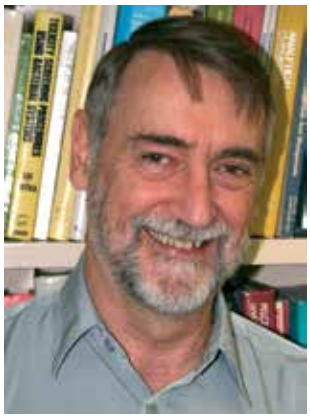
The millennium bugs



Paul R. Fisher

Department of Microbiology
La Trobe University
Vic 3086 Australia
Tel +61 3 9479 2229
Fax +61 3 9479 1222
Email Fisher@latrobe.edu.au
Web http://fishfam.homeip.net:100/mcbg

In the first decade of the new millennium, microorganisms continued to have a disproportionately large impact on biomedical science, not so much because they cause disease but because they provide tractable models in which to study fundamental cellular processes. They have featured in Nobel Prizes every year this century except 2004 and 2010. Of the nine non-mammalian model organisms recognised in the first years of the millennium by the NIH for their value in biomedical research, four were microbes. Collectively, a nation's effort in studying these model organisms correlates more strongly with its worldwide scientific impact than research in other fields. Compared to other fields, research on model organisms generates three to four times more publications per funding dollar and is three to four times more likely to be sufficiently significant for publication in *Nature* or *Science*. Given the scientific impact and value for money of model organism research, Australia in the third millennium should invest significantly more funds in this type of research.

The Nobel microbes of the third millennium

Microbes matter. For millennia they were one of the main causes of human mortality and for much of humanity, in many parts of the world, this remains true. For the average person, infectious disease is still the major association made with the words "germs", bacteria" or "viruses". However, microbiologists know that microbes matter for many more reasons than this. Microbes made the planet habitable for almost all other forms of life, continue to play vital roles in the ecology, fertility and habitability of the earth and we use them in food manufacture and in waste disposal.

Yet even microbiologists sometimes overlook the impact that microbes have had on the rest of biology and medicine – microbes were arguably at the heart of most of our advances in understanding biology and medicine at the cellular and molecular level. Our present understanding of plant, animal and human metabolism, physiology, genetics, molecular and cell biology depended on vital contributions made by the study and use of microorganisms. So significant has the role of microorganisms been that they have featured in a disproportionate number of Nobel Prizes in Chemistry and in Physiology and Medicine[1,2]. In fact it has been said that to win a Nobel Prize "you must cultivate a quiet career in the backwaters of microbiology"[3]. This has been true throughout the history of the Nobel Prizes and continues to the present with microorganisms featuring in Nobel Prizes every year of the third millennium (Table 1) except 2004 and 2010 (taking 2001 to be the first year of the millennium).

What can account for this disproportionate impact of microorganisms on biology and medicine? The answer is twofold. Firstly, the fundamental processes of life at the cellular and molecular level were "invented" by microorganisms in the course of the first few billion years of evolution and were conserved and selectively elaborated upon in the subsequent evolution of multicellular plants and animals. The result is that the fundamental mechanisms of life are similar across the living world and can be studied in microbes as well as in plants or animals or humans. Secondly, the study of fundamental processes in cells at the molecular level depends upon readily accessible biological material to work with and the ability to manipulate the organism experimentally. With their short generation times, capacity to be grown clonally, rapidly and cheaply in large quantities, small

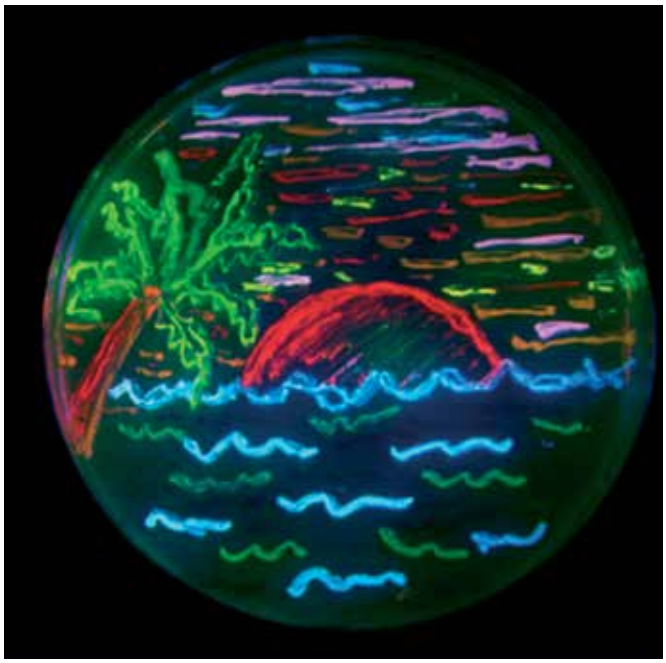
Table 1. Nobel Prizes of the third millennium featuring microorganisms.

Year	Nobel Prize	Winner(s)	Discovery	Microorganism(s)	Role
2001	Physiology or Medicine	Leland H. Hartwell Tim Hunt Sir Paul Nurse	Key regulators of the cell cycle	*Saccharomyces cerevisiae* *Schizosaccharomyces pombe*	Model
2002	Chemistry	John B. Fenn Koichi Tanaka Kurt Wüthrich	Development of methods for identification and structure analyses of biological macromolecules	Prions	Model
2003	Chemistry	Peter Agre Roderick MacKinnon	Discovery of water channels and Structural and mechanistic studies of ion channels	*Streptomyces lividans*	Model
2005	Physiology or Medicine	Barry J. Marshall, J. Robin Warren	*Helicobacter pylori* and its role in gastritis and peptic ulcer disease	*Helicobacter pylori*	Subject
2006	Chemistry	Roger D. Kornberg	The molecular basis of eukaryotic transcription	*Saccharomyces cerevisiae*	Model
2007	Physiology or Medicine	Mario R. Capecchi Sir Martin J. Evans Oliver Smithies	Principles for introducing specific gene modifications in mice by the use of embryonic stem cells	SV40 virus (mammalian origin of replication); Herpes virus (*tk* gene); *Escherichia coli* (*neo* gene and pBR322 plasmid vector)	Tool
2008	Physiology or Medicine	Harald zur Hausen Françoise Barré-Sinoussi Luc Montagnier	Human papilloma viruses causing cervical cancer and Human Immunodeficiency Virus	Human Papilloma Viruses; Human Immunodeficiency Virus	Subject
2008	Chemistry	Osamu Shimomura Martin Chalfie Roger Y. Tsien	Discovery and development of the green fluorescent protein, GFP	*Escherichia coli*	Model and tool
2009	Physiology or Medicine	Elizabeth H. Blackburn Carol W. Greider Jack W. Szostak	How chromosomes are protected by telomeres and the enzyme telomerase	*Tetrahymena thermophila;* *Saccharomyces cerevisiae*	Model
2009	Chemistry	Venkatraman Ramakrishnan Thomas A. Steitz Ada E. Yonath	The structure and function of the ribosome	*Geobacillus stearothermophilus; Haloarcola arismortui; Thermus thermophilus; Deinococcus radiodurans; Escherichia coli*	Model
2011	Physiology or Medicine	Bruce A. Beutler Jules A. Hoffmann Ralph M. Steinman	The activation of innate immunity and the dendritic cell and its role in adaptive immunity	*Aspergillus fumigatus;* Gram-negative bacteria (Lipopolysaccharide)	Model and tool

genomes and tractable genetics, biochemistry, cell biology and molecular biology, selected microorganisms still offer enormous advantages that are reflected in the list of Nobel prizes in Table 1.

Most of the Nobel microbes in Table 1 were not the subject of the investigation in and of themselves, that is, they were not under study because, for example, they caused a specific disease. In some cases they were used primarily as a main tool in the generation of materials for studying something else. For example, in the case of the Physiology and Medicine Prize in 2007, the *tk* gene encoding herpesvirus thymidine kinase and a neomycin resistance gene from a bacterial plasmid[4,5] were exploited as selectable markers in mouse cells and the vector DNA was cloned and prepared using *E. coli* as a host and *E.coli* plasmid (pBR322) to provide the vector backbone. Much of modern molecular biology uses microorganisms this way.

In most of the Nobel Prize-winning work featured in Table 1, microorganisms were being used as a model for the study of broader biological issues. For example, the work on GFP leading to the 2008 Chemistry Prize used *E. coli* as the first heterologous host to test the idea that the GFP would fold and fluoresce properly when expressed in organisms other than the jellyfish *Aequoria victoria* from which it originated. *E. coli* was being used as a model. This work heralded a revolution in molecular cell biology that exploited GFP and derivatives of it and other fluorescent proteins (Figure 1) to reveal the dynamic localisation of tagged proteins in living cells in an enormous variety of circumstances. The Physiology and Medicine Prize in the following year involved an Australian Nobel Laureate, Professor Elizabeth Blackburn, and two microbial models – the ciliate *Tetrahymena* and the yeast *Saccharomyces cerevisiae.* Professor Blackburn and colleagues, Carol W. Greider and Jack W. Szostak, used chromosome ends (telomeres) from *Tetrahymena* (because they were readily cloned) placed into the yeast *Saccharomyces cerevisiae* (because of its experimental tractability) to discover the role of telomerase in replicating chromosome ends[6,7]. The resulting telomere extension process limits the degree to which telomeres are progressively "whittled" away by the inability of DNA polymerase in the replication fork to copy both strands of the linear chromosomal DNA molecule right to the end. The absence of telomerase in a cell leads to progressive telomere shortening and ultimately cell senescence. Not surprisingly, alterations in the telomerase activity of cells is associated with disease and contributes to the ageing process[8]. Tumour cells frequently have either elevated telomerase levels or an alternative telomere lengthening mechanism in order to increase the stability of their chromosome ends during replication and division.



Description A San Diego beach scene drawn with an eight colour palette of bacterial colonies expressing fluorescent proteins derived from GFP and the red-fluorescent coral protein dsRed.
The colours include BFP, mTFP1, Emerald, Citrine, mOrange, mApple,mCherry and mGrape.
Artwork by Nathan Shaner, photography by Paul Steinbach, created in the lab of Roger Tsien in 2006.
Date 2006
Source transferred from en:Image: FPbeachTsien.jpg
Author Nathan Shaner
http://upload.wikimedia.org/wikipedia/commons/7/7b/FPbeachTsien.jpg

Figure 1. Fluorescent proteins expressed in bacteria.

Models for biomedical science

It was the successful use of microorganisms and a few other notable organisms, both mammals such as the mouse and non-mammals such as the vinegar fly *Drosophila*, that led to the concept of the "model" organism. In the latter half of the 20th century, scientists consciously looked for new eukaryotic model organisms that would extend the range of problems able to be profitably tackled using this approach. By the early years of the new millennium, two mammals (the mouse and the rat) and nine non-mammalian models were recognised (and funded) by the NIH for their value in biomedical research. The number has since been expanded by addition of the microcrustacean *Daphnia*[9]. Four of these are microorganisms – two yeast species (*S. cerevisiae* and *Schizosaccharomyces pombe*), a filamentous fungus *(Neurospora crassa*) and an amoebozoan (the cellular

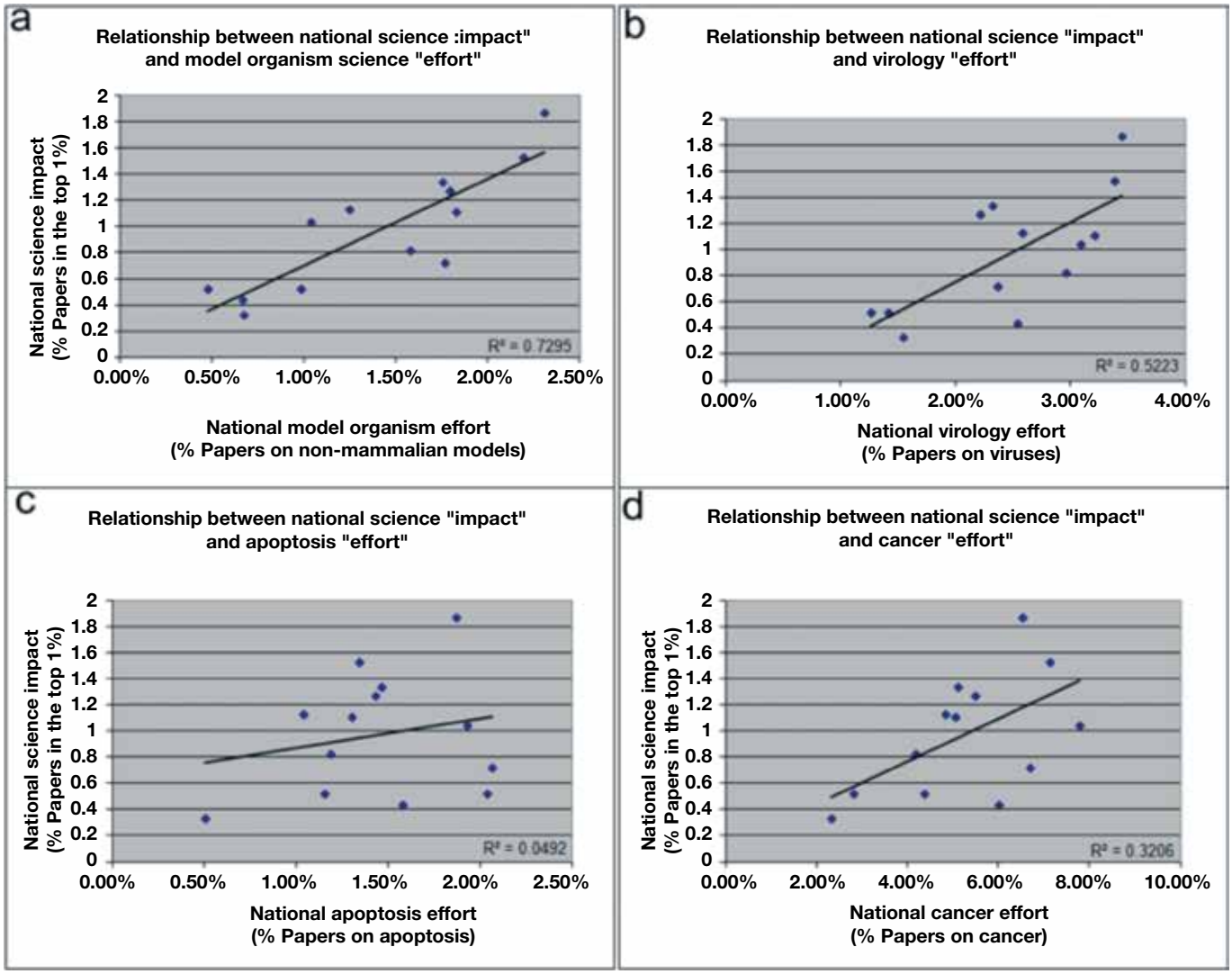


Figure 2. Relationship between national scientific effort in different fields and national scientific impact between 1996 and 2006.

slime mould or social amoeba *Dictyostelium discoideum*).

To investigate the scientific impact of these biomedical model organisms around the world, I began by asking how the collective scientific impact of the top science nations in the world correlates with the proportion of scientific effort they expend on model organisms. In 2007, Christopher King had published a citation analysis of the top 13 science nations' papers published in the decade spanning the beginning of the new millennium (1996 to 2006)[10]. As a measure of national scientific impact, this particular study used the proportion of each nation's papers that were ranked in the top 1% of all cited articles. To estimate each nation's relative scientific effort on the model organisms in the same period, I searched Current Contents Online (Ovid) to determine for each nation the proportion of its scientific publications mentioning any of the NIH biomedical model organisms (except *Daphnia*) in the title, abstract or keywords (Figure 2). The Ovid database was used for this purpose because its coverage was slightly better than that of the Thomson's ISI database (*circa* 7.85M versus 7.77M publications from the top 13 nations in 1996–2006). However, the results of searching the two databases were tightly correlated with one another (ρ=0.94, *data not shown*). The proportion of each nation's publications on viruses, apoptosis and cancer were also determined for purposes of comparison. Intriguingly the scientific impact of national publications as a whole correlated more tightly with its collective effort in model organism research than with its contribution to other more apparently "valuable" fields of study. Nations who devote a higher proportion of their scientific effort to areas that correlate strongly with the nation's overall scientific impact must devote correspondingly less of their effort to areas that do not contribute to overall scientific impact. Indeed, a corollary of these findings is that there must be other fields of scientific research for which the national scientific effort correlates negatively with national scientific impact. In any case,

a nation's effort in model organism research seems to be a better indicator of its overall scientific impact than its effort in other research areas.

Given the small proportion of scientific papers in any one field of study, a nation's effort in model organism research (or indeed in any other single field of research) cannot be the sole source of its national scientific impact. However, it could well reflect national funding priorities and attitudes to fundamental research – the kind of research that has the greatest scientific impact and long term benefit as reflected in Nobel Prizes.

The Nobel Prizes notwithstanding, the foregoing results beg the question of whether research on the "NIH Nine" is generally of greater scientific significance than other types of research. To investigate this question I used as an indicator of scientific significance, the proportion of articles of sufficient significance to be published in the top two general science journals in the world – *Nature* and *Science*. This index reflects both the significance and quality of work as judged by the editors and referees of *Nature* and *Science* and, like any other research metric, has its own weaknesses. However, it compensates at least partly for the effect of the size of the research field internationally on other measures such as simple citation rates. Citation rates in very large research fields can be higher than in smaller research fields because of the much larger pool of citation opportunities. The results in Table 2 revealed that compared to articles in all fields, articles on the model organisms are 4.3 times more likely to be of sufficient import to be published in *Nature* or *Science*. Although they could not match the heavyweights in the model organism world, the nematode *C. elegans* and the fly *Drosophila,* articles on the microbial models – yeast, *Neurospora* and *Dictyostelium* were about three times more likely than articles in other scientific fields to be published in *Nature* or *Science*. By comparison, articles on viruses, cancer or apoptosis were not much more or less likely to be published in these journals than articles in all other fields combined.

Since model organism research provides so much scientific "bang", I was led to ask how many "bucks" are committed to achieving this. As an indicator, I searched the funding data to which I had access – the annual funding outcomes for ARC Discovery and NHMRC Project grants in the years 2003 to 2007 inclusive (applications submitted in the years 2002–2006). Over

Table 2. Scientific significance of articles on non-mammalian model organisms.

Topic (Articles 1996–2006)	All articles top 13 countries	Per cent of all articles	*Science* articles	Per cent of all articles on the topic	Per cent of articles in the journal	*Nature* articles	Per cent of all articles on the topic	Per cent of articles in the journal	Per cent of all *Nature*+ Science papers	Significance ratio = per cent *Nature*/*Science*/ per cent all papers
All topics	8173375	100.00%	18178	0.22%	100.00%	19894	0.24%	100.00%	100.00%	1.00
D. discoideum	2921	0.04%	21	0.72%	0.12%	15	0.51%	0.08%	0.09%	2.65
S. cerevisiae/S. pombe	46139	0.56%	327	0.71%	1.80%	356	0.77%	1.79%	1.79%	3.18
N. crassa	2802	0.03%	18	0.64%	0.10%	18	0.64%	0.09%	0.09%	2.76
C.elegans	15321	0.19%	216	1.41%	1.19%	275	1.79%	1.38%	1.29%	6.88
D. melanogaster	39681	0.49%	506	1.28%	2.78%	633	1.60%	3.18%	2.99%	6.16
X. laevis	20952	0.26%	111	0.53%	0.61%	165	0.79%	0.83%	0.72%	2.83
G. gallus	1627	0.02%	0	0.00%	0.00%	3	0.18%	0.02%	0.01%	0.40
D. rerio	7018	0.09%	50	0.71%	0.28%	63	0.90%	0.32%	0.30%	3.46
All models	122529	1.50%	1102	0.90%	6.06%	1335	1.09%	6.71%	6.40%	4.27
Virology	195400	2.39%	554	0.28%	3.05%	460	0.24%	2.31%	2.66%	1.11
Apoptosis	110472	1.35%	270	0.24%	1.49%	338	0.31%	1.70%	1.60%	1.18
Cancer	409660	5.01%	505	0.12%	2.78%	780	0.19%	3.92%	3.38%	0.67

Searches conducted using Current Contents Online (OvidSP, Ovid Technologies, Inc., *http://ovidsp.ovid.com/)*. *D. discoideum* = *Dictyostelium discoideum* (cellular slime mould); *S. cerevisiae* = *Saccharomyces cerevisiae* (budding yeast); *S. pombe* = *Schizosaccharomyces pombe* (fission yeast); *N. crassa* = *Neurospora crassa* (filamentous fungus); *C. elegans* = *Caenorhabditis elegans (*nematode); *D. melanogaster* = *Drosophila melanogaster; X. laevis* = *Xenopus laevis* (African Clawed Frog); *G. gallus* = *Gallus gallus* (chicken); *D. rerio* = *Danio rerio* (zebrafish).

Table 3. Value of research on non-mammalian model organisms.

Field of research	Scientific output (Australia) (per cent of all Australian Papers)	Funding (Australia) (per cent of Australian ARC/ NHMRC project grant funds)	Productivity index (Australia) (per cent output/per cent funds)	Significance index (all countries) (per cent Papers in the field in *Nature* or *Science*/per cent Papers in all fields in *Nature* or *Science*)	Scientific value for money (Productivity index x Significance index)
Model organisms	1.25	0.34	3.64	4.27	15.54
Virology	2.58	2.20	1.17	1.11	1.30
Apoptosis	1.04	0.40	2.58	1.18	3.04
Cancer	4.83	4.46	1.08	0.67	0.73
All fields	100	100	1.00	1.00	1.00

this period, I identified 10 NHMRC projects and 14 ARC projects using one of the NIH non-mammalian model organisms – roughly 1 project in 400. The total funding for these projects was $11.1m, or about 0.34% of the total of $3,255m (Table 3). For this money, what proportion of the nation's scientific publications were generated? From 1996 to 2006 Australian authors published 3091 articles on model organisms, 2.2% of all model organism papers in the world and about 1.25% of our total scientific output. The scientific productivity (articles per dollar) for model organism research in Australia was thus about 3.7-fold greater than that for all fields combined. The scientific productivity of research on apoptosis was about 2.6-fold higher than average while that for virology and cancer was not very different from the average across all fields. Not only is the scientific significance of model organism research three- or fourfold higher than other fields, it is achieved with nearly fourfold less funding per paper. Together these comparisons indicate that research on non-mammalian model organisms provides at least an order of magnitude better scientific "bang" for the "buck" than other fields (Table 3).

The foregoing conclusions support the conviction gained from history that research on model organisms, including microorganisms, is both highly significant and cost-effective. One wonders why the ARC and NHMRC do not invest in non-mammalian models in proportion to the scientific significance and productivity of the research that uses them. Perhaps both organisations should target around 1% of their funds to these organisms to match the proportion of Australia's research output they generate. In the first years of the third millennium, the millennium bugs have continued to match the enormous scientific impact they have had in the past and Australian science could profit from exploiting them more.

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Biography

Paul Fisher is Professor of Microbiology and Associate Dean (Research) in the Faculty of Science, Technology and Engineering at La Trobe University. His research interests are in using *Dictyostelium* to study the pathological dysregulation of signal transduction networks in mitochondrial, neurological and neurodegenerative diseases.