Bugge, T. H., Q. Xiao, K. W. Kombrinck, M. J. Flick, K. Holmback, M. J. Danton, M. C. Colbert, D. P. Witte, K. Fujikawa, E. W. Davie, and J. L. Degen. 1996b. Fatal embryonic bleeding events in mice lacking tissue factor, the cell-associated initiator of blood coagulation. *Proceedings of the National Academy of Sciences (USA)* 93: 6258–63.

Cavalier-Smith, T. 1997. The blind biochemist. *Trends in Ecology and Evolution* 12: 162–3.

Coyne, J. A. 1996. God in the details. *Nature* 383: 227–8.

Darwin, C. 1859. *The Origin of Species*. New York: Bantam Books.

Dawkins, R. 1986. *The Blind Watchmaker*. New York: Norton.

DeRosier, D. J. 1998. The turn of the screw: The bacterial flagellar motor. *Cell* 93: 17–20.

Doolittle, R. F. A delicate balance. *Boston Review*, February/March 1997, pp. 28–9.

Dorit, R. 1997. Molecular evolution and scientific inquiry, misperceived. *American Scientist* 85: 474–5.

Dutcher, S. K. 1995. Flagellar assembly in two hundred and fifty easy-to-follow steps. *Trends in Genetics* 11: 398–404.

Gavin, A. C., et al. 2002. Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature* 415: 141–7.

Greenspan, N. S. 2002. Not-so-intelligent design. *The Scientist* 16: 12.

Halkier, T. 1992. *Mechanisms in Blood Coagulation Fibrinolysis and the Complement System.* Cambridge: Cambridge University Press.

Harold, F. M. 2001. *The Way of the Cell.* Oxford: Oxford University Press.

Hueck, C. J. 1998. Type III protein secretion systems in bacterial pathogens of animals and plants. *Microbiology and Molecular Biology Reviews* 62: 379–433.

Kauffman, S. A. 1995. *At Home in the Universe: The Search for Laws of Self-Organization and Complexity.* New York: Oxford University Press.

Miller, K. R. 1999. *Finding Darwin's God: A Scientist's Search for Common Ground between God and Evolution.* New York: Cliff Street Books.

Pomiankowski, A. 1996. The God of the tiny gaps. *New Scientist,* September 14, pp. 44–5.

Ruse, M. 1998. Answering the creationists: Where they go wrong and what they're afraid of. *Free Inquiry,* March 22, p. 28.

Shapiro, J. 1996. In the details . . . what? *National Review,* September 16, pp. 62–5.

Shapiro, L. 1995. The bacterial flagellum: From genetic network to complex architecture. *Cell* 80: 525–7.

Suh, T. T., K. Holmback, N. J. Jensen, C. C. Daugherty, K. Small, D. I. Simon, S. Potter, and J. L. Degen. 1995. Resolution of spontaneous bleeding events but failure of pregnancy in fibrinogen-deficient mice. *Genes and Development* 9: 2020–33.

Sun, W. Y., D. P. Witte, J. L. Degen, M. C. Colbert, M. C. Burkart, K. Holmback, Q. Xiao, T. H. Bugge, and S. J. Degen. 1998. Prothrombin deficiency results in embryonic and neonatal lethality in mice. *Proceedings of the National Academy of Sciences USA* 95: 7597–602.

Yonekura, K., S. Maki, D. G. Morgan, D. J. DeRosier, F. Vonderviszt, K. Imada, and K. Namba. 2000. The bacterial flagellar cap as the rotary promoter of flagellin self-assembly. *Science* 290: 2148–52.

Exhibit 10

Protein Science journal article

Simulating evolution by gene duplication of protein features that require multiple amino acid residues

MICHAEL J. BEHE[1] AND DAVID W. SNOKE[2]

[1]Department of Biological Sciences, Lehigh University, Bethlehem, Pennsylvania 18015, USA
[2]Department of Physics and Astronomy, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, USA

(RECEIVED April 8, 2004; FINAL REVISION June 17, 2004; ACCEPTED June 17, 2004)

Abstract

Gene duplication is thought to be a major source of evolutionary innovation because it allows one copy of a gene to mutate and explore genetic space while the other copy continues to fulfill the original function. Models of the process often implicitly assume that a single mutation to the duplicated gene can confer a new selectable property. Yet some protein features, such as disulfide bonds or ligand binding sites, require the participation of two or more amino acid residues, which could require several mutations. Here we model the evolution of such protein features by what we consider to be the conceptually simplest route—point mutation in duplicated genes. We show that for very large population sizes N, where at steady state in the absence of selection the population would be expected to contain one or more duplicated alleles coding for the feature, the time to fixation in the population hovers near the inverse of the point mutation rate, and varies sluggishly with the λ^{th} root of $1/N$, where λ is the number of nucleotide positions that must be mutated to produce the feature. At smaller population sizes, the time to fixation varies linearly with $1/N$ and exceeds the inverse of the point mutation rate. We conclude that, in general, to be fixed in 10^8 generations, the production of novel protein features that require the participation of two or more amino acid residues simply by multiple point mutations in duplicated genes would entail population sizes of no less than 10^9.

Keywords: gene duplication; point mutation; multiresidue feature; disulfide bonds; ligand binding sites

Although many scientists assume that Darwinian processes account for the evolution of complex biochemical systems, we are skeptical. Thus, rather than simply assuming the general efficacy of random mutation and selection, we want to examine, to the extent possible, which changes are reasonable to expect from a Darwinian process and which are not. We think the most tractable place to begin is with questions of protein structure. Our approach is to examine pathways that are currently considered to be likely routes of evolutionary development and see what types of changes Darwinian processes may be expected to promote along a particular pathway.

Reprint requests to: Michael J. Behe, Department of Biological Sciences, Lehigh University, 111 Research Drive, Bethlehem, PA 18015, USA; e-mail: mjb1@lehigh.edu; fax: (610) 758-4004.

Abbreviation: MR, multiresidue

Article published online ahead of print. Article and publication date are at http://www.proteinscience.org/cgi/doi/10.1110/ps.04802904.

A major route of evolutionary innovation is thought to pass through gene duplication (Ohno 1970; Lynch and Conery 2000; Wagner 2001; Chothia et al. 2003). Because one copy of the gene can continue to fulfill the original function, in this view a duplicate, redundant copy of a gene is substantially free from purifying selection, allowing it to freely accumulate mutations. Although the great majority of nonneutral mutations to duplicated genes are expected to result in a null allele (Walsh 1995; Lynch and Walsh 1998), that is, a gene that no longer codes for a functional protein, occasionally one might confer a novel function on the incipient paralog. If this occurs, then the duplicated gene can be refined by mutation and positive selection, independent of the parent gene.

In most models of the development of evolutionary novelty by gene duplication, it is implicitly assumed that a single, albeit rare, mutation to the duplicated gene can confer a new selectable property (Ohta 1987, 1988a,b; Walsh 1995). However, we are particularly interested in the ques-

tion of how novel protein structural features may develop throughout evolution; not all structural features of a protein may be attainable by single mutations. In particular, some protein features require the participation of multiple amino acid residues. Perhaps the simplest example of this is the disulfide bond. In order to produce a novel disulfide bond, a duplicated gene coding for a protein lacking unmatched cysteines would require at least two mutations in separate codons, and perhaps as many as six mutations, depending on the starting codons. We call protein characteristics such as disulfide bonds which require the participation of two or more amino acid residues "multiresidue" (MR) features.

A more general example of an MR feature is that of a protein binding site. A ligand bound to a protein interacts with multiple amino acid residues (Janin and Chothia 1990; Cunningham and Wells 1993; Braden and Poljak 1995; Lo et al. 1999; Chakrabarti and Janin 2002). In general, therefore, in order to produce a binding site for a new ligand in a protein originally lacking the ability to bind it, multiple mutational events would be necessary. Li (1997) drew attention to this fact in his textbook *Molecular Evolution*. Prefacing a discussion of the evolutionary development of the 2,3-diphosphoglycerate binding site of hemoglobin, he wrote, "acquiring a new function may require many mutational steps, and a point that needs emphasis is that the early steps might have been selectively neutral because the new function might not be manifested until a certain number of steps had already occurred" (Li 1997).

In this paper, we report the results of the stochastic simulation of the time to fixation of new MR features by what we consider to be the conceptually simplest route: point mutation in the absence of recombination in a duplicated gene that is free of purifying selection. It can be seen that, for very large populations, the expected time to fixation resides near the inverse of the mutation rate per nucleotide and decreases only slowly with the λ^{th} root of increasing population size, where λ is the number of nucleotide positions that must be mutated to produce the feature. For smaller populations, the time varies linearly with $1/N$.

Results

The model

The model presented here assumes that newly duplicated genes encode a full-length protein with the signals necessary for its proper expression. It is further assumed that all duplicate genes are selectively neutral. (This postulate is examined in the Discussion.) Any given organism in the population may be thought to have anywhere from zero to multiple extra copies of the gene; that is, duplicate copy number is considered to have no selective effect. However, the model presupposes that there are a total of N duplicate copies of the gene, equal to the number of organisms in the population. The model assumes that either copy of a newly duplicated gene can be the one to undergo mutation and that either copy can retain the original function. That is, the original gene is not necessarily the one to retain the original function. Because the model does not include recombination, all copies of the gene accumulate point mutations independently of each other. The basic "task" that the model asks a duplicate gene to perform is to accumulate λ mutations at the correct nucleotide positions to code for a new selectable feature before suffering a null mutation. Because the model presented here does not include recombination, the results can be considered to be most applicable to a haploid, asexual population. However, as will be discussed, implications can also be made for the evolution of diploid, sexual species.

The process we envision for the production of a multiresidue (MR) feature is illustrated in Figure 1, where a duplicate gene coding for a protein is represented as an array of squares that stand for nucleotide positions. A gene coding for a duplicate, redundant protein would contain many nucleotides. The majority of nonneutral point muta-

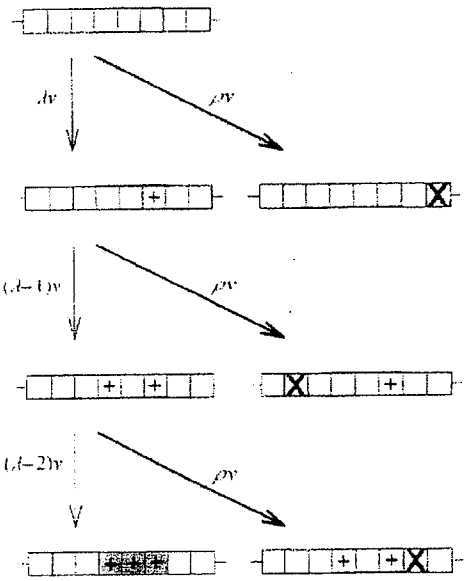


Figure 1. A freshly duplicated gene must accrue several compatible mutations without suffering a null mutation in order to code for the multiresidue (MR) feature. Each box in an array represents a nucleotide position in the duplicated gene. The three boxes outlined in blue are the positions that must be changed in order to produce the new MR feature. (Although they are contiguous in the drawing, they do not necessarily represent contiguous positions in the gene.) A "+" labels a compatible mutation. A red "X" labels a null mutation. The green-shaded box represents the gene coding for the MR feature, where the several necessary changes have all been acquired. The forward mutation rate is ν times the number of incompatible loci λ remaining to be changed. The null mutation rate is $\rho\nu$.

tions to the gene will yield a null allele (again, by which we mean a gene coding for a nonfunctional protein) because most mutations that alter the amino acid sequence of a protein effectively eliminate function (Reidhaar-Olson and Sauer 1988, 1990; Bowie and Sauer 1989; Lim and Sauer 1989; Bowie et al. 1990; Rennell et al. 1991; Axe et al. 1996; Huang et al. 1996; Sauer et al. 1996; Suckow et al. 1996). However, if several point mutations (indicated by a "+" in the figure) accumulate at specific nucleotide positions (indicated by the three squares outlined in blue in the figure) in the gene coding for the protein before a null mutation occurs elsewhere in the gene (indicated by a red "X"), then several amino acid residues will have been altered and the new selectable MR feature will have been successfully built in the protein (indicated by the green-shaded area). By hypothesis, the gene is not selectable for the new feature when an intermediate number of mutations has occurred, but only when all sites are in the correct state.

