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Emerging Technologies

Genetic Engineering and Biological Weapons

Summary

Emerging diseases are often discussed as a global public health threat; but the threat of these diseases is paralleled by another, that posed by emerging technologies. Rapid developments in biotechnology, genetics and genomics pose a variety of environmental, ethical, political, and social questions. And because they open up tremendous new possibilities for biological warfare, these technological developments have grave implications for peace and security.

In this report, we give a systematic overview of the impact of biotechnology on biological weapons (BW) development, focussing on existing technologies and recent discoveries whose implications are still poorly understood. Much of what we present may sound like science fiction, but in fact it is far more science than fiction – and in some cases it is already a reality. The most frightening developments can currently be witnessed in the US, where new technology is being exploited to create new types of biological and biochemical weapons, including material degrading microorganisms and psychoactive chemicals, raising the spectre of a new biological and chemical arms race.

Genetic engineering can contribute to offensive BW programs in a variety of ways. With genetic manipulation, classical biowarfare agents such as anthrax or plague may be made more efficient weapons. Barriers to access to agents such as smallpox, Ebola or the Spanish flu[1] are being lowered by genetic and genomic techniques.

Completely new types of weapons are also becoming possible, including the use of food crops as tools for biological warfare. Even ethnically specific weapons, hitherto thought to be impossible, have become a real possibility. We present data here showing that ethnic specific genetic sequences do exist in considerable high numbers.

Alarmed by the rapidly increasing technical possibilities, the International Committee of the Red Cross recently appealed to governments to take concrete steps to avert the hostile use of biotechnology. A broad array of political measures will be needed to counter the threat of hostile exploitation of biotechnology. First and foremost, the Biological Weapons Convention needs to be strengthened through multilaterally agreed, legally binding verification measures. In addition, three immediate steps are of specific importance:

- All projects that violate the Chemical and Biological Weapons Conventions must be immediately abandoned, specifically development of so-called "non-lethal" chemical weapons, anti-material biowarfare agents, and fungi for the war on drugs. Failure to do so will encourage other countries to follow suit with R & D projects on biotechnological weapons, leading to an unravelling of two key disarmament treaties.
- There is an urgent need to ensure that governments restrict themselves and ensure maximum transparency in their biodefense programs, to prevent a race for offensive capabilities under cover of defense. All governments should adopt the 'Government Undertaking on Biodefense Programs', recently brought forward by the Sunshine Project[2]. It contains, among others, a provision that "*biodefense programs will not, for any purpose, utilize or construct, including single-gene changes, novel biological agents with an enhanced offensive potential"* such as treatment resistance, environmental stability, or enhanced pathogenicity.
- For some particularly dangerous technologies, restrictions on research are required. These research prohibitions, which are an inherently more effective approach than imposing limits on publication, should apply in specific fields that a) may easily be abused for hostile purposes, b) where no

[1] In 1918, a particularly aggressive influenza virus spread around the globe and killed 20 – 40 million people. This influenza pandemia was dubbed the 'Spanish flu'.

[2] See www.sunshine-project.org. In Germany, a petition supported by a variety of organisations including the Sunshine Project is currently underway to encourage the government to formally adopt high transparency and strict limits for its biodefense program.

effective global arms control or non-proliferation efforts are presently feasible, and c) where other technical avenues to reach the same peaceful scientific goal are available.

1. Introduction

Biological arms control is currently in one of its worst crisis since before the signing of the Bioweapons Convention (BWC) in 1972. Efforts to strengthen the BWC through comprehensive declaration and verification measures failed in 2001 due to US resistance.[3] At the same time, the US has massively expanded its biodefense program and embarked on the exploitation of biotechnology for weapons development.[4]

Mark Wheelis and Malcolm Dando, biologists and biological weapons experts, recently warned that "*the US may already be plunging recklessly forward into the military applications of biotechnology, whose legacy, we predict, will be as troubling to our children as is our parents' nuclear legacy to us*" (Wheelis & Dando 2002). Wheelis and Dando further argue the imminent danger of a new biological arms race: "*This U.S. exploration of the utility of biotech for bioweapons development is unwise, for the rest of the world will be obliged to follow suit. In its rush to stay ahead technologically, the United States runs the risk of leading the world down a path toward much-reduced security*" (Wheelis & Dando 2003). We concur and here present further discussion of specific technologies and civilian and military research that endangers security.

The danger that such experiments in biotechnology and biomedicine will lower the threshold for a BW use is also seen by government researchers: "*The wide range of effects that can be designed into [biowarfare] agents will expand options for [their] employment significantly and ultimately may decrease the current threshold for use of biological warfare… advances in biotechnology research may lead to a coming revolution in BW development for technologically proficient rogue nations…*".[5] The authors –from the US Defense Intelligence Agency – fail to mention that the threshold is most obviously and aggressively being lowered by the US itself.

Alarmed by the failure of the BWC Verification Protocol, rapidly increasing technical possibilities, and the renewed interest in biological warfare capabilities, the International Committee of the Red Cross recently issued an appeal to all political and military authorities *"to work together to subject potentially dangerous biotechnology to effective controls"*. It continues: "*We urge you to consider the threshold at which we all stand and to remember our common humanity*."[6]

This dramatic appeal is based on the inescapable facts that the revolution in biotechnology does indeed lead to a dramatically increased biowarfare risk and that governments have achieved little in reigning in these risks. Whereas thirty years ago, biotechnology was restricted to a small number of advanced research laboratories, today it is ubiquitous This global distribution of modern biotechnology has led to a worldwide availability of knowledge and facilities useful in biowarfare programs. In some countries, even high school students now conduct experiments in genetic engineering. High-tech facilities for the production of vaccines, single-cell-protein or biocontrol agents are widely distributed and will continue to spread as biotechnology or, at least, certain biotechnologies, find commercial uses in a larger number of markets.

Within the more generalized spread of biotechnology, there are specific new applications that are particularly troublesome. A relatively clear-cut problem is the genetic engineering of classical

[3] See http://fas.org/bwc/index.html for further reading on recent BWC developments.
[4] Specifically, it has become public in the past months that the US is pursuing development of so called non-lethal chemical weapons, material degrading microorganisms and an array of questionable 'biodefense' activities. See www.sunshine-project.org, Wheelis & Dando (2002, 2003) and Steinbrunner & Harris (2003) for further reading.
[5] Petro JB, Plasse TR, McNulty JA (2003) Biotechnology: Impact on biological warfare and biodefense. Biosecurity and Bioterrorism Volume 1, Number 3
[6] Appeal of the International Committee of the Red Cross on Biotechnology, Weapons and Humanity. September 2002 (online at www.icrc.org).

biowarfare agents to make them more effective. But new genetic and genomic techniques provide for additional, new, warfare possibilities. Once eradicated, viruses such as smallpox or the deadly 1918 influenza virus (which killed 20-40 million people in a global epidemic) may now be synthesised in the laboratory. Genetically engineered crops and insects can be used for the production – and secret delivery – of harmful biological substances and, in human genomics, even ethnically specific biological weapons are becoming a real possibility.

The following chapters give a systematic overview on these issues – some of the examples are already a reality, others are hypothetical in the sense that they have not, to our knowledge, been utilized for hostile purposes; but the science behind them is very real.

2. Single Gene Transfer and Similar Genetic Engineering of BW Agents

In the debate about genetic engineering and biological weapons it has often been stated that natural pathogens are sufficiently dangerous and deadly so that genetic engineering is not necessary for effective biological warfare. This is true: biological weapons can indeed be used without even any systematic knowledge on microbiology, as shown by their effective use in past centuries.[7]

Genetic engineering, however, has been employed in offensive biowarfare programs in order to make biowarfare agents more effective. In the former Soviet Union a variety of such experiments were undertaken. Three examples:

- **Bacteria causing unusual symptoms**: Researchers from Obolensk near Moscow inserted a gene into the bacterium *Francisella tularensis*, the causative agent of tularemia and a well known biological weapon agent. The gene made the bacteria produce beta-endorphin, an endogenous human drug, which caused changes in the behaviour of mice when infected with the transgenic bacteria.[8] According to the published results, the endorphin gene was not introduced into a fully virulent strain, but only into a vaccine strain.

  If inserted into virulent *F. tularensis*, the victims would not show the usual symptoms of tularemia, but instead unusual symptoms that could obscure diagnosis and delay therapy. Development of symptom-altered BW agents has been identified as one possible application of genetic engineering by the US Department of Defense.[9]
- **'Invisible' Anthrax***:* In the 1990s, Russian researchers altered the immunological properties of anthrax, making existing vaccines and detection methods ineffective against a new genetically engineered type.[10] They also developed a new vaccine against the artificial strain. Following the Russians, the US Department of Defense is now also genetically engineering anthrax.[11] According to the US, the classified experiments are to test if the Russian microbe can defeat the US anthrax vaccine.
- **Treatment resistant plague***:* According to scientists involved in offensive biowarfare research in the former Soviet Union, plague bacteria (*Yersinia pestis*) were developed in the former Soviet BW program that were resistant to 16 different antibiotics.[12] Today, the genetic introduction of

[7] Before Pasteur and Koch discovered bacteria as disease causing agents in the late 19th century, biological weapons were used. For example, in the 14th century, Mongol invaders catapulted plague victims into besieged cities. In the 18th century Britain distributed smallpox-infected blankets to native Americans.

[8] Borzenkov VM, Pomerantsev AP, Ashmarin IP (1993) The additive synthesis of a regulatory peptide in vivo: the administration of a vaccinal Francisella tularensis strain that produces beta-endorphin Biull Eksp Biol Med 116(8):151-3 (Article in Russian)

[9] Jane's Defence Weekly, 13. August 1997, page 6: US DoD reveals horrific future of biological wars

[10] Pomerantsev AP, Staritsin NA, Mockov YV, Marinin LI (1997) Expression of cereolysine ab genes in Bacillus anthracis vaccine strain ensures protection against experimental hemolytic anthrax infection. Vaccine 15:1846-1850

[11] New York Times, 4. September 2001

[12] A. Hay, quoted in 'The bugs of war', news feature in Nature 411:232-235

antibiotic resistance into bacterial pathogens is routine work in almost any microbiology laboratory.

These are some of the examples of genetic engineering in offensive biowarfare programs that have become public. It is safe to assume that theses are only a portion of what has been attempted, as offensive bioweapons programs are obviously not publicized.

Despite these examples, it should not be assumed that genetic engineering will play a major role in the early steps of a national biowarfare program.[13] The development of reliable, effective biological weapons requires an intense and ressource demanding research program that must solve – step by step – three increasingly complex problems: procurement of virulent strains of suitable agents, mass production of agents without loss of pathogenicity, and development of effective means of delivery. The third step is especially demanding and has rarely been solved (with notable exceptions such as the former biowarfare programs of the US and USSR). Even after several years of an active biowarfare program, in the early 1990s, Iraq possessed only rudimentary means of delivery. From this perspective, genetic engineering is simply another step in the development of a biowarfare potential, which may not be taken before the first three essential steps are solved.

On the other hand, the limited biowarfare suitability of almost any natural pathogen should not be dismissed. In the classic military point of view, a microorganism must fulfil a variety of demands. It must be producible in large amounts, act quickly, and be environmentally robust. The disease also needs to be treatable, to permit protection of an aggressor's own troops. *Bacillus anthracis*, for example, essentially fulfills the military specification, although anthrax victims may be treated up to several days after exposure with antibiotics. Therefore, only a minority of the infected persons will die from an anthrax attack in circumstances where appropriate medical response is possible, as was shown by the anthrax attacks in 2001 in the USA.

A very simple genetic intervention such as increased antibiotic resistance, however, could provoke much more deadly results by impairing timely and effective treatment. The technical possibilities for such manipulations are many, and are growing by the day. In many basic science research projects, methods to overcome current technical limitations in the military use of pathogenic agents have been demonstrated – sometimes unwittingly. Countless examples from the daily work of molecular biologists could be presented here, but one particularly interesting example is the transfer of "suntanning" genes: Many microorganisms are rapidly destroyed by bright sunshine (hence the Sunshine Project) and are thus only of limited use as a biowarfare agent. Many biological weapons are much more effectively used at night or dawn in order to avoid the destructive effect of the ultraviolet light. But "suntanning" genes may be introduced into microorganisms to confer UV resistance. In one experiment, genes coding for the synthesis of carotinoids have been transferred into harmless bacteria (Sandmann et al. 1998). Another possibility would be to engineer toxins into microorganisms that are naturally UV-protected (Manasherob et al. 2002).

[13] An exception may be sophisticated non-state actors which may seek to apply modern genetics for their own hostile interests, especially for low level or private conflicts. This refers less to non-state actors such as Al Qaeda but rather to companies and/or single individuals which due to their professional background have the capability to do so.

3. Emerging Technologies I: Novel infectious agents

More complex genetic interventions, such as multiple gene transfers and "tailor-made" novel agents are becoming possible. Harmless bacteria may be equipped the capability to cause illness and death, and even inter-species hybrids ('chimera') involving large gene sequences are a real possibility.

Two years ago, Australian scientists inadvertently created a virus that turned out to be lethal for mice. In a genetic experiment, mousepox virus was altered to create a sort of fertility control vaccine, intended to be used to control mouse infestations in Australia. In a first experiment, proteins from the surface of mouse egg cells were inserted into the virus to trigger an immune response against the egg cells. Because the immune response was insufficient, the researcher tried to boost it in a second experiment by adding another gene. Completely unintended and unforeseen, all mice infected with the new virus strain died, even if they had been vaccinated against mousepox. It turned out that the additional gene had the unforeseen effect of turning off the immune system of the mice, making them vulnerable to lethal infection by the otherwise harmless virus (Jackson et al. 2001).

It is safe to assume that many other experiments have unwittingly created more pathogenic variants, without that fact having become public. In most instances, the result of such "failed" experiments end up in the laboratory freezer or simply go down the sink. According to a British government paper from 2001 the mousepox experiment exemplifies that "*the risk of unexpected outcomes with genetically modified micro-organisms must increase with the increase in the number of laboratories both in developed and developing countries that routinely apply recombinant technologies to micro-organisms. (...) unforeseen consequences (...) could be disastrous for example if such organisms escaped from the laboratory. This emphasises the importance of careful risk analysis and appropriate procedural and physical containment measures.*"[14]

In the Australian experiment, a new way to enhance the pathogenicity of usually harmless viruses had been demonstrated. The researchers were aware of the potential military abuse of their work and directly contacted the Australian ministry of defense, to discuss how to proceed with their findings. When they decided to foster transparency and publish the work, it started a global debate about possible abuse of genetic engineering.

While the Australian research group accidentally stumbled across this effect, US scientists wittingly repeated the same experiment and deliberately took the lethal approach further. In October 2003, Mark Buller of the University of St Louis told a scientific conference that his group performed the same experiments with cowpox virus – a virus that may also affect humans. Buller also increased the lethal efficacy of the engineered virus by 'optimizing' the genetic insert. Buller's mousepox strain killed 100 per cent of infected mice, even when they were vaccinated and also treated with the antiviral drug cidofovir.[15]

In another example, British researchers pled guilty in 2001 to charges that they improperly handled a genetically engineered hybrid of the viruses causing hepatitis C and dengue fever. British authorities characterized the virus as "more lethal than HIV"[16] "Dengatitis" was deliberately created by researchers who wanted to use fewer laboratory animals in a search for a vaccine for Hepatitis C. Under unsafe laboratory conditions, the researchers created and nearly accidentally released a new hybrid human disease whose effects, fortunately, remain unknown; but which may have displayed different symptoms than its parents and thus been difficult to diagnose, and have required a new, unknown treatment regime.

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[14] Background paper on new scientific and technological developments relevant to the convention on the prohibition of the development, production and stockpiling of bacteriological (biological) and toxin weapons and on their destruction. BWC/CONF.V/4/Add.1, 26 October 2001.

[15] US develops lethal new viruses. New Scientist, 29 October 2003.

[16] Arthur C "Scientists made virus 'more lethal than HIV', The Independent, 24 July 2001.

Pathogenicity factors

A key research area for biomedicine – and biodefense – is the identification of pathogenicity or virulence factors, meaning those proteins or genes that contribute to an infectious microorganism's ability to cause illness or spread from host to host. It is a scientifically challenging area and it is still far from easy to determine what makes one bacterium so deadly while a close relative is completely harmless or even beneficial.[17] While the field remains difficult, research applications can already be witnessed. As early as 1986, a US-based research team transferred the lethal factor from anthrax bacteria into harmless gut bacteria (*E. coli*). As expected, the gut bacteria started to produce the corresponding protein that turned out to be as lethal as the natural toxin from anthrax bacteria.

And in the view of an ever increasing number of bacterial genomes that are completely sequenced – including some of the most deadliest organisms such as *Yersinia pestis, Variola major*, or *Bacillus anthracis*, the causative agents of plague, smallpox and anthrax, respectively – it can be expected that in coming years genes will be identified that may turn harmless bacteria into deadly weapons. A lot of effort is currently put into unravelling virulence related genes, many of them in the course of officially defensive military sponsored research. Earlier this year, for example, the US Department of Energy, which runs US weapons laboratories such as Lawrence Livermore and Los Alamos, solicited grant proposals for "*the identification … of proteins expressed from virulence genes in biological pathogens relevant to the [Chemical Biological Nonproliferation Program] mission."*[18]

Also possible are genetic alterations that increase the ability of a microorganism to invade human cells. As early as 1997, a US patent was granted for a US Department of Defense funded project on 'invasive microorganisms'.[19] This patent describes how innocuous bacteria may be genetically altered to invade cells and deliver "*molecules of interest*" into these cells. While the patent probably aims at beneficial "*molecules of interest*", i.e. pharmaceutical substances, it may also be used in other ways.

4. Emerging technologies II: Synthesis of biowarfare agents

Today access to highly virulent agents and strains is increasingly regulated and restricted. Smallpox viruses, eradicated outside the laboratory more than 20 years ago, are today (most likely) present in only two high security laboratories in the US and Russia. But it is only a question of time before the artificial synthesis of agents or agent combinations becomes possible.

Artificial poliovirus

Poliovirus was recently synthesized by a US research team at the State University of New York in Stony Brook. The researchers built poliovirus "from scratch" through chemical synthesis (Cello et al. 2002). Starting with the gene sequence of the agent, which is available online, the researchers synthesized virus sequences in the lab and ordered other tailor-made DNA sequences from a commercial source. They then combined them to form the full polio genome. In a last step, the DNA-sequence was brought to life by adding a chemical cocktail that initiated the production of a living, pathogenic virus. The experiment was funded by the US Defense Advanced Research Projects Agency (DARPA).

In principle, this method may be used with other viruses that have a similarly short genetic sequence (genome). This is true for at least five viruses that are considered to be potential biowarfare agents, including Ebola, Marburg and Venezuelan Equine Encephalitis. Ebola and Marburg are very rare viruses that may be difficult to acquire for potential bioweaponeers. Using the method that has now

[17] For review, see the complete volume 264 of Curr Top Microbiol Immunol (2002), edited by Hacker J & Kaper JB, which focuses on 'Pathogenicity Islands and the Evolution of Pathogenic Microbes'.
[18] http://www.science.doe.gov/sbir/Solicitations/FY%202003/NN.htm#T1
[19] US Patent 5662908 from 2 Sept. 1997, assigned to Stanford University in Palo Alto, California.

been published for polio, Ebola might be synthesized in a laboratory. At present the method is mastered by only a few highly trained experts, although this is unlikely to remain so for long.

Another route to smallpox

Poliovirus is not terribly well suited to be a biological weapon,[20] but the experiment exemplifies possibilities that generate real problems if similar techniques become applicable to agents such as smallpox. Today it is unlikely (though not completely impossible) that countries apart from Russia and the USA have access to smallpox virus. This is the basis of the current threat assessments with regard to smallpox, which rate the likelihood of a smallpox attack very low. Should it become possible in a few years to build smallpox virus in the laboratory, the situation would be turned upside down. The relative security that can be assumed today (at least for most countries in the world) will evaporate.

The method to artificially create poliovirus can not be directly transferred to smallpox virus. The smallpox genome, with more than 200,000 base pairs, is far larger than that of poliovirus, and even if it would be possible to create the full smallpox sequence in vitro, it cannot be as easily be "brought to life" as poliovirus. But there may be other ways to build smallpox artificially. It would, for example, be possible to start with a closely related virus such as monkeypox or mousepox and to alter specifically those base pairs and sequences that differ from the human smallpox.

In 2002, the first steps in such a technique were demonstrated. It was documented for the first time that the sequence of a (pathogenicity related) gene in the smallpox-related Vaccinia virus can be transformed into the sequence of the corresponding smallpox gene through a targeted mutation of 13 base pairs (Rosengard et al. 2002). It is probably only a matter of a few years until this kind of technique may be applicable to full genomes, meaning the current smallpox threat assessment (and that for some other agents) will have to be reconsidered.

Currently, the full sequences of at least two different smallpox strains are available in the internet[21], and most recently a new internet site dedicated to poxvirus genomic sequences has been launched (Upton et al. 2003). According to a spokesperson[22] of the National Center for Biotechnology Information in the USA, there appears to be a view in the scientific community that the smallpox sequences 'are already out there' and withdrawing it from databases like GenBank would rather hinder vaccine research than provide any additional security.

Recreating the Spanish flu

Influenza as a bioweapon does not sound like a particularly grave threat. Annual outbreaks kill many people, particularly the elderly; but a case of the flu is generally perceived as an uncomfortable nuisance rather than a grave threat. But flu viruses can be devastating. In 1918 and 1919, the so-called 'Spanish flu' killed an estimated 20-40 million people worldwide and, since then, the highly changeable flu virus has resurfaced in a variety of particularly virulent forms.

The strain of influenza virus that caused the 1918 global epidemic ('pandemic') was exceptionally aggressive. It showed a high capacity to cause severe disease and a propensity to kill fit young adults rather than the elderly. The mortality rate among the infected was over 2.5%, as compared to less than 0.1% in other influenza epidemics (Taubenberger et al. 1997). This high mortality rate, especially amongst the younger, lowered the average life expectancy in the USA by almost 10 years (Tumpey et al. 2002). Creation of this particularly dangerous influenza strain, as it is currently pursued by a US research team, may thus pose a serious biowarfare threat.

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[20] In more than 95% of infected persons, only mild flu-like symptoms – if any – are caused by the virus. With only about 1% of the infected having the risk of severe illness, polio does not rank high on a bioweaponeer's wish list.

[21] One sequence of smallpox (Variola virus) with the GenBank code X69198 (identical with NC_001611) was published by a team from Russia's former offensive biowarfare program, and a second sequence (Variola major virus strain Bangladesh 1975) with the GenBank code L22579 was published by an American team.

[22] Personal communication on 26 June 2003 by Dr D. Wheeler, NCBI, to Jan van Aken, Sunshine Project

A recent commentary in the Journal of the Royal Society of Medicine (Madjid et al. 2003) noted that influenza is readily transmissible by aerosol and that a small number of viruses can cause a full-blown infection. The authors continued: "*...the possibility for genetic engineering and aerosol transmission* [of influenza] *suggests an enormous potential for bioterrorism"* The possible hostile abuse of influenza virus is seen as a very real threat by public health officials in the USA. In September 2003, a total of 15 million dollar was granted by the US National Institutes of Health to Stanford University to study how to guard against the flu virus "*if it were to be unleashed as an agent of bioterrorism*".[23]

US scientists led by a Pentagon pathologist recently began to genetically reconstruct this specifically dangerous influenza strain. In one experiment a partially reconstructed 1918 virus killed mice, while virus constructs with genes from a contemporary flu virus had hardly any effect.

Attempts to recover the Spanish flu virus date to the 1950s when scientists unsuccessfully tried to revive the virus from victims buried in the permafrost of Alaska.[24] In the mid 1990s, Dr Jeffrey Taubenberger from the US Armed Forces Institute of Pathology started to screen preserved tissue samples from 1918 influenza victims. It appears that this work was not triggered by a search for flu treatments, or the search for a new biowarfare agent, but by a rather simple motivation: Taubenberger and his team were just able to do it. In previous experiments they had developed a new technique to analyse DNA in old, preserved tissues and for now looking for new applications: "*The 1918 flu was by far and away the most interesting thing we could think of*"[25] explained Taubenberger the reason why he started to unravel the secrets of one of most deadliest viruses known to humankind.

A sample of lung tissue from a 21-year-old soldier who died in 1918 at Fort Jackson in South Carolina[26], yielded what the Army researchers were looking for: intact pieces of viral RNA that could be analysed and sequenced. In a first publication in 1997, nine short fragments of Spanish flu viral RNA were revealed (Taubenberger et al. 1997). Due to the rough tissue preparation procedure in 1918, no living virus or complete viral RNA sequences were recovered.

Genetic techniques helped to isolate more Spanish flu RNA from a variety of sources. By 2002, four of the eight viral RNA segments had been completely sequenced, including the two segments that are considered to be of greatest importance for the virulence of the virus: the genes for hemagglutinin (HA) and neuraminidase (NA).

The project did not stop at sequencing the genome of the deadly 1918 strain. The Armed Forces Institute of Pathology teamed up with a microbiologist from the Mount Sinai School of Medicine in New York. Together, they started to reconstruct the Spanish flu. In a first attempt, they combined gene fragments from a standard laboratory influenza strain with one 1918 gene.[27] They infected mice with this chimera, and it turned out that the 1918 gene made the virus less dangerous for mice (Basler et al. 2001).[28]

In a second experiment, published in October 2002 (Tumpey et al. 2002), the scientists were successful in creating a virus with two 1918 genes. This virus was much more deadly to mice than other constructs containing genes from contemporary influenza virus[29]. This experiment is only one

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[23] Stanford University News Release 17 September 2003, online at http://mednews.stanford.edu/news_releases_html/2003/septrelease/bioterror%20flu.htm

[24] Spanish flu keeps its secrets. Nature science update at www.nature.com/nsu/990304/990304-5.html

[25] Profile: Jeffery Taubenberger at www.microbeworld.org/htm/aboutmicro/what_m_do/profiles/taubenberger.htm

[26] AFIP scientists discover clues to 1918 Spanish flu, www.dcmilitary.com/army/stripe/archives/mar28/str_flu032897.html

[27] The so called 'nonstructural' gene (NS)

[28] It should be noted that for this experiments, a standard influenza strain was used that was specifically adapted to mice and that was lethal to mice. The scientists reasoned that the 1918 gene probably weakened the lethality for the mice as it stemmed from a human-adapted strain.

[29] This time, the 1918 genes for hemagglutinin (HA), neuraminidase (NA) and matrix (M) were used, single and in combination. Only the combination of the 1918 HA and NA genes caused a dramatic increase in lethality if compared to constructs containing genes from a more recent human influenza virus. The scientists concluded: "*These data suggest that the 1918 HA and NA genes might possess intrinsic high-virulence properties.*"(Tumpey et al. 2002:13853)

step away from taking the 1918 demon entirely out of the bottle and bringing the Spanish flu back to life.

The scientists were aware of the dangers of their creation. The experiments were conducted under high biosafety conditions at a laboratory of the US Department of Agriculture in Athens, Georgia. Possible hostile use of their work was an issue considered by the scientists: "*…the available molecular techniques could be used for the purpose of bioterrorism"* (Tumpey et al. 2002:13849).

There is no sound scientific reason to conduct these experiments. The most recent experiments (Tumpey et al. 2002) allegedly seeked to test the efficacy of existing antiviral drugs on the 1918 construct – but there is little need for antiviral drugs against the 1918 strain if the 1918 strain would not have been sequenced and recreated in the first place. It is true that biodefense research – and any kind of civilian medical research – is always a race with its counterpart, the evolution of naturally occurring infectious agents or the development of biowarfare agents. But in this race it should be avoided to create the threats that are allegedly the motivation for the research. A self-made vicious circle is created: "*The technologies are in place with reverse genetics to generate any influenza virus we wish … studies are envisaged using genes of the 1918 Spanish Influenza virus…"*[30] These arguments were recently brought forward to justify another maximal biosafety laboratory for biological defense work in Texas, USA. Without Taubenberger's pioneering work, the money for the lab could have been saved and better invested in combatting naturally occurring diseases such as tuberculosis, malaria or HIV.

Other papers argued that the experiments may help to elucidate the mechanisms of influenza evolution and virulence (Taubenberger et al. 1997, Basler et al. 2001), but this argument is deeply flawed, too. Since 1918, a large amount of different influenza viruses with different virulence and pathogenicity properties have been isolated and characterised by researchers around the world – a more than abundant source for generations of scientists to study influenza evolution and virulence. A resuscitation of the Spanish flu is neither necessary nor warranted from a public health point of view.

There may be many reasons for the individual scientists to work on this project, not least the scientific prestige – the 'Spanish flu' subject matter practically guaranteed a series of publications in prestigious journals. From an arms control perspective it appears to be particularly sensitive if a military research institution embarks on a project that aims at constructing more dangerous pathogens – if Jeffery Taubenberger worked in a Chinese, Russian or Iranian laboratory, his work might well be seen as the 'smoking gun' of a biowarfare program.

5. Emerging technologies III: New types of weapons

Many other new weapons may become possible in the decades to come. The deciphering of the human genome, synthetic genes and organisms, new approaches to gene therapy and drug delivery, and the sheer volume of genetic engineering experiments with potentially pathogenic microorganisms will increase the availability of much more sophisticated biological agents with a potential for hostile use, not only in classical warfare scenarios, but also for "peacekeeping", "military operations other than war", "low intensity conflict", and covert operations. To illustrate the possibilities, examples of future weapons based on current technologies follow:

Food Weapons

So called "edible vaccines" and "biopharming" (i.e. the production of vaccines or other bioactive substances in edible crops) can be put to hostile use. In the past decade, genetically engineered plants have been investigated as a means to produce and deliver vaccines. There are already a variety of

[30] Letter (4 February 2003) from Robert G. Webster, Professor of Virology at St. Jude Children's Research Hospital to Stanley Lemon, Dean, School of Medicine, University of Texas Medical Branch (UTMB), in support of the UTMB application to construct a National Biosafety Laboratory.

research reports demonstrating that engineered plants can elicit an immune response in humans (Haq et al. 1995, for review see Streatfield/Howard 2003), and clinical trials on humans are currently underway to test vaccines produced in edible crops.[31] These vaccines may be isolated from the plant for further processing or directly delivered to the patients by consumption of the engineered plant.

Vaccines are only one type of bioactive substances being produced in edible crops. Several US companies are using genetically engineered crops to produce industrial enzymes, growth hormones, and other potent pharmaceutical compounds. These techniques pose a serious risk to human health and the environment, especially when the highly active pharmaceuticals are introduced into edible crops.[32]

The possibility of abuse of these crops and/or the underlying technology for hostile purposes is serious. In long term conflicts, it may be tempting to weaponize engineered crops, spiking them with, for example, disease-inducing (e.g. cancer) or debilitating compounds (e.g. affecting human or animal fertility) or built-in deficiencies that could lead to crop failure. Such "weaponized" germplasm may thereafter be introduced in the target country's seed supply and consequently its food supply through covert actions or simply by means of seed sales or humanitarian aid. This may not be possible with crops that are exported by the target country, as, given today's global market, the spiked food/feed could end up in the aggressor's food supply. But for most countries it will be possible to identify food or feed crops in the target country that are not exported.

There are routes to possibly achieve similar effects without sophisticated knowledge to engineer a specific crop with a specific compound. Theft of a few corn kernels from one of the many trials with edible plants producing bioactive substances may be enough. Pharmaceuticals such as blood clotters or blood thinners may not be a weapon of choice, but introduction into the food supply would not be technically difficult. Profusion of such artificial traits would likely produce panic and could be very difficult and expensive to eradicate. Public concern would be amplified if the trait in question was a potent growth hormone, which has been field trialed in the US, or a drug called trichosanthin, which has also been tested. Trichosanthin, considered to be a potential anti-cancer agent, has the same mode of action as the biowarfare agent ricin[33] and is a strong abortion-inducing compound. In the US, trichosanthin production in tobacco plants was induced by a genetically engineered plant virus. That same virus also easily infects crops such as tomatoes and peppers.

A 'contraceptive corn" developed by the US company Epicyte is unlikely to be usable for hostile purposes; but illustrates the potential abuse of pharming. Epicyte engineered corn to produce an antibody against human sperm. The company wants to produce large amounts of the antibody in order to extract it for use in a contraceptive gel. Consumption of the engineered corn or the extracted antibodies is unlikely to confer sterility – but a similar approach would yield dramatically different results. Introduction of a gene for human sperm cell antigens into a crop could create (an easily abused) contraceptive vaccine, preventing women who eat the engineered corn from reproducing.

Edible weapons pose a serious problem for BW non-proliferation efforts. No biological arms control effort could stop a person from stealing a handful of kernels, growing more, and introducing them into a country's food supply. The technology and especially its products are inherently difficult to control – the past years witnessed a variety of cases where specific genetically engineered crop varieties showed up in unexpected places. In one case, a corn variety that was not permitted for human consumption by US regulatory agencies showed up in a broad variety of human food supplies – despite it being approved for animal feed only.[34]

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[31] See, for example, ProdiGene press release, 12 August 2002: ProdiGene and NIH beginning phase I study on oral vaccine derived from transgenic corn. At www.prodigene.com.

[32] For a detailed discussion of possible effects on the environment and human health see the background paper "Manufacturing drugs and chemicals in crops" published by Friends of the Earth; http://www.foe.org/camps/comm/safefood/biopharm/BIOPHARM_REPORT.pdf

[33] Both, ricin and trichosanthin, are ribosomal inhibitor proteins.

[34] For an overview on the escape and potential risks of StarLink corn see Washington Post, 19. March 2001, 'Biotech Corn Is Test Case For Industry', http://www.washingtonpost.com/ac2/wp-dyn/A23092-2001Mar18?language=printer

Considering how easy and effective the hostile abuse of these genetically engineered crops is once they are developed, a complete ban on the production of hazardous compounds in edible crops appears to be justified. This may not stop a criminal from willfully creating an 'edible weapon', but it would tremendously raise the threshold compared to wandering into a corn field and grabbing some cobs. In addition, it will be technologically more challenging for a future biowarfare program to develop its own 'food weapon' if the technology is not further developed. With each experiment and each field trial, more knowledge on how to turn food crops into dangerous weapons will be accumulated, simultaneously creating pathways to weapons.

A complete ban on this particular technology will not cause severe scientific or industrial setbacks. All bioactive compