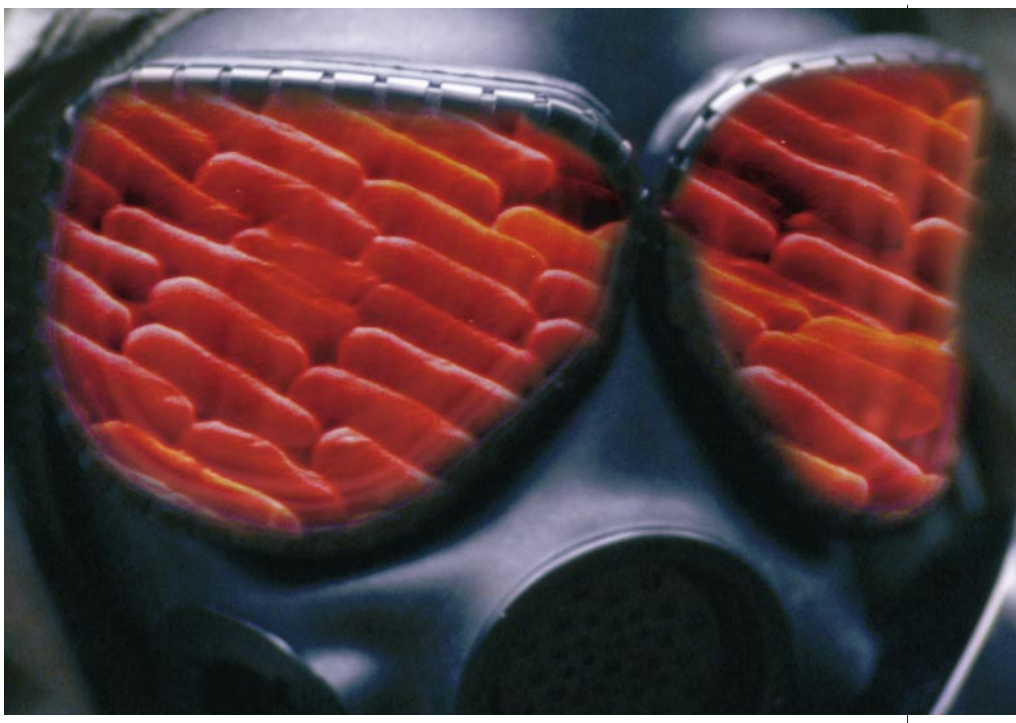
The bugs of war

Could our knowledge of microbial genomics and skill in genetic engineering be used to create 'enhanced' bioweapons? Carina Dennis assesses the threat, and the efforts to counter it.



Present arms: anthrax, portrayed in this montage, is a formidable weapon. But could it be made worse?

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Ron Jackson and Ian Ramshaw weren't looking for trouble. Jackson, who works at the Pest Animal Control Cooperative Research Centre in Canberra, and Ramshaw, who is in the same city at the Australian National University, were searching for a way to control the mice that are serious pests in Australia. They wanted to make a contraceptive vaccine by altering the genes of the mousepox virus.

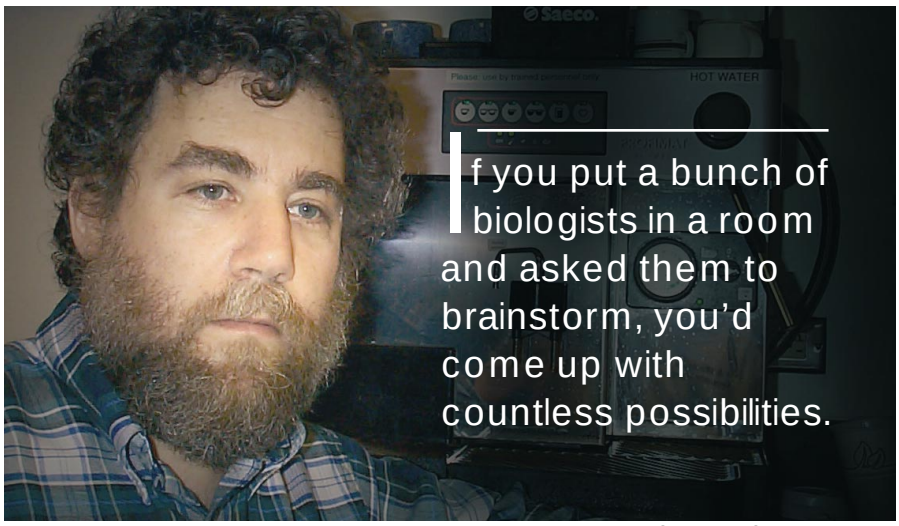
But in January the project gained notoriety after the pair inadvertently created an unusually virulent strain of mousepox. If a similar genetic manipulation were applied to smallpox, the scientists realized, this feared killer could be made even more dangerous. When they published their paper[1], it was only after much discussion about the wisdom of drawing attention to the findings. "It has to be brought out into the public arena so the situation can be addressed," argues Ramshaw.

The incident has heightened concerns about the potential for using genetic engineering to create biological weapons that surpass the destructive potential of natural pathogens. With the decoding of a pathogen's entire genome now commonplace, and transgenic techniques advancing all the time, some researchers believe that the sinister potential of biology can no longer be ignored.

Most experts feel that the hype surrounding bioengineered weapons still outweighs the threat, and argue that the main focus for concern should remain on conventional biological agents. But government agencies are already working on methods of detecting disease outbreaks caused by genetically engineered organisms.

The potential for bioengineering agents of destruction was considered in 1997 by JASON, a group of mostly academic scientists that provides technical advice to the US government. In theory, it might be possible to build novel bacteria or viruses from a set of component parts — although most experts don't yet see that as a realistic scenario for creating bioweapons (see 'Starting from scratch', overleaf).

But making subtle genetic alterations to existing pathogens to increase their virulence or durability in the environment, or to make them harder to detect or to treat with drugs, is within the limits of today's technology[2]. "If you put a bunch of biologists in a room and asked them to brainstorm, you'd come up with countless possibilities," says Steven Block, a biophysicist at Stanford University in California who led the JASON study.



Steven Block believes some genetic technologies have serious implications for biowarfare.

Resistance is fertile

Perhaps the simplest way to 'enhance' a bacterial bioweapon is to make it resistant to antibiotics. Bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* can evolve drug resistance at a startling rate. Some of the genes that convey this antibiotic resistance lie in the bacteria's genomes. But others can be carried on plasmids, circular pieces of DNA that replicate themselves independently — and the same is true of genes for other important traits such as virulence and infectivity. Plasmids can move between bacteria, and genes carried on plasmids can be incorporated into the

genome, which means that genes conferring antibiotic resistance, or any other advantageous trait, can spread rapidly.

Molecular geneticists have long exploited plasmids as a means of cloning DNA and creating transgenic bacteria. So splicing genes for antibiotic resistance into plasmids and introducing them into a bacterium being developed as a biological weapon would, by the standards of today's top biology labs, be child's play. Anthrax, for instance, is typically treated using derivatives of penicillin, but they could be rendered ineffective by introducing a gene for the enzyme β-lactamase, which disables the antibiotics, into the anthrax pathogen, *Bacillus anthracis*.

According to Alastair Hay, an expert on biological warfare at the University of Leeds in the UK, manipulations of this type may already have been done. Hay helped to debrief defectors from Biopreparat, a clandestine network of facilities spread across Russia and Kazakhstan that worked on biological warfare until 1992. The scientists claimed that Biopreparat had developed a form of *Yersinia pestis*, the causal agent of plague, that was resistant to 16 different antibiotics.

Using the same techniques, it might be possible to transfer genes for pathogenicity. For example, the gene for the deadly toxin produced by the food-poisoning organism *Clostridium botulinum* could be introduced into ubiquitous bacteria such as *Escherichia coli*. More likely, says Block, bioengineers could take an existing biowarfare agent and add further virulence genes from other microorganisms.

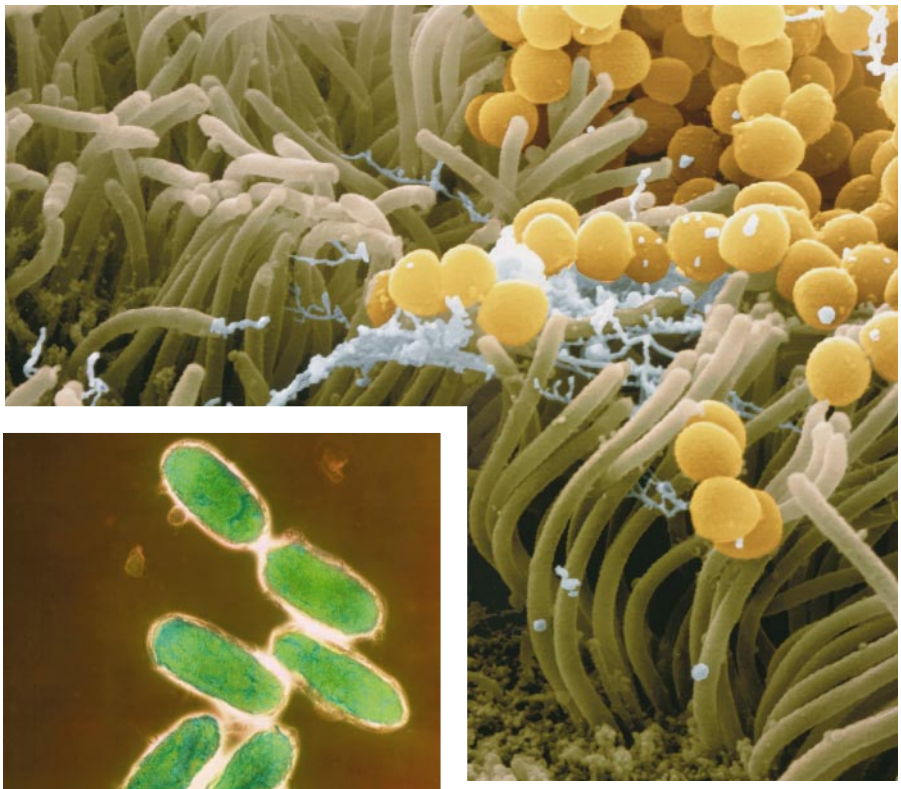
Mix and match

Advances in genomics have greatly increased the possibilities of mixing and matching traits from different microorganisms. Among the complete pathogen genetic sequences now available are *P. aeruginosa*[3], plus the bacteria responsible for tuberculosis[4] and cholera[5]. This year's crop includes the leprosy bacterium[6] and *E. coli* O157:H7 (ref. 7), a strain rendered deadly by the acquisition of a gene for a toxin that damages the kidneys. Only last month, a Japanese team published the genome sequences of two antibiotic-resistant strains of *S. aureus*[8]. The complete sequences of *B. anthracis* and *Y. pestis* are expected to follow later this year.

Some researchers are concerned about potential abuses of DNA sequence data. But Tim Read, who leads the team sequencing *B. anthracis* at The Institute for Genomic Research in Rockville, Maryland, is confident that a policy of releasing the sequence information into publicly accessible databases is best. "My feeling is that releasing the data will tilt the scientific advantage towards biodefence and force malevolent interests to work harder," he says. "The release of the data has stimulated research into vaccines, drugs and diagnostics."

One disturbing possibility is that knowledge of pathogen genomics could be combined with insights gleaned from human genetics to target particular ethnic groups. But most experts are sceptical of the potential for bioweapons as agents of ethnic cleansing. Although it is true that certain ethnic groups are unusually susceptible to particular pathogens, genetic variation in susceptibility to disease is likely to be greater within ethnic groups than between them. "You'd have a lot of collateral damage," predicts Paul Ewald, an expert on the evolution of disease at Amherst College in Massachusetts.

The accumulation of genomic and other biological data is also spawning a boom in computational biology, which uses mathematical modelling to help understand how networks of genes and proteins work. Although such approaches might yield information on drug targets, they could also highlight vulnerabilities that could be



JÜRGEN BERGER/SPL

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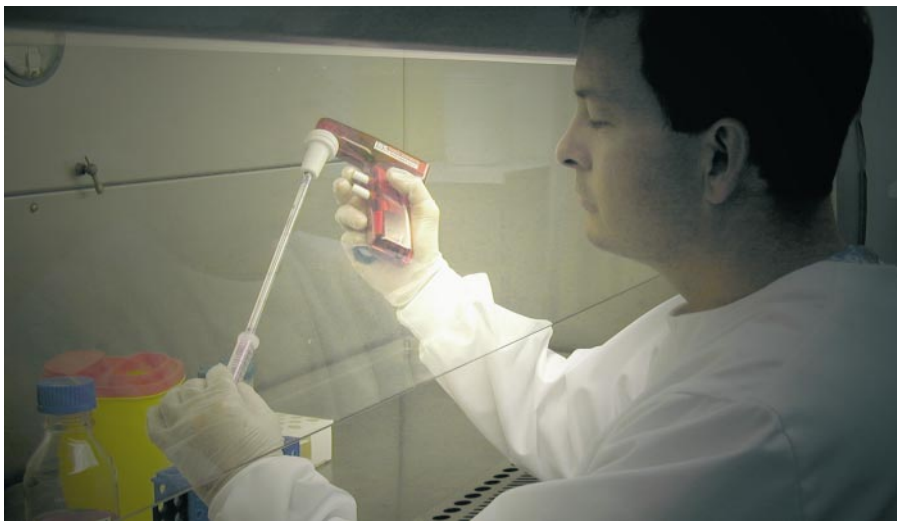
Customized killers: in theory, genes for antibiotic resistance taken from *Staphylococcus aureus* (above) could be transferred to *Yersinia pestis* (left), the plague pathogen.



CSIRO

Accidental architect: Ron Jackson co-engineered a particularly virulent form of mousepox.

exploited by malevolent biologists.

But the information being generated by genome projects is not the only concern. Thanks to recent advances in biotechnology, would-be weapons bioengineers need not limit themselves to working with genetic sequences evolved through natural selection. Several companies are developing techniques of 'directed molecular evolution', which can be used to accelerate the evolution of desired traits by deliberately introducing genetic variation and then applying artificial selection.

Unnatural selection

One of the most powerful of these methods is DNA shuffling, developed by Willem Stemmer, chief scientist with the company Maxygen in Redwood City, California. Multiple copies of a given gene are first shattered into fragments, then reassembled using a variation of the polymerase chain reaction — a standard tool for copying sequences of DNA. This produces a range of 'daughter' genes with the fragments stitched together in subtly different ways. The enzymes involved in the reassembly process are also prone to errors, which introduce point mutations, further adding to the genetic diversity. The daughter genes can then be reintroduced into bacteria, which are selected to identify those with the desired traits[9,10].

Stemmer has also refined the technique to reassemble fragments taken from families of related genes from different bacteria[11]. Most recently, his team started to shuffle entire genomes in commercially valuable microorganisms. "We have recapitulated what could be accomplished in 15 years of classical recombination and selection in about six months," says Stemmer.

Maxygen is using the technique to develop better drugs and other proteins. But if it were to get into the wrong hands, Block considers the method to have "serious implications for biowarfare". Stemmer believes DNA shuffling is too sophisticated to be used by a lone bioterrorist. But it might not be beyond the capabilities of a bioweapons lab sponsored by a 'rogue' state.

Indeed, the potential of DNA shuffling as a tool for bioweapons development was demonstrated in its first application. In this, Stemmer focused on a gene for β-lactamase, creating strains of *E. coli* that were 32,000 times less sensitive than wild-type bacteria to the antibiotic cefotaxime. Shortly after his paper was published[9], Stemmer says he received a letter from the American Society for Microbiology expressing concerns about potential misuse and asking that he destroy the strain — which he did.

AP

Protect and survive: emergency services in South Carolina practise decontamination techniques.

Designer destruction

Other approaches that might be used to develop bioweapons include the deliberate hybridization of related viral strains. Although most crosses of viruses are less potent than the parent strains, sometimes virulence increases — some virulent strains of flu, for instance, arise as naturally occurring recombinants between different influenza viruses[12].

Potential bioweapons designers might also be watching developments in gene therapy. Attempts to introduce therapeutic genes into patients' tissues rely mostly on weakened forms of various viruses. These vectors have yet to introduce genes efficiently and reliably. But if researchers can make them do so, similar vectors might also be used to ferry harmful genes into unsuspecting victims.

The experience of Jackson and Ramshaw, meanwhile, shows that scientists can stumble across possibilities for bioweapons quite by accident. To create their mouse contraceptive vaccine, they took a relatively benign strain of the mousepox virus, and added genes for proteins carried on the surface of mouse eggs. The idea was that cells infected by the viruses would churn out the proteins, causing female mice to produce antibodies against their own eggs. To maximize the vaccine's effectiveness, Jackson and Ramshaw also engineered the virus so that it contained the gene for interleukin-4 (IL-4), a protein

Starting from scratch

In January 1999, at the American Association for the Advancement of Science's annual meeting in Anaheim, California, genomics pioneer Craig Venter announced that scientists at his institute were contemplating creating novel bacteria. He claimed that they could chemically synthesize a 'minimal' genome and insert it into a bacterial cell stripped of its own DNA.

But Venter said the project had been put on hold, pending an ethical review. In addition to worries about scientists 'playing God', Venter cited fears that bioterrorists might adapt synthetic bacteria to make weapons that would evade conventional diagnostic tests.

The work on which Venter's claim was based was published later that year[14]. Researchers at The Institute for Genomic Research in Rockville, Maryland, had taken the smallest known bacterial genome, that of *Mycoplasma genitalium*, and started to knock out its genes to find out which were essential to survival. They predicted that a set of about 300 genes would be sufficient to maintain a single-celled organism in the laboratory.

Arthur Caplan of the Center for Bioethics at the University of Pennsylvania, who led the panel that reviewed the project, believes the creation of artificial life is a "certainty". The panel's review[15] acknowledged the bioweapons potential of the minimal genome project and other genomics efforts, concluding that "we need to give serious thought to monitoring at the level of national and international public policy".

Synthetic pathogens — probably viruses, rather than bacteria — are on the list of long-term threats being considered by experts in biodefence. "It's not out of the question that we'll be able to produce artificial viruses," says Mark Wheelis of the University of California, Davis, a member of the Federation of American Scientists' working group on bioweapons.

But even if 'artificial' pathogens can be created, most experts are sceptical of their relevance to biowarfare — at least given our current understanding of ecological genetics. If genetically engineered bacteria and viruses would struggle to survive if released into the environment (see main story), the likelihood of a completely synthetic pathogen prospering in the wild seems slim.

that boosts antibody production.

But the IL-4 gene also effectively shut down the cellular arm of the animals' immune systems, rendering them unable to fight off mousepox. Most disturbing was that mice previously vaccinated against the virus also succumbed, being killed within days[1]. Given that mousepox is a relative of smallpox, the potential for using similar techniques to develop an enhanced bioweapon is all too obvious. "If one were to use a bioengineered virus of this nature, it may not be possible to vaccinate against it," says Ramshaw.

Such examples reveal that some developments in biology have nightmarish potential. But many experts say that at present the reality of the threat posed by bioengineered weapons is probably much less than that from conventional biological agents. "The worst that you can imagine is probably not a very realistic scenario," says Albert Osterhaus, a virologist at Rotterdam University in the Netherlands. One reason for optimism is that pathogens engineered in the lab may struggle to survive, or quickly lose their imbued characteristics, if they were ever released. Evolution, argues Ewald, is on our side. "People don't think about natural selection. If they did, they would have a clearer idea of what the dangers would be."

Trading places

Because evolution is all about trade-offs between the costs and the benefits of different traits in particular environments, Ewald suspects that it would be extremely difficult to engineer all of the desired 'attributes' into a bioweapon and still have an organism that is transmitted effectively and predictably. In naturally occurring pathogens, he points out, traits such as virulence and transmissibility often counteract one another[13].

Microbial evolution is also usually a matter of use it or lose it. Traits such as toxin production usually impose costs on their bearers, and so are likely to be lost quickly by engineered organisms unless they confer a selective advantage. The key question is how much damage an engineered pathogen might inflict before losing its added genes.

Even if introduced genes can persist, pathogens grown in culture tend to adapt to their new environment and lose the characteristics that made them pathogenic. Indeed, this strategy has been used to create harmless strains of viruses, such as polio, for use in vaccines. A bacterium engineered in culture to resist antibiotics may soon become similarly benign, suggests Stemmer: "You may end up with something antibiotic-resistant but no longer pathogenic."

The sinister implication is that the most effective means of developing an enhanced bioweapon would be to use human subjects in its development and testing. "If anyone were to use humans as guinea pigs, they could make some very nasty weapons," says Ewald.

The worst that you can imagine is probably not a very realistic scenario.

Such horrific possibilities underscore the need to strengthen the 1972 international Biological Weapons Convention, say experts.

Counterstrike

Despite the evolutionary arguments against assuming a worst-case scenario, researchers working on biological defence take the threat of bioengineered weapons seriously, and are trying to develop the means to counter it. "Most detection technology is based on knowing what you are looking for," says Duane Lindner, who works on biodefence at Sandia National Laboratories in Livermore, California. "You could imagine engineering a pathogen so that detectors would be blind to that particular threat."

With this in mind, Sandia's Center for National Security and Arms Control is developing an Internet-based system to detect early signs of bioweapon exposure, irrespective of the agent responsible. The idea behind the Rapid Syndrome Validation Project is that doctors would enter details of unusual symptoms or disease outbreaks into the system. Neural network software would then be used to identify suspicious outbreaks of disease, without the need to wait for diagnostic laboratory data (see News, page 228).

In collaboration with the Lawrence Livermore National Laboratory in California, Sandia is also trying to develop generic methods to detect biological agents without needing to know their identity. Lindner and his colleagues have used computational techniques to identify conserved regions of biological toxins, and they are now moving on to other proteins involved in pathogenicity. The knowledge gained could then be used to develop sensors to detect these molecular signatures.

GEOFF TOMPKINSON/SPL

The US Defense Advanced Research Projects Agency (DARPA), meanwhile, is developing biosensors based on living tissues that should provide physiological responses to a wide spectrum of both known and unknown pathogens. The biosensors are three-dimensional matrices containing cells including neurons, muscle cells, immune cells, and cells from the skin and the endothelia that line our guts and nasal passages.

DARPA is also investing heavily in the development of new antibiotics and vaccines that could target a broad range of pathogens. Some of DARPA's strategies target common mechanisms of bacterial growth, such as genes essential to cell division and those encoding enzymes central to evolutionarily conserved metabolic pathways. Maxygen, with funding from DARPA, is applying its DNA shuffling technology to combine proteins from related pathogens in the hope of developing vaccines that could provide broad protection.

In other words, the techniques that could produce bioweapons are also being deployed to set up countermeasures against them. This neatly illustrates the point that legitimate and malevolent applications of biology are merely two sides of the same coin.

Although bioengineered weapons may currently be less of a concern than their conventional counterparts, the threat they pose can only increase as technologies develop. "It's time for biologists to begin asking what means we have to keep the technology from being used in subverted ways," says Matthew Meselson, a molecular biologist at Harvard University who has spoken out frequently on the dangers of biowarfare. ■

Carina Dennis is a senior biology editor with *Nature*.

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