In our computer model of the process described above, the nucleotide positions that must be changed from the sequence of the parent gene to be compatible with the developing MR feature (we call states of nucleotide positions "compatible" if they are consistent with what is necessary to code for the MR feature, and "incompatible" if they are not) are explicitly represented as elements of an array (see Materials and Methods for details). These correspond to the squares outlined in blue in Figure 1. (Although the positions are next to each other in the figure, they are not necessarily contiguous in the gene.) These may be considered to be nucleotide positions in the same codon, separate codons, or a combination. The pertinent feature of the model is that multiple changes are required in the gene before the new, selectable feature appears. Changes in these nucleotide positions are assumed to be individually disruptive of the original function of the protein but are assumed either to enhance the original function or to confer a new function once all are in the compatible state. Thus, the mutations would be strongly selected against in an unduplicated gene, because its function would be disrupted and no duplicate would be available to back up the function.

The other nucleotide positions in the gene, corresponding to the black squares in Figure 1, which if they were changed would yield a null allele, are represented only implicitly in our computer model by the constant ρ, which is the ratio of the number of mutations of the original duplicated gene that would produce a null allele to the number of mutations of the original duplicated gene that would yield a compatible residue. (Definitions of terms are given in Table 1.) As an example, consider a gene of a thousand nucleotides. If a total of 2400 point mutations of those positions would yield a null allele, whereas three positions must be changed to build a new MR feature such as a disulfide bond, then ρ would be 2400/3, or 800. (Any possible mutations which are neutral are ignored.) In each generation of the simulation, each of the three positions that must be changed to yield the MR feature is sequentially given a chance to mutate with a probability governed by the mutation rate. However, although a mutation may occur in a position needed for an MR feature, it would nonetheless be unproductive if a null mutation had first occurred at a separate position. To simulate this possibility in our model, when an explicitly represented position does mutate, then we take a further probabilistic step to decide if a null mutation has in the meantime occurred elsewhere in the gene, in positions not explicitly represented. In the earlier example, if one of the three positions mutates, then a further step decides with probability $\rho / (1 + \rho)$ (which in the example would be 800/801) that one or more null mutations have already occurred somewhere in the gene, and the gene is considered to be irrecoverably lost. (The likelihood of a null mutation reverting and the gene then successfully developing an MR feature before other null mutations occur is much lower than if the first λ mutations to the duplicate gene yield compatible residues; thus, we ignore that possibility.) With probability $1 / (1 + \rho)$ (in the example this would be 1/801), the gene is considered to be free of null mutations and continues in the simulation.

The starting point of the simulation (see Materials and Methods for a more complete description) is a population of organisms that already contains N exact duplicates of the parent gene, which then begin to undergo mutation. For simplicity, each position in an array, representing sites which must be changed to yield an MR feature, can be in either of just two states—the original incompatible state or the mutated, compatible state. Mutations can change a site

Table 1. *Definitions of terms*

N	Number of organisms/duplicate genes in the population
λ	Number of initially incompatible nucleotide loci in a duplicate gene that must be changed to form the selectable, multiresidue feature
ν	Point mutation rate per nucleotide per generation
ρ	Ratio of the number of possible mutations of the original duplicated gene that would produce a null allele to the number of possible mutations of the original duplicated gene that would yield a compatible residue. Neutral mutations, such as those that produce synonymous codons, are disregarded.
ϕ	Fraction of a particular nucleotide position that is in the incompatible state. $(1 - \phi)$ is the fraction in the compatible state.
t	Time, in generations
T_f	Time in generations to the first occurrence of a particular multiresidue, selectable features
T_{fx}	Time in generations to fixation in the population of a particular multiresidue, selectable feature
s	Selection coefficient

either forward from incompatible to compatible or backward from compatible to incompatible. (Unlike for null mutations, reversions of compatible mutations back to incompatible ones must be explicitly considered because the probability of reversion in this case is significant.) These transitions occur with equal intrinsic probabilities.

Starting from a uniform population in which all sites that must be changed are in a state incompatible with the MR feature, then there are three processes in our model which affect the rate of approach of the population to steady state, which in turn affect the time required to generate the new MR feature:

1. Sites in the incompatible state can mutate to the compatible state before any null mutation has occurred. This takes place at a rate equal to the mutation rate per site times the fraction of sites that are in the incompatible state (since only that fraction can mutate directly to the compatible state) times the probability that no null mutation has already occurred. That is, at a rate equal to

$$\nu\phi\left(\frac{1}{1+\rho}\right),$$

where ν is the mutation rate per site per generation, ϕ is the fraction of nucleotide sites in the population that are in the incompatible state, ρ (as mentioned above) is the ratio of possible null to compatible mutations over the entire protein, and $1/(1+\rho)$ is the probability that a compatible mutation occurs before a null mutation. (Definitions of terms are given in Table 1.)

2. A site in the compatible state can mutate back to the incompatible state before a null mutation occurs. This takes place at a rate equal to

$$\nu(1-\phi)\left(\frac{1}{1+\rho}\right).$$

3. A mutation can occur in any one of the λ sites, but a stochastic check at this point decides with probability $\rho/(1+\rho)$ that one or more detrimental mutations have already occurred somewhere else in the protein, rendering it nonfunctional. The gene is then considered to be null, and it no longer counts in the model. However, the model allows for the occurrence of new gene duplication events, which recent estimates have shown to happen at a rate comparable to that of point mutation (Lynch and Conery 2000). Because the rates of point mutations and gene duplication are similar, in the model a gene that is determined to be null is replaced by a new gene duplication event, with a new copy of the original gene (which is presumed to be still under selection) with all sites in the original, incompatible state. In the computer model, this process effectively results in all λ sites of a null gene being reset to the original, incompatible state from whatever state they were in. This will happen at rate

$$\nu\lambda(1-\phi)\left(\frac{\rho}{1+\rho}\right).$$

The number of nucleotide positions λ appears in this expression because the more compatible positions that were contained in a discarded null gene, the more that are replaced with incompatible ones in a new gene duplication event. The protocol of checking for null mutations in the model only when a mutation first occurs in one of the λ array sites has the intended effect of ensuring that gene duplication occurs in the population at a rate that is comparable to the rate of point mutation.

The overall net rate of change of the fraction ϕ of sites from the incompatible state will be a sum of these three processes:

$$\frac{d\phi}{dt}=\frac{-\nu\phi}{1+\rho}+\frac{\nu(1-\phi)}{1+\rho}+\frac{\nu\rho\lambda(1-\phi)}{1+\rho} \qquad (1)$$

The first term of the right-hand side of the equation is negative because it is a process in which incompatible sites are removed. The second and third terms are positive because they describe processes where incompatible sites are gained.

Integration yields:

$$(1-\phi)=\frac{1-\exp\left(-\nu t\left(\frac{2+\rho\lambda}{1+\rho}\right)\right)}{2+\rho\lambda} \qquad (2)$$

The numerator of the right-hand term is the degree of saturation of the population with compatible mutations—the degree to which it has approached steady state. The value of $(1-\phi)$ is the population-wide fraction of nucleotide positions that are in a state compatible with the MR feature.

Because of computing limitations, the values of 0.01–0.0001 used for the mutation rate ν in the simulations presented following are much higher than the biologically realistic value of about 10^{-8} (Drake et al. 1998), and the values of 1–100 used here for ρ are lower than the value of a thousand or greater expected for biologically realistic situations (Walsh 1995). However, the fact that Figure 2 shows that the fraction $(1-\phi)$ of compatible mutants in our simulations follows equation 2 very closely over a wide range of values for λ and ρ in populations that reproduce either deterministically or stochastically makes us more confident

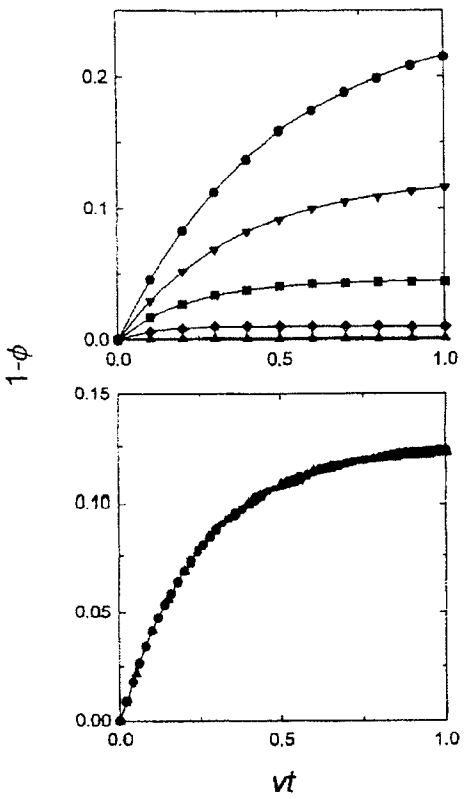


Figure 2. Fraction $(1-\phi)$ of a nucleotide position in a compatible state versus time (generations) normalized for the mutation rate (νt). In all cases, the curves are determined from equation 2. (*Top*) $N = 10{,}000$, $\nu = 0.001$, deterministic reproduction. Circles: $\rho = 1$, $\lambda = 2$; inverted triangles: $\rho = 2$, $\lambda = 3$; squares: $\rho = 4$, $\lambda = 5$; diamonds: $\rho = 10$, $\lambda = 10$; triangles: $\rho = 100$, $\lambda = 10$. Each point is the average of 100 repetitions. (*Bottom*) $N = 100$, $\nu = 0.001$, $\rho = 1$, $\lambda = 6$. Circles are for deterministic reproduction; each point is the average of 100 repetitions. Triangles are for stochastic reproduction; each point is the average of 1024 repetitions.

when we extrapolate the model to biologically realistic values of ν and ρ.

In the following paragraphs, we develop from simple considerations an equation which gives the same quantitative behavior as the numerical model. In Appendix 1, we derive the same form of equation more rigorously by considering coupled equations representing different segments of the population.

What is the probability that a duplicated gene will give rise to a particular MR feature? Consider a gene with λ sites all originally in the incompatible state. As discussed previously, the probability of one of those sites mutating to a compatible state before the occurrence of a null mutation elsewhere in the gene is

$$\frac{1}{(1+\rho)}.$$

Because any one of the λ sites can mutate first, we can write this as

$$\frac{\lambda}{\lambda}\frac{1}{(1+\rho)}.$$

To mutate another residue to a compatible state, we must choose among the remaining $(\lambda - 1)$ possibilities. Thus, the probability for the second position is

$$\frac{(\lambda-1)}{\lambda}\frac{1}{(1+\rho)}.$$

The multiplied probability of all λ sites mutating to compatible states before a null mutation occurs and before a back mutation occurs is thus

$$\frac{\lambda!}{\lambda^{\lambda}}\frac{1}{(1+\rho)^{\lambda}}.$$

(If a back mutation occurs at any point, the likelihood of successfully developing an MR feature is much lower than if the first λ mutations to the duplicate gene yield compatible residues; thus, we ignore that possibility.)

If the probability of an event is P, then of course on average $1/P$ opportunities will be required before the event occurs. Thus, to produce an MR feature in our model will require an average number of opportunities equal to the inverse of the probability discussed earlier, or

$$(1+\rho)^{\lambda}\left(\frac{\lambda^{\lambda}}{\lambda!}\right).$$

At steady state, the number of opportunities to produce an MR function in a given time period in a population will be equal to the number of point mutations that occur in the potential MR site across the population—that is, to the time multiplied by the mutation rate per nucleotide ν, the number of nucleotide positions λ that must mutate to compatible residues, and the population size N—that is, equal to $N\nu\lambda t$. To produce a gene with λ compatible mutations, the incompatible residue in a gene with $\lambda - 1$ compatible mutations has to be mutated, so that the time to produce an MR function with λ compatible sites will be proportional to the degree of saturation of the system with genes containing $\lambda - 1$ compatible sites. However, as exemplified by Figure 2, our model does not start at steady state; it starts with all sites in the incompatible state. Thus, the time required to produce an event will also depend on the degree to which the system has approached steady state, as follows. If the degree of saturation for one compatible site is in general S, then the degree of saturation for n compatible sites is S^n.

Thus, the degree of saturation with $\lambda - 1$ compatible sites at any given time is equal to the degree of saturation given in equation 2 raised to the $\lambda - 1$ power. Because the degree of saturation changes in time, to find the total number of opportunities for producing an MR feature, this value must be integrated over time.

These considerations can be combined to yield a quantitative description of the behavior of the model with time. The expected average time T_f to the first occurrence of an MR feature for a population of duplicate genes initially in a uniform state, needing λ positions mutated to acquire the MR feature, and with a ratio ρ of null-to-compatible mutations, can be evaluated by equation 3.

$$N\nu\lambda \int_0^{T_f} \left(1 - \exp\left(\frac{-\nu t(2+\rho\lambda)}{1+\rho}\right)\right)^{(\lambda-1)} dt = (1+\rho)^{\lambda}\left(\frac{\lambda^{\lambda}}{\lambda!}\right) \quad (3)$$

The right-hand side of equation 3 is the inverse of the probability discussed earlier. The left-hand side gives the number of opportunities for production of the MR feature in the nonequilibrium system starting with no nucleotide positions in compatible states. The preintegral term of the left-hand side of the equation, $N\nu\lambda$, is the number of point mutations occurring in the population per unit time at steady state. The integrand of equation 3, which is the numerator from the right-hand side of equation 2 raised to the power of $\lambda - 1$, is the degree of saturation of the system with "preselectable" mutants—that is, mutants that are one step from being selectable, with $\lambda - 1$ sites in the compatible state.

Figure 3 shows the result of simulations in which the number of sites λ in an MR feature was varied along with the ratio ρ of null-to-compatible mutations and the haploid population size N. As can be seen, the curves generated by equation 3 match the results of the simulations very closely for a wide range of values of N, ρ, and λ.

The effect of selection

The simulations shown in Figure 3 examined the number of generations required to produce just the first occurrence of

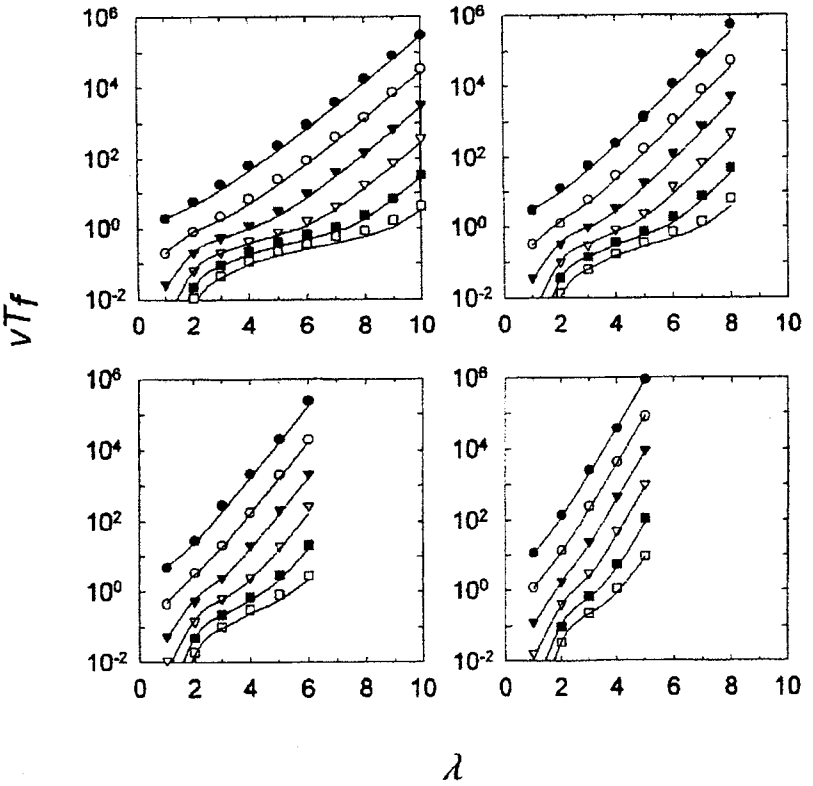


Figure 3. Normalized time (generations) to first appearance (νT_f) versus number of loci λ required to be changed to yield the multiresidue (MR) feature. In all cases, the curves are determined from equation 3. $\nu = 0.01$. Reproduction was deterministic. Filled circles, $N = 1$; open circles, $N = 10$; filled inverted triangles, $N = 100$; open inverted triangles circles, $N = 1000$; filled squares, $N = 10{,}000$; open squares, $N = 100{,}000$. (*Upper left*) $\rho = 1$; (*upper right*) $\rho = 2$; (*lower left*) $\rho = 4$; (*lower right*) $\rho = 10$. Each point is the average of 100 repetitions.

an MR feature in a population. However, beneficial mutations are frequently lost from a population by stochastic processes before fixation (Kimura 1983). In Figure 4, we present the results of simulations which determine the time to fixation T_{fx} of the MR feature in the population as a function of the strength of the selection coefficient s. The simulation results are well fit by equation 4.

$$N\nu\lambda\int_0^{T_{fx}}\left(1-\exp\left(\frac{-\nu t(2+\rho\lambda)}{1+\rho}\right)\right)^{(\lambda-1)}dt=\frac{(1+\rho)^{\lambda}}{2s}\left(\frac{\lambda^{\lambda}}{\lambda!}\right) \quad (4)$$

Equation 4 is a minor modification of equation 3, where the right-hand side of the equation is divided by twice the selection coefficient. This result follows from the dependence of the fixation probability on the selection coefficient (Li 1997).

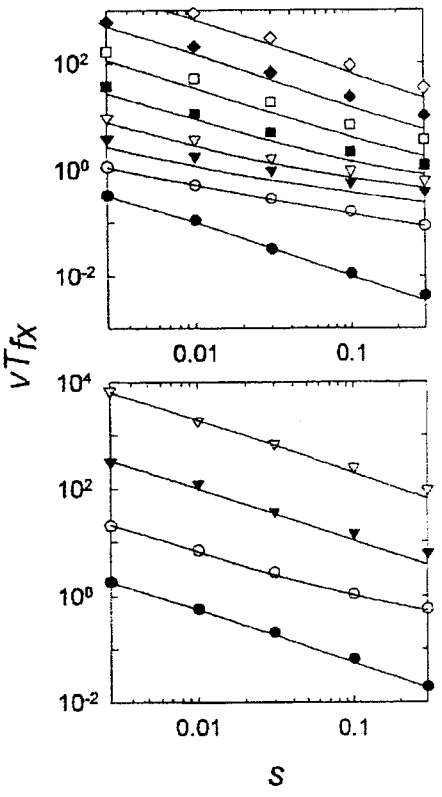


Figure 4. Normalized time (generations) to fixation (vT_{fx}) versus the selection coefficient s. In all cases, the curves are determined from equation 4. Reproduction was stochastic. N = 1000; v = 0.01–0.0001. Each point is the average of 100 repetitions. (*Top*) ρ = 1. Filled circles, λ = 1; open circles, λ = 2; filled inverted triangles, λ = 3; open inverted triangles circles, λ = 4; filled squares, λ = 5; open squares, λ = 6; filled diamonds, λ = 7; open diamonds, λ = 8. (*Bottom*) ρ = 10. Filled circles, λ = 1; open circles, λ = 2; filled inverted triangles, λ = 3; open inverted triangles, λ = 4.

Pre-equilibration of the population

Thus far, the starting point for the model has been a uniform population in which all genes are initially present as exact duplicates of the parent gene. Mutations then begin to accumulate and the program immediately starts to check for the presence of the MR feature, simulating the presence of selective pressure from the start. However, a different situation can also be considered, in which the duplicate gene begins to undergo mutation, but selective pressure arises only at a later time, perhaps as a result of environmental changes. In that case, the population of duplicate genes will be at least part of the way toward its steady-state frequency before selection affects the population. This can be modeled in the simulation by neglecting to check for the presence of the MR feature, treating it as a neutral property, until a predetermined number of generations have passed.

Figure 5 shows the result of simulations in which all duplicate genes began in a uniform state, identical to the parent gene, but the population was allowed to undergo mutation and reproduction for varying periods of time before starting to check for the MR feature. It can be seen that as the length of the pre-equilibration period increases, the average time from the start of selection to observation of the duplicate gene coding for the new MR feature decreases for population sizes, where, at steady state in the absence of selection, at least one duplicated gene with the feature is expected to already be present in the population, that is, where the population size is greater than the inverse of the probability of producing the MR feature, $N > (1+\rho)^{\lambda}(\lambda^{\lambda}/\lambda!)$. In Figure 5, this occurs at $\lambda \leq 5$. For the case where $N < (1+\rho)^{\lambda}(\lambda^{\lambda}/\lambda!)$ (at $\lambda \geq 6$ in Fig. 5), however, the expected time is essentially unaffected by pre-equilibration of the population. Because it follows from equation 3 that $N < (1+\rho)^{\lambda}(\lambda^{\lambda}/\lambda!)$, when v times the evaluated integral is >1, then T_f will be substantially unaffected by pre-equilibration when $T_f \geq 1/v$.

Discussion

The model and its limits

Some features of proteins, such as disulfide bonds and ligand binding sites, which here we call MR features, are composed of multiple amino acid residues. As Li (1997) points out, the evolutionary origins of such features must have involved multiple mutations that were initially neutral with respect to the MR feature. We have attempted to model such a process. In doing so, one might examine a number of possible routes to an MR feature, for example, looking at a unique gene that is under selective constraints, or looking at mutations caused by insertions and deletions or recombination in a duplicate gene. Our model is restricted to the development of MR features by point mutation in a dupli-

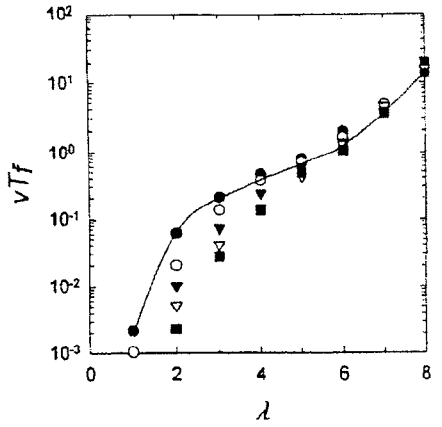


Figure 5. Effect of pre-equilibration of the population on normalized time (generations) to first appearance (νT_f) versus number of loci λ required to be changed to yield the MR feature. $N = 1000$; $\nu = 0.001$; $\rho = 1$. Each point is the average of 100 repetitions. The curve is determined from equation 3. Reproduction was deterministic. The simulation was pre-equilibrated (that is, the population was subject to mutation and reproduction without checking for the appearance of the multiresidue (MR) feature, regarding it as neutral) for filled circles, 0 generations; open circles, $0.1 / \nu$ generations; filled inverted triangles, $0.3 / \nu$ generations; open inverted triangles, $1 / \nu$ generations; filled squares, $3 / \nu$ generations.

cated gene. We strongly emphasize that results bearing on the efficiency of this one pathway as a conduit for Darwinian evolution say little or nothing about the efficiency of other possible pathways. Thus, for example, the present study that examines the evolution of MR protein features by point mutation in duplicate genes does not indicate whether evolution of such features by other processes (such as recombination or insertion/deletion mutations) would be more or less efficient.

There are several reasons, both practical and theoretical, for examining this limited model. First, as mentioned earlier, gene duplication is considered to be a major route to evolutionary novelty (Ohno 1970; Lynch and Conery 2000; Wagner 2001; Chothia et al. 2003) and therefore it is important to explore its potential in regard to MR features. Second, a duplicated gene can be considered to be largely free of the effects of purifying selection (but see following) and therefore selective effects, which are difficult to estimate, can be ignored, simplifying the task at hand. Third, point mutations are well-defined events, where transitions occur among a limited set of states. In contrast, insertions and deletions vary in size and composition, making them difficult to model for our purposes. Thus, we confine our model of the development of MR features to what we consider to be the conceptually simplest and computationally most tractable route, of point mutations in a duplicated gene that is free of purifying selection.

Is the assumption of the selective neutrality of duplicated genes either a realistic or a useful one? On the one hand, the assumption appears not to be correct in at least some situations. For example, although the vast majority of neutral duplicated genes are expected to result in null alleles, studies of polyploid organisms showed that more duplicate genes survived over long periods of time than expected (Ferris and Whitt 1977, 1979; Hughes and Hughes 1993; White and Doebley 1998). This has provoked the suggestion that gene dosage effects in polyploids might slow the decay of duplicate gene copies and that more duplicates may be preserved than expected by the process of subfunctionalization, where a gene with two or more functions duplicates and each copy subsequently loses one of the functions and then goes on to specialize in the preserved function (Force et al. 1999; Lynch et al. 2001). Although the assumption of the selective neutrality of duplicated genes does not fit data from some polyploid species (Ferris and Whitt 1977, 1979; Hughes and Hughes 1993; White and Doebley 1998), it may yet be a good model for individual gene duplication events (Lynch and Conery 2000). In support of this view, recent studies have shown that genes that have been recently duplicated seem to be under relaxed selection, as indicated by the similar number of synonymous and nonsynonymous mutations they have acquired (Lynch and Conery 2000; Kondrashov et al. 2002).

On the other hand, it should be emphasized that the utility of the idealized model presented here—where there is no selective effect from duplicate genes or from intermediate states of the gene until the MR feature is completely in place in a gene and where the only mutagenic process considered is point mutations—is not dependent on a comprehensive accounting for all relevant biological processes. Rather, its usefulness lies in its ability to indicate when processes in addition to those described in the model are required to account for a feature. If the development of an MR feature by means of point mutation in an ideal, neutral, duplicated gene would require unrealistically large population sizes or unrealistically long times, then one can conclude that other factors (such as recombination, selection of intermediate states, and/or other factors) must be examined to account for the feature. Because neutral gene duplication and point mutation is often invoked to account for complex features of proteins, it would be useful to have a quantitative understanding for what such scenarios would entail in order to assess their reasonableness.

In our simulations, the model starts in a uniform initial state, with the population already in possession of N exact duplicates of the parent gene. This, of course, is biologically unrealistic but can be considered to approximate the end result of either of two processes: (1) the spread of a duplicate gene through a population by random drift until it is fixed or (2) the occurrence of a phylogenetic branching point, where after the branch point, a small population that is homogeneous with respect to the duplicate gene expands to a population size N. Although mutations will occur in

copies of the duplicate gene during the period of either drift of the gene or expansion of the population, there will be fewer mutations—and thus fewer opportunities to produce the MR feature—than in a population already at size N, each with on average one copy of the duplicate gene, for the same period of time. In either case, the time to reach the initial state is neglected, so the time obtained from the simulations can be considered to be an underestimate of the time to fixation T_{fx} of the MR feature. Although we envision each organism of the population as having one duplicate gene per haploid genome, because recombination is disallowed and each duplicate accumulates mutations independently, it does not affect the model (as represented by equation 4) if there is variation in copy number of the duplicate gene in organisms, as long as the total number of duplicate gene copies in the population is N.

Figure 3 shows that the results of the simulation closely match those predicted from equation 3, which gives us confidence to extrapolate to biologically realistic values of the parameters of the equation. The curves in Figure 3 exhibit two regions: (1) a nonlinear region at larger population sizes and/or smaller numbers of sites and (2) a linear region at smaller population sizes and/or higher numbers of loci. These regions represent, respectively, (1) the situation where in the absence of selection for the MR feature the steady-state population would be expected to contain one or more copies of the duplicate gene with an MR feature [that is, where $N > (1 + \rho)^{\lambda}(\lambda^{\lambda}/\lambda!)$] and (2) the situation where in the absence of selection, the population on average at steady state is not expected to contain a copy with the MR feature [that is, where $N < (1 + \rho)^{\lambda}(\lambda^{\lambda}/\lambda!)$].

The expected time for the nonlinear region largely reflects the amount of time necessary for the population to approach steady state. This time is on the order of the inverse of the rate of point mutation and is relatively insensitive to either the number of loci involved in the MR feature or the population size, varying inversely with only the λ^{th} root of N (see Appendix 2). Thus, the ability to decrease the time required to produce an MR feature much below $1/\nu$ by increasing population size is greatly constrained by the nonlinearity of the model, reflecting the slow equilibration of the population when multiple mutations are required.

As shown in Figure 4, the effect of changes in the selection coefficient on the behavior of the model are closely fit by equation 4. It should be noted that the time calculated from equation 4 reflects the average time required simply to produce the MR mutant that will go on to become fixed in the population; it does not explicitly include the time required for the mutation to spread and become fixed in the population once it has been produced. The close fit of the simulation results of Figure 4—which does include both the time to produce the MR mutation that will be fixed plus the time required for the mutation to spread through the population to fixation—to the curve predicted from equation 4 emphasizes the fact that the timescale for fixation of the mutation is negligible compared with the timescale required to produce the mutation that will go on to become fixed.

As shown in Figure 5 for the nonlinear region, if the population has been accumulating mutations for a period of time before selection for the MR feature is applied (perhaps representing a population approaching steady state where the environment then changes, making a feature selectable that previously had been neutral), then the expected time, measured from the start of selection to the appearance of the MR feature, decreases. On the other hand, as also shown in Figure 5, for situations where the population is not expected to have a copy of the duplicate gene with the MR feature at steady state (in Figure 5, for $\lambda \geq 6$), then the expected time to its fixation is essentially unaffected by pre-equilibration of the population. This is the case whenever $T_f \geq 1/\nu$. It should be noted that pre-equilibration explicitly allows for the occurrence of rare, "lucky" alleles whose sequence is closer to that of the MR feature than is the sequence of the starting, predominant gene. Such rare alleles could thus be poised to give rise to the MR feature in perhaps one or two steps. The result shown in Figure 5—that, for $\lambda \geq 6$, pre-equilibration has no effect on T_f—demonstrates that on average the opportunity for the serendipitous occurrence of rare alleles does not alter the expected time.

Estimation of T_{fx} for several cases

Estimated values for parameters of our model can be garnered from the literature. Drake et al. (1998) estimate the deleterious mutation rate to be about 0.2–2.0 per generation per effective genome size of 10^8 bp for a variety of multicellular organisms, both vertebrate and invertebrate. We use that number to approximate the effective nucleotide point mutation rate per generation ν in coding regions to be on the order of 10^{-8}. Lynch and Conery (2000) calculate the rate of duplication of a given gene to be 0.01 per million years—in other words, 10^{-8} per year, which for our purposes we consider to be roughly equal to the estimated nucleotide point mutation rate (Lynch and Conery 2000; see also the discussion of that work [Long and Thornton 2001; Lynch and Conery 2001; Zhang et al. 2001]). Although here we assign single values to the parameters, one must keep in mind that there is significant uncertainty in estimating them and that the rates may vary with time, species, region of the genome, and other factors.

An estimate of ρ can be inferred from studies of the tolerance of proteins to amino acid substitution. Although there is variation among different positions in a protein sequence, with surface residues in general being more tolerant of substitution than buried residues, it can be calculated that on average a given position will tolerate about six different amino acid residues and still maintain function

(Reidhaar-Olson and Sauer 1988, 1990; Bowie and Sauer 1989; Lim and Sauer 1989; Bowie et al. 1990; Rennell et al. 1991; Axe et al. 1996; Huang et al. 1996; Sauer et al. 1996; Suckow et al. 1996). Conversely, mutations to an average of 14 residues per site will produce a null allele, that is, one coding for a nonfunctional protein. Thus, in the coding sequence for an average-sized protein domain of 200 amino acid residues, there are, on average, 2800 possible substitutions that lead to a nonfunctional protein as a result of direct effects on protein structure or function. If several mutations are required to produce a new MR feature in a protein, then ρ is roughly of the order of 1000. This value for ρ is on the low end used by Walsh (1995), who considered values for ρ up to 10^5. (Walsh, however, defined ρ as the ratio of advantageous-to-null mutations—the inverse of our definition.)

It should be emphasized that the value of ρ is not the ratio of mutations to an organismal genome that would be lethal to those that would be mildly deleterious. Rather, it is the ratio of the number of mutations that would inactivate a typical protein to the number that would lead to a new MR feature for that particular protein. Many genes can be silenced with small or moderate ill effect on the organism (for example, the gene for myoglobin can be inactivated in mice with little ill effect in adult mice [Garry et al. 1998, 2003; Meeson et al. 2001]). However, if a mutation inactivates a protein, then it is counted in the model as a null mutation for purposes of calculating ρ, whether or not it may have severe phenotypic effects. The best estimate for this number comes not from studies of mutations in organisms, but rather from studies of the tolerance of specific proteins to mutation (Reidhaar-Olson and Sauer 1988, 1990; Bowie and Sauer 1989; Lim and Sauer 1989; Bowie et al. 1990; Rennell et al. 1991; Axe et al. 1996, 1998; Huang et al. 1996; Sauer et al. 1996; Suckow et al. 1996).

The uncertainties involved in estimating ρ should be kept in mind. On the one hand, although as just discussed, studies selecting for activity of mutant proteins show most substitutions to reduce function below that required for a biological assay, a study searching for inactivating mutations to the autotoxic ribonuclease barnase showed that comparatively few substitutions reduced activity to that of uncatalyzed reactions (Axe et al. 1998). This consideration may lower the estimate of ρ. On the other hand, duplicate genes might also be lost by processes other than point mutation, such as deletion or recombination. Additionally, null mutations in the coding sequence or flanking sequences might occur because of indirect effects such as, for example, altering the stability of the mRNA. These considerations might effectively increase the value of ρ.

Figure 6 uses equation 4 and the values for ν and ρ estimated earlier to plot the expected time in generations to the fixation of an MR protein feature for populations of different sizes. In addition, we use a value of 0.01 for the

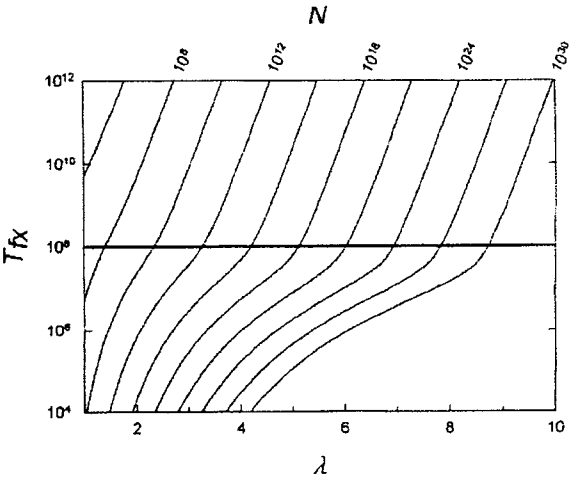


Figure 6. Time to fixation T_{fx} versus number of loci λ required to be changed to yield the multiresidue (MR) feature. $\nu = 10^{-8}$; ρ = 1000; $s = 0.01$. Values for population sizes N are given across the *top* axis. In all cases the curves are determined from equation 4. A line is drawn across the figure at $T_{fx} = 1/\nu$, which is 10^8 generations. *Above the line*, values for T_{fx} are essentially unaffected by pre-equilibration of the population in the absence of selection.

selection coefficient s. Figure 6 shows that the fixation of specific MR features by point mutation in duplicated genes is a long-term phenomenon that requires populations of considerable size. For example, consider a case where three nucleotide changes must be made to generate a novel feature such as a disulfide bond. In that instance, Figure 6 shows that a population size of approximately 10^{11} organisms on average would be required to give rise to the feature over the course of 10^8 generations, and this calculation is unaffected by pre-equilibration of the population in the absence of selection. To produce the feature in one million generations would, on average, require an enormous population of about 10^{17} organisms, although this number would change if the population had pre-equilibrated in the absence of selection.

For features requiring more participating residues, the expected population sizes are even larger. As Li (1997) noted, the binding site for diphosphoglycerate in hemoglobin requires three residues. The population size required to produce an MR feature consisting of three interacting residues by point mutation in a duplicated gene initially lacking those residues would depend on the number of nucleotides that had to be changed—a minimum of three and a maximum of nine. If six mutations were required then, as indicated by Figure 6, on average a population size of $\sim 10^{22}$ organisms would be necessary to fix the MR feature in 10^8 generations, and a population of $\sim 10^{30}$ organisms would be expected to fix the mutation in one million generations. In a recent in vitro study intended to mimic evolution, a re-

combinant amphioxus insulin-like peptide was altered by site-directed mutagenesis at seven nucleotide positions to contain five altered amino acid residues that would allow interaction with mammalian insulin receptor (Guo et al. 2002). In order for such a process to occur in vivo by gene duplication and point mutation within a hundred million generations would be expected on average to require $>10^{25}$ organisms.

Such numbers seem prohibitive. However, we must be cautious in interpreting the calculations. On the one hand, as discussed previously, these values can actually be considered underestimates because they neglect the time it would take a duplicated gene initially to spread in a population. On the other hand, because the simulation looks for the production of a *particular* MR feature in a *particular* gene, the values will be overestimates of the time necessary to produce *some* MR feature in *some* duplicated gene. In other words, the simulation takes a prospective stance, asking for a certain feature to be produced, but we look at modern proteins retrospectively. Although we see a particular disulfide bond or binding site in a particular protein, there may have been several sites in the protein that could have evolved into disulfide bonds or binding sites, or other proteins may have fulfilled the same role. For example, Matthews' group engineered several nonnative disulfide bonds into lysozyme that permit function (Matsumura et al. 1989). We see the modern product but not the historical possibilities.

We should also notice which parameters the model is particularly sensitive to and which not. The model is least sensitive to the point mutation rate ν and the selection coefficient s because both of those appear only as linear terms in equation 4. Thus, for example, if we consider an organism where the point mutation rate is increased by a factor of 10^3, then the numbers calculated from equation 4 will decrease by only that factor. For the case discussed earlier in which six nucleotide changes were required, the population size needed to fix the feature in 10^8 generations would then decrease from 10^{22} to just 10^{19}.

The model is more sensitive to the value of ρ, because ρ appears with an exponent in equation 4. If ρ were less by a factor of 10 (100 instead of 1000), then the population size needed to fix the feature in the preceding example in 10^8 generations would decrease from 10^{22} to 10^{16}. The number of possible null mutations—the numerator of ρ—arises from basic considerations of protein structure so that it is unlikely to vary significantly. The number of possible compatible mutations λ—the denominator of ρ—is more difficult to estimate. However, the value of one thousand that we use for ρ in Figure 6 is conservative compared with the range of values used by other workers (Walsh 1995). It should be noted that as λ becomes larger, the number of possible null mutations—and thus implicitly the length of the gene—must increase to maintain a constant value of ρ.

The model is most sensitive to the value of λ—the number of loci that must mutate before a new MR function occurs—which appears as an exponent in equation 4. If in the case just mentioned, because of the particular initial sequence of the parent gene, either three or nine nucleotide changes were necessary instead of six, then the population sizes required to fix the feature in 10^8 generations would vary from 10^{11} to 10^{31} organisms. The dependence on λ may encourage speculation that perhaps MR mutations could develop by point mutation in duplicate genes if the parent gene giving rise to the duplication were serendipitously poised to lead to the new feature with only one mutation in the precursor gene. Although this is certainly possible, it is unlikely to be the general case. As one example, Li (1997) has argued that the precursor to modern hemoglobins that can bind diphosphoglycerate did not have any of the three amino acid residues involved in the interaction. As shown in Figure 5, for the average case, pre-equilibration, which allows for the occurrence of rare, fortunate alleles, does not affect the expected time T_f in the linear portion of the curve.

The lack of recombination in our model means it is most directly applicable to haploid, asexual organisms. Nonetheless, the results also impinge on the evolution of diploid sexual organisms. The fact that very large population sizes—10^9 or greater—are required to build even a minimal MR feature requiring two nucleotide alterations within 10^8 generations by the processes described in our model, and that enormous population sizes are required for more complex features or shorter times, seems to indicate that the mechanism of gene duplication and point mutation alone would be ineffective, at least for multicellular diploid species, because few multicellular species reach the required population sizes. Thus, mechanisms in addition to gene duplication and point mutation may be necessary to explain the development of MR features in multicellular organisms.

Although large uncertainties remain, it nonetheless seems reasonable to conclude that, although gene duplication and point mutation may be an effective mechanism for exploring closely neighboring genetic space for novel functions, where single mutations produce selectable effects, this conceptually simple pathway for developing new functions is problematic when multiple mutations are required. Thus, as a rule, we should look to more complicated pathways, perhaps involving insertion, deletion, recombination, selection of intermediate states, or other mechanisms, to account for most MR protein features.

Materials and methods

A duplicated gene in a population was represented by an array of integer elements that could take the values of either zero or one. The number of elements λ in the array corresponded to the number

of nucleotide positions that would have to mutate in a particular gene to yield a hypothesized MR protein feature. In all cases, we begin the simulation in a uniform initial state, with N duplicate copies of the parent gene in the population, represented by N identical arrays. This simplification of starting with the duplicate gene already fixed in the population ignores the time needed for the duplicate copy to initially spread in the population; thus the average times we calculate from this model can be considered underestimates of the time for fixation of a gene with a new MR function. All elements of the array were initially set to a value of one, which represented the initial, incompatible state of the position, which could not contribute to the MR feature. Each position in an array was then allowed to mutate sequentially with a probability set by the mutation rate ν. A value of zero represented the state that could potentially contribute to an MR feature. Back mutations were permitted, so that a position with a value of zero could revert to a value of one. The equal rates of forward and backward point mutations should not be confused with the very different rates at which a gene will acquire null mutations versus acquiring a new, selectable MR feature.

After each step in which a mutation occurred at an array position, a further probabilistic step was taken to simulate the possible occurrence of one or more null mutations elsewhere in the gene. With probability $1/(1+\rho)$, where ρ is the ratio of null-to-compatible mutations in the gene (neutral mutations are ignored), the gene was considered to be free of null mutations and continued in the simulation. With probability $\rho/(1+\rho)$ the gene was deemed to have suffered one or more null mutations at positions not explicitly represented in the array and consequently to have become a pseudogene. In this case, the array was replaced in the population by a new array in which all loci were again set to one. This is intended to simulate replacement of the nonfunctional duplicate gene by a new duplication of the original gene, whose sequence is considered to remain constant under selection. Checking for null mutations only when mutations occur at an array position has the intended effect of making the gene duplication rate similar to the rate of point mutation ν. It has recently been shown that those two rates are in fact similar (Lynch and Conery 2000). It should be noted that the model purposely does not replace the duplicate gene immediately whenever a null mutation would occur anywhere in the gene, rather than waiting for one of the λ array sites to mutate, because that would have the effect of making the gene duplication rate much faster than it is estimated to be.

After the mutation step, the population was checked for the number of selectable organisms—those whose array elements all had a value of zero. Arrays in which some but not all elements were in a compatible state had no advantage. (This models MR features where, by hypothesis, the selectable feature does not exist until all contributing amino acids are in the correct state.) For most simulations, the run was halted when the first selectable array was discovered and the time in generations to the first occurrence of the selectable MR mutant recorded. For other runs, the simulation was continued and selection was applied at the reproduction step. In these cases, the simulation was continued until >50% of the population carried the MR feature, which was then considered to be "fixed" in the population, and the time to fixation recorded. The time for the selectable MR mutation to spread is generally much less than the time for it to be produced by the population.

After each array was subjected to the mutation step, the next generation was populated, either by deterministic reproduction or by simulated stochastic reproduction. In deterministic reproduction, the next generation was taken clonally from the previous; that is, the composition of the next generation was identical to the previous generation after the mutation step. In stochastic reproduction, the subsequent generation was populated by copying randomly chosen arrays, with or without selection, from the previous generation until the subsequent generation was fully populated.

In some simulations, the process was "prerun" for a selected number of generations, undergoing mutation and reproduction but not selection. This was done to model populations that had to varying degrees approached steady state with respect to the occurrence of the MR mutation in the population in the absence of selection for it.

All equations were evaluated using *Mathematica*.

Acknowledgments

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

Appendix 1

Consider a population in which the number of duplicated genes that has none of the necessary mutations to produce an MR feature is n_0, the number that has one required mutation is n_1, the number with two required mutations is n_2, and so forth. As in the text, ν is the mutation rate per nucleotide per generation, ρ is the ratio of possible null mutations to compatible mutations in a gene, and λ is the number of initially incompatible nucleotide loci in a duplicate gene that must be changed to form the selectable, MR feature. Also as in the text, genes suffering a null mutation are presumed to be replaced by a new duplicate of the original gene, with all loci in the incompatible state. Then we can write:

$$\frac{\partial n_0}{\partial t} = -\lambda\nu\alpha n_0 + \nu\alpha n_1 + \nu\beta n_1 + \nu\beta n_2 + \ldots + \nu\beta n_{\lambda-1} \quad (5)$$

$$\frac{\partial n_1}{\partial t} = \lambda\nu\alpha n_0 - \nu\alpha n_1 - \nu\beta n_1 - (\lambda-1)\nu\alpha n_1 + 2\nu\alpha n_2 \quad (6)$$

$$\frac{\partial n_2}{\partial t} = (\lambda-1)\alpha n_1 - 2\nu\alpha n_2 - \nu\beta n_2 - (\lambda-2)\nu\alpha n_2 + 3\nu\alpha n_3 \quad (7)$$

$$\vdots$$

$$\frac{\partial n_\lambda}{\partial t} = \nu\alpha n_{\lambda-1} \quad (8)$$

where

$$\alpha \equiv \frac{1}{1+\rho}$$

and

$$\beta \equiv \frac{\rho\lambda}{1+\rho}$$

Terms representing processes in which an additional compatible mutation is gained without a null mutation first occurring are multiplied by the factor $\lambda - m$, where m is the number of compatible mutations a gene has already acquired, to account for the decreasing number of sites that are available for potentially beneficial mutation, and terms representing processes in which a compatible mutation is lost without a null mutation first occurring are multiplied by m to account for the increasing number of sites that can revert. Equation 8 represents the transition to an absorbing state that is under selection, where all required mutations necessary for the new MR feature are present. Once in the selectable state, the gene is presumed not to leave by mutation to another state. (This is similar to the situation described by equation 3, where the time to just the first appearance of an MR feature is estimated, which thus does not allow for back mutation of the gene with the MR feature.)

If $\rho \gg 1$, then $n_0 \gg n_1 \gg n_2 \ldots$ and $\beta \gg \alpha$. In this limit, equations 5–8 become simply:

$$\frac{\partial n_0}{\partial t} = -\lambda\nu\alpha n_0 + \nu\beta n_1 \tag{9}$$

$$\frac{\partial n_1}{\partial t} = \lambda\nu\alpha n_0 - \nu\beta n_1 \tag{10}$$

$$\frac{\partial n_2}{\partial t} = (\lambda-1)\nu\alpha n_1 - \nu\beta n_2 \tag{11}$$

$$\frac{\partial n_\lambda}{\partial t} = \nu\alpha n_{\lambda-1} \tag{12}$$

These can be solved in sequence by successive approximations. For equation 10, we approximate $n_0 = N$ (the total population size), which for initial condition $n_1 = 0$ gives:

$$\frac{\partial n_1}{\partial t} = \lambda\nu\alpha N - \nu\beta n_1 \tag{13}$$

which has the solution

$$n_1 = N\lambda\frac{\alpha}{\beta}(1 - e^{-\nu\beta t}). \tag{14}$$

Using this solution for n_1 in the next equation, with initial condition $n_2 = 0$, we have

$$n_2 = N\lambda(\lambda - 1)\left(\frac{\alpha}{\beta}\right)^2(1 - e^{-\nu\beta t})^2 \tag{15}$$

Therefore, using the same approximation successively, we obtain

$$n_{\lambda-1} = N(\lambda)!\left(\frac{\alpha}{\beta}\right)^{\lambda-1}(1 - e^{-\nu\beta t})^{\lambda-1}. \tag{16}$$

Last, we integrate equation 16 to get $n\lambda$. Setting $n\lambda = 1$ (the first appearance of the MR feature) yields

$$1 = \int_0^{T_f}(\lambda!)\nu\alpha N\left(\frac{\alpha}{\beta}\right)^{\lambda-1}(1 - e^{-\nu\beta t})^{\lambda-1}\,dt, \tag{17}$$

or

$$N\nu\lambda\int_0^{T_f}(1 - e^{\nu\beta t})^{\lambda-1}\,dt = \frac{\lambda(1+\rho)(1+\lambda\rho)^{\lambda-1}}{\lambda!}. \tag{18}$$

When ρ is small (<10), the values of T_f given by equation 18 do not give a good match to the results of the simulations, which are nonetheless closely matched by equation 3 in the text. When ρ is large ($\gtrsim$10), however, which will be the case in biologically realistic situations, then equation 18 is approximately equal to equation 3 and both equations closely match the results of the simulations because, for large ρ, for the exponent of the integrand on the left-hand sides of the equations

$$\frac{2+\rho\lambda}{1+\rho} \approx \frac{\rho\lambda}{1+\rho} \approx \lambda$$

and for the right-hand sides of the equations

$$(1+\rho)^\lambda\left(\frac{\lambda^\lambda}{\lambda!}\right) \approx \frac{\lambda(1+\rho)(\lambda\rho)^{\lambda-1}}{\lambda!} \approx \frac{\rho^\lambda\lambda^\lambda}{\lambda!}.$$

Appendix 2

In the limit of $T_f \ll 1/\nu\lambda$, the left-hand sides of both equation 3 and equation 18 are proportional to $(T_f)\lambda$. This is seen in the following:

$$\int_0^{T_f}(1 - e^{\gamma t})^{(\lambda-1)}\,dt = \int_0^{T_f}(1-(1-\gamma t))^{(\lambda-1)}\,dt \tag{19}$$

$$= \gamma^{(\lambda-1)}\int_0^{T_f} t^{(\lambda-1)}\,dt \tag{20}$$

$$= \frac{\gamma^{(\lambda-1)}}{\lambda}T_f^\lambda \tag{21}$$

where $\gamma = \nu\lambda$ for both equations in the limit $\rho \gg 1$. In this limit, equations 3 and 18 therefore become

$$N\nu\lambda\left(\frac{(\nu\lambda)^{\lambda-1}}{\lambda}T_f^\lambda\right) \approx \frac{\rho^\lambda\lambda^\lambda}{\lambda!} \tag{22}$$

which implies

$$\nu T_f \approx \left(\frac{\rho^\lambda\lambda^\lambda}{\lambda!N}\right)^{1/\lambda}, \tag{23}$$

where we have used the approximations of Appendix 1 for the case of $\rho \gg 1$. The limit $T_f \ll 1/\nu\lambda$ therefore corresponds to the limit $N \gg (1+\rho)^\lambda(\lambda^\lambda/\lambda!)$, or $\rho \ll N^{1/\lambda}$.

For $\rho \ll N^{1/\lambda}$, then, the time required to produce a selectable state is inversely proportional to the λ^{th} root of the population size, that is, $T_f \propto N^{-1/\lambda}$.

References

Axe, D.D., Foster, N.W., and Fersht, A.R. 1996. Active barnase variants with completely random hydrophobic cores. *Proc. Natl. Acad. Sci.* **93**: 5590–5594.

———. 1998. A search for single substitutions that eliminate enzymatic function in a bacterial ribonuclease. *Biochemistry* **37:** 7157–7166.
Bowie, J.U. and Sauer, R.T. 1989. Identifying determinants of folding and activity for a protein of unknown structure. *Proc. Natl. Acad. Sci.* **86:** 2152–2156.
Bowie, J.U., Reidhaar-Olson, J.F., Lim, W.A., and Sauer, R.T. 1990. Deciphering the message in protein sequences: Tolerance to amino acid substitutions. *Science* **247:** 1306–1310.
Braden, B.C. and Poljak, R.J. 1995. Structural features of the reactions between antibodies and protein antigens. *FASEB J.* **9:** 9–16.
Chakrabarti, P. and Janin, J. 2002. Dissecting protein-protein recognition sites. *Proteins* **47:** 334–343.
Chothia, C., Gough, J., Vogel, C., and Teichmann, S.A. 2003. Evolution of the protein repertoire. *Science* **300:** 1701–1703.
Cunningham, B.C. and Wells, J.A. 1993. Comparison of a structural and a functional epitope. *J. Mol. Biol.* **234:** 554–563.
Drake, J.W., Charlesworth, B., Charlesworth, D., and Crow, J.F. 1998. Rates of spontaneous mutation. *Genetics* **148:** 1667–1686.
Ferris, S.D. and Whitt, G.S. 1977. Loss of duplicate gene expression after polyploidisation. *Nature* **265:** 258–260.
———. 1979. Evolution of the differential regulation of duplicate genes after polyploidization. *J. Mol. Evol.* **12:** 267–317.
Force, A., Lynch, M., Pickett, F.B., Amores, A., Yan, Y.L., and Postlethwait, J. 1999. Preservation of duplicate genes by complementary, degenerative mutations. *Genetics* **151:** 1531–1545.
Garry, D.J., Ordway, G.A., Lorenz, J.N., Radford, N.B., Chin, E.R., Grange, R.W., Bassel-Duby, R., and Williams, R.S. 1998. Mice without myoglobin. *Nature* **395:** 905–908.
Garry, D.J., Kanatous, S.B., and Mammen, P.P. 2003. Emerging roles for myoglobin in the heart. *Trends Cardiovasc. Med.* **13:** 111–116.
Guo, Z.Y., Shen, L., Gu, W., Wu, A.Z., Ma, J.G., and Feng, Y.M. 2002. In vitro evolution of amphioxus insulin-like peptide to mammalian insulin. *Biochemistry* **41:** 10603–10607.
Huang, W., Petrosino, J., Hirsch, M., Shenkin, P.S., and Palzkill, T. 1996. Amino acid sequence determinants of β-lactamase structure and activity. *J. Mol. Biol.* **258:** 688–703.
Hughes, M.K. and Hughes, A.L. 1993. Evolution of duplicate genes in a tetraploid animal, *Xenopus laevis*. *Mol. Biol. Evol.* **10:** 1360–1369.
Janin, J. and Chothia, C. 1990. The structure of protein-protein recognition sites. *J. Biol. Chem.* **265:** 16027–16030.
Kimura, M. 1983. *The neutral theory of molecular evolution*. Cambridge University Press, Cambridge, UK.
Kondrashov, F.A., Rogozin, I.B., Wolf, Y.I., and Koonin, E.V. 2002. Selection in the evolution of gene duplications. *Genome Biol.* **3:** RESEARCH0008.
Li, W.H. 1997. *Molecular evolution*. Sinauer Associates, Sunderland, MA.
Lim, W.A. and Sauer, R.T. 1989. Alternative packing arrangements in the hydrophobic core of λ repressor. *Nature* **339:** 31–36.
Lo, C.L., Chothia, C., and Janin, J. 1999. The atomic structure of protein-protein recognition sites. *J. Mol. Biol.* **285:** 2177–2198.
Long, M. and Thornton, K. 2001. Gene duplication and evolution. *Science* **293:** 1551.
Lynch, M. and Conery, J.S. 2000. The evolutionary fate and consequences of duplicate genes. *Science* **290:** 1151–1155.
———. 2001. Gene duplication and evolution. *Science* **293:** 1551.
Lynch, M. and Walsh, B. 1998. *Genetics and analysis of quantitative traits*. Sinauer, Sunderland, MA.
Lynch, M., O'Hely, M., Walsh, B., and Force, A. 2001. The probability of preservation of a newly arisen gene duplicate. *Genetics* **159:** 1789–1804.
Matsumura, M., Becktel, W.J., Levitt, M., and Matthews, B.W. 1989. Stabilization of phage T4 lysozyme by engineered disulfide bonds. *Proc. Natl. Acad. Sci.* **86:** 6562–6566.
Meeson, A.P., Radford, N., Shelton, J.M., Mammen, P.P., DiMaio, J.M., Hutcheson, K., Kong, Y., Elterman, J., Williams, R.S., and Garry, D.J. 2001. Adaptive mechanisms that preserve cardiac function in mice without myoglobin. *Circ. Res.* **88:** 713–720.
Ohno, S. 1970. *Evolution by gene duplication*. Springer-Verlag, Berlin, Germany.
Ohta, T. 1987. Simulating evolution by gene duplication. *Genetics* **115:** 207–213.
———. 1988a. Further simulation studies on evolution by gene duplication. *Evolution* **42:** 375–386.
———. 1988b. Time for acquiring a new gene by duplication. *Proc. Natl. Acad. Sci.* **85:** 3509–3512.
Reidhaar-Olson, J.F. and Sauer, R.T. 1988. Combinatorial cassette mutagenesis as a probe of the informational content of protein sequences. *Science* **241:** 53–57.
———. 1990. Functionally acceptable substitutions in two α-helical regions of λ repressor. *Proteins* **7:** 306–316.
Rennell, D., Bouvier, S.E., Hardy, L.W., and Poteete, A.R. 1991. Systematic mutation of bacteriophage T4 lysozyme. *J. Mol. Biol.* **222:** 67–88.
Sauer, R.T., Milla, M.E., Waldburger, C.D., Brown, B.M., and Schildbach, J.F. 1996. Sequence determinants of folding and stability for the P22 Arc repressor dimer. *FASEB J.* **10:** 42–48.
Suckow, J., Markiewicz, P., Kleina, L.G., Miller, J., Kisters-Woike, B., and Muller-Hill, B. 1996. Genetic studies of the Lac repressor. XV: 4000 single amino acid substitutions and analysis of the resulting phenotypes on the basis of the protein structure. *J. Mol. Biol.* **261:** 509–523.
Wagner, A. 2001. Birth and death of duplicated genes in completely sequenced eukaryotes. *Trends Genet.* **17:** 237–239.
Walsh, J.B. 1995. How often do duplicated genes evolve new functions? *Genetics* **139:** 421–428.
White, S. and Doebley, J. 1998. Of genes and genomes and the origin of maize. *Trends Genet.* **14:** 327–332.
Zhang, L., Gaut, B.S., and Vision, T.J. 2001. Gene duplication and evolution. *Science* **293:** 1551.

Exhibit 11

article on evolutionary potential
by Barry Hall

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 2004, p. 1032–1033 Vol. 48, No. 3
0066-4804/04/$08.00+0 DOI: 10.1128/AAC.48.3.1032–1033.2004

In Vitro Evolution Predicts that the IMP-1 Metallo-β-Lactamase Does Not Have the Potential To Evolve Increased Activity against Imipenem

Barry G. Hall*

Biology Department, University of Rochester, Rochester, New York

Received 31 July 2003/Returned for modification 20 October 2003/Accepted 11 November 2003

In vitro evolution was used to predict whether the IMP-1 metallo-β-lactamase has the potential to evolve an increased ability to confer resistance to imipenem. Screening of eight libraries containing $9.8 \times 10^6 \pm 1.4 \times 10^6$ (mean ± standard error) variants per library, with an average of 1.2 mutations per variant, detected no increased resistance to imipenem. The results predict, with >99.9% confidence, that even under intense selection the IMP-1 β-lactamase will not evolve to confer increased resistance to imipenem.

Carbapenems are among the most potent agents for treatment of gram-negative bacterial infections (9, 12) and are hydrolyzed by a wide variety of metallo-β-lactamases (9). Widespread clinical use of carbapenems (8, 12) has led to several reports of resistance associated with the presence of metallo-β-lactamases (7). The IMP family of metallo-β-lactamases is a particular source of concern, because the IMP enzymes are typically plasmid-borne and typically found in integron cassettes (5, 10). IMP genes are therefore easily transferred among diverse bacterial species. A number of researchers have advised careful clinical use to prevent proliferation of carbapenem-resistant strains that produce metallo-β-lactamases (7, 12). Yano et al. (12) recently reported that IMP-6, which differs from IMP-1 by a single amino acid substitution, increases the MIC of meropenem 128-fold but does not increase the resistance to imipenem. If the rapid evolution of the class A extended-spectrum β-lactamases is typical, then we should indeed be concerned about the evolution of metallo-β-lactamases in response to the clinical use of imipenem and other carbapenems.

Instead of assuming that metallo-β-lactamases will evolve rapidly, it would be highly desirable to accurately predict their evolution in response to carbapenem selection.

The Barlow-Hall in vitro evolution model has been shown to accurately mimic the natural evolution of the TEM β-lactamases (4) and has been used to predict that both the class A TEM and class C CMY-2 β-lactamases will soon evolve to provide high levels of resistance to cefepime (2, 3). Here I have used that method to predict the evolution of the IMP-1 β-lactamase in response to clinical selection with imipenem.

The bla_{IMP-1} gene was amplified from genomic DNA of *Serratia marcescens* strain AK9373 (7) and cloned into the expression vector pACSE3 (4) where it is expressed from the plasmid's pTAC promoter upon induction with 100 μM IPTG (isopropyl-β-D-thiogalactopyranoside). The sequence of the bla_{IMP-1} allele in the resulting plasmid, pIMP1, was determined to be identical to the published sequence (1) (GenBank accession no. AF416297) except for a silent T-to-C mutation at bp 15 of the coding sequence. Sequencing of the original amplicon confirmed that the polymorphism is present in *S. marcescens* strain AK9373.

The IMP-1 gene of pIMP1 was mutagenized by amplification with the highly error-prone polymerase Mutazyme (Stratagene). The mutagenesis reaction yielded 9.85 μg of amplicon, or 1.2×10^{13} mutant molecules. The mutagenized amplicon was digested with restriction endonucleases *Bsp*HI and *Sac*I, and in eight separate reactions, the digested amplicons were ligated to plasmid pACSE3 and transformed into *Escherichia coli* strain DH5αE ([F^- ϕ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169 *endA1 recA1 hsdR17*(r^- m^+) *deoR thi-1 phoA supE44* λ^- *gyrA96 relA1* Gal^-) by selecting for tetracycline resistance to produce eight libraries containing $9.8 \times 10^6 \pm 1.4 \times 10^6$ (mean ± standard error) insert-bearing transformants per library. Because each library contained $<10^{-6}$ molecules of the original pool of mutant molecules, the libraries were essentially independent samples of the pool of mutant molecules, and the probability that sibling molecules were present in different libraries is negligible. The bla_{IMP-1} genes of plasmids extracted from 10 randomly chosen transformants were sequenced. There was an average of 1.2 mutations per gene.

In pIMP1, the bla_{IMP-1} gene is expressed under control of the inducible pTAC promoter that is regulated by the plasmid-borne $lacI^q$ repressor. In the presence of 100 μM IPTG, pIMP1 conferred an imipenem MIC of 2 μg/ml on the host strain DH5αE compared with the imipenem MIC of 0.125 μg/ml for DH5αE carrying only the pACSE3 vector. That is the same level of resistance that has previously been reported for IMP-1 in *E. coli* (12).

Each of the eight libraries was expanded by growth overnight in L broth containing tetracycline (in 1 liter, 10 g of tryptone, 5 g of yeast extract, 10 g of NaCl, 1 g of glucose, 15 mg of tetracycline). Bottles containing 50 ml of L broth with 100 μM IPTG, with twofold serial dilutions of imipenem from 8 μg/ml down to 0.0625 μg/ml, were inoculated with 1.1×10^8 cells (10 times the largest library size) of the libraries. For a control, a similar imipenem dilution series was inoculated with 1.1×10^8 DH5αE carrying the unmutagenized plasmid pIMP1.

After 48 h of incubation at 37°C, the control series and each of the library series grew at imipenem concentrations of 2 μg/ml and below, but none grew at concentrations in excess of

* E-mail: drbh@mail.rochester.edu.

2 μg/ml. Plasmid was prepared from each of the cultures growing in the presence of 2 μg of imipenem per ml and was transformed into *E. coli* strain DH5αE by selection for tetracycline resistance. The resulting populations of transformants and *E. coli* DH5αE/pIMP1 after imipenem selection were used to measure the MIC of imipenem in Muller-Hinton broth (Difco) containing 100 μM IPTG using an inoculum of 10^5 cells per ml by broth serial dilution as previously described (4). For seven of the eight mutant populations and for the control, the imipenem MIC was 2 μg/ml, and for one mutant population, the MIC was 1 μg/ml. Thus, neither the initial selections nor the MIC measurements provided any evidence of mutants that exhibited increased resistance to imipenem.

Because MICs can detect only twofold increases in resistance, a more sensitive disk diffusion test was used to detect small improvements in resistance. Forty cultures were grown overnight in L broth containing tetracycline. The cultures were grown from individual colonies of transformed library 8 after imipenem selection. For a control, 10 cultures were grown from individual colonies of *E. coli* DH5αE/pIMP1. One hundred microliters of each culture was spread on a Muller-Hinton broth plate containing 100 μM IPTG, a BBL antibiotic disk containing 10 μg of imipenem was placed onto the center of each plate, and after 24 h of incubation at 37°C, the zone of inhibition was measured. The diameters of the zones of inhibition were 17.2 ± 0.2 mm for pIMP1 and 17.1 ± 0.1 mm for cells carrying plasmids from library 8 after imipenem selection. The results of the disk diffusion test thus confirm that the mutants failed to confer increased resistance to imipenem, leading to the strong prediction that the IMP-1 metallo-β-lactamase does not have the potential to evolve increased activity against imipenem.

Confidence in that prediction is based on a simulation of the in vitro evolution process using the program In vitro Evolution Simulator (6, 11). The program simulates the random mutation of the input sequence and determines the fraction of possible single and double amino acid substitutions that are obtained in a library of a given size. It is important to consider the effects of only one or two independent amino acid substitution mutations, because in nature mutations almost always arise one at a time, and each mutation must be fixed into microbial populations by selection. The input sequence was the IMP-1 sequence, the mutation frequency was 1.2 mutations per molecule, and the fraction of possible single and double amino acid substitutions obtained was calculated separately for each library. The mean fractions per library were 0.897 ± 0.009 of the single amino acid substitutions and 0.670 ± 0.01 of the double amino acid substitutions (mean ± standard error). For the eight libraries taken together, the probability of having failed to screen any particular single amino acid substitution enzyme is 1.0×10^{-8}, and the probability of having failed to screen any particular double amino acid substitution enzyme is 1.3×10^{-4}. These results predict, with >99.9% confidence, that bla_{IMP-1} will not evolve to confer increased resistance to imipenem. That prediction depends on the sensitivity with which we can detect increased resistance in the laboratory. I cannot eliminate the possibility that increased resistance, below the level of laboratory detection, could be selected in nature.

It is clear from this study that the risks associated with the presence of bla_{IMP-1} do not include the risk of evolving increased activity against imipenem. This study, alone, is not sufficient to justify reconsideration of policies concerning the use of imipenem. In order to understand the risks posed by metallo-β-lactamases, it will be necessary to conduct similar studies on representative members of each of the three metallo-β-lactamase subfamilies and to include all clinically relevant carbapenems in those studies.

I am grateful to Fred Tenover for the gift of *Serratia marcescens* strain AK9373.

This study was supported in part by grant GM60761 from the National Institutes of Health.

REFERENCES

1. **Arakawa, Y., M. Murakami, K. Suzuki, H. Ito, R. Wacharotayankun, S. Ohsuka, N. Kato, and M. Ohta.** 1995. A novel integron-like element carrying the metallo-β-lactamase gene bla_{IMP}. Antimicrob. Agents Chemother. **39:** 1612–1615.
2. **Barlow, M., and B. G. Hall.** 2003. Experimental prediction of the evolution of cefepime resistance from the CMY-2 AmpC β-lactamase. Genetics **164:** 23–29.
3. **Barlow, M., and B. G. Hall.** 2003. Experimental prediction of the natural evolution of antibiotic resistance. Genetics **163:**1237–1241.
4. **Barlow, M., and B. G. Hall.** 2002. Predicting evolutionary potential: *in vitro* evolution accurately reproduces natural evolution of the TEM β-lactamase. Genetics **160:**823–832.
5. **Docquier, J. D., M. L. Riccio, C. Mugnaioli, F. Luzzaro, A. Endimiani, A. Toniolo, G. Amicosante, and G. M. Rossolini.** 2003. IMP-12, a new plasmid-encoded metallo-β-lactamase from a *Pseudomonas putida* clinical isolate. Antimicrob. Agents Chemother. **47:**1522–1528.
6. **Hall, B. G.** 2002. In vitro Evolution Simulator, version 1.2. University of Rochester, Rochester, N.Y.
7. **Ito, H., Y. Arakawa, S. Ohsuka, R. Wacharotayankun, N. Kato, and M. Ohta.** 1995. Plasmid-mediated dissemination of the metallo-β-lactamase gene bla_{IMP} among clinically isolated strains of *Serratia marcescens*. Antimicrob. Agents Chemother. **39:**824–829.
8. **Minami, S., M. Akama, H. Araki, Y. Watanabe, H. Narita, S. Iyobe, and S. Mitsuhashi.** 1996. Imipenem and cephem resistant Pseudomonas aeruginosa carrying plasmids coding for class B beta-lactamase. J. Antimicrob. Chemother. **37:**433–444.
9. **Rasmussen, B. A., and K. Bush.** 1997. Carbapenem-hydrolyzing beta-lactamases. Antimicrob. Agents Chemother. **41:**223–232.
10. **Riccio, M. L., N. Franceschini, L. Boschi, B. Caravelli, G. Cornaglia, R. Fontana, G. Amicosante, and G. M. Rossolini.** 2000. Characterization of the metallo-β-lactamase determinant of *Acinetobacter baumannii* AC-54/97 reveals the existence of bla_{IMP} allelic variants carried by gene cassettes of different phylogeny. Antimicrob. Agents Chemother. **44:**1229–1235.
11. **Salipante, S., and B. G. Hall.** 2003. Determining the limits of the evolutionary potential of an antibiotic resistance gene. Mol. Biol. Evol. **20:**653–659.
12. **Yano, H., A. Kuga, R. Okamoto, H. Kitasato, T. Kobayashi, and M. Inoue.** 2001. Plasmid-encoded metallo-β-lactamase (IMP-6) conferring resistance to carbapenems, especially meropenem. Antimicrob. Agents Chemother. **45:** 1343–1348.

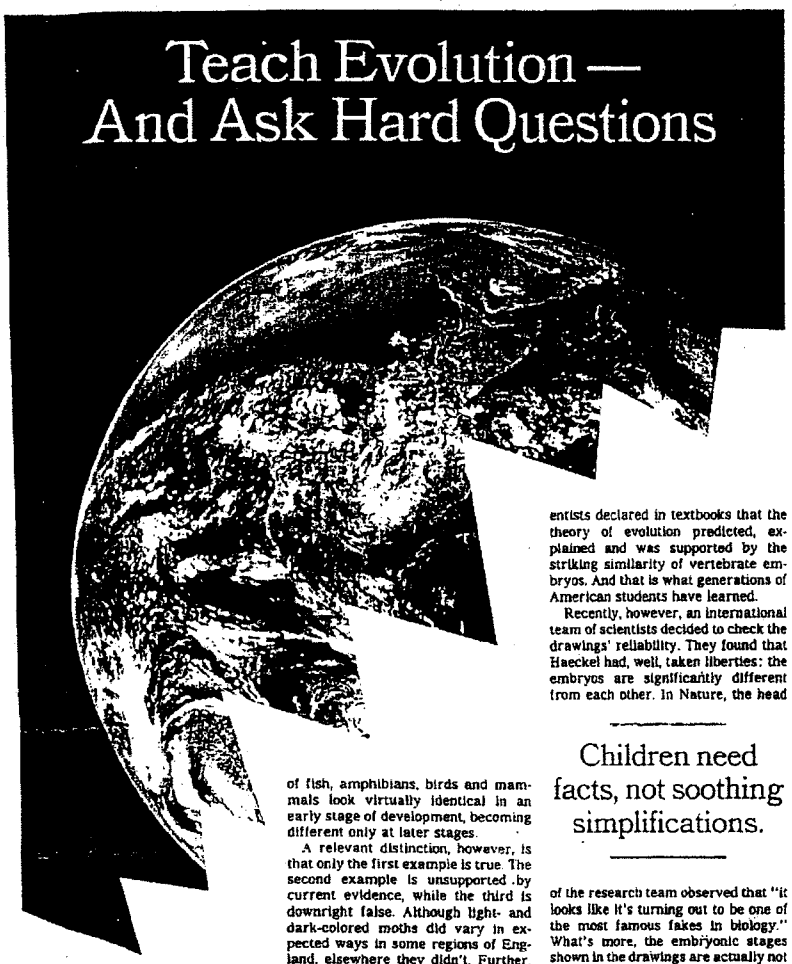
Exhibit 12

New York Times op-ed containing my views on teaching problems with Darwinian theory

The New York Times

FRIDAY, AUGUST 13, 1999

Teach Evolution — And Ask Hard Questions



By Michael J. Behe

BETHLEHEM, Pa.

The debate leading the Kansas Board of Education to abolish the requirement for teaching evolution has about the same connection to reality as the play "Inherit the Wind" had to the actual Scopes trial. In both cases complex historical, scientific and philosophical issues gave way to the simplifying demands of the morality play. If the schoolchildren of Kansas and other states are to receive a good science education, however, then we'll have to forgo the fun of demonizing each other, take a deep breath and start making a few distinctions.

Regrettably, the action of the Kansas board makes that much more difficult. Not only are teachers there now discouraged from discussing evidence in support of Darwin's theory, results questioning it won't be heard either.

For example, let's look at three claims of evidence for Darwinian evolution often cited by high school textbooks. First, as the use of antibiotics has become common, mutant strains of resistant bacteria have become more common, threatening public health. Second, dark-colored variants of a certain moth species evaded predation by birds because their color matched the sooty tree trunks of industrial England. Third, the embryos of fish, amphibians, birds and mammals look virtually identical in an early stage of development, becoming different only at later stages.

A relevant distinction, however, is that only the first example is true. The second example is unsupported by current evidence, while the third is downright false. Although light- and dark-colored moths did vary in expected ways in some regions of England, elsewhere they didn't. Further, textbook photographs showing moths resting on tree trunks in the day, where birds supposedly ate them, run afoul of the fact that the moths are active at night and don't normally rest on tree trunks. After learning about the problems with this favorite Darwinian example, an evolutionary scientist wrote in the journal Nature that he felt the way he did as a boy when he learned there was no Santa Claus.

The story of the embryos is an object lesson in seeing what you want to see. Sketches of vertebrate embryos were first made in the late 19th century by Ernst Haeckel, an admirer of Darwin. In the intervening years, apparently nobody verified the accuracy of Haeckel's drawings. Prominent scientists declared in textbooks that the theory of evolution predicted, explained and was supported by the striking similarity of vertebrate embryos. And that is what generations of American students have learned.

Recently, however, an international team of scientists decided to check the drawings' reliability. They found that Haeckel had, well, taken liberties: the embryos are significantly different from each other. In Nature, the head of the research team observed that "it looks like it's turning out to be one of the most famous fakes in biology." What's more, the embryonic stages shown in the drawings are actually not the earliest ones. The earliest stages show much greater variation.

> Children need facts, not soothing simplifications.

If I were teaching a high school biology course, I certainly would want my students to understand Darwin's theory of evolution by natural selection, which explains antibiotic resistance and a lot of other things. I would want them to know the many similarities among organisms that are interpreted in terms of common descent, as well as to understand the laboratory experiments that show organisms changing in response to selective pressure.

But I would also want them to learn to make distinctions and ask tough questions. Questions we might discuss include these:

If it's so difficult to demonstrate that small changes in modern moths are the result of natural selection, how confident can we be that Darwinian selection drove large changes in the distant past? If supposedly identical embryos were touted as strong evidence for evolution, does the recent demonstration of variation in embryos now count as evidence against evolution? If some scientists relied for a century on an old, mistaken piece of data because they thought it supported the accepted theory, is it possible they might even now give short shrift to legitimate contrary data or interpretations?

Discussing questions like these would help students see that sometimes a theory actively shapes the way we think, and also that there are still exciting, unanswered questions in biology that may require fresh ideas.

It's a shame that Kansas students won't get to take part in such a discussion. We should make sure that the students of other states do.

Emotions run very deep on the subject of evolution, and while the morality play generally casts religious people as the ones who want to limit discussion, some scientists on the "rational" side could fit that role, too. But if we want our children to become educated citizens, we have to broaden discussion, not limit it.

Teach Darwin's elegant theory. But also discuss where it has real problems accounting for the data, where data are severely limited, where scientists might be engaged in wishful thinking and where alternative — even "heretical" — explanations are possible. □

Michael J. Behe, professor of biological sciences at Lehigh University, is author of "Darwin's Black Box: The Biochemical Challenge to Evolution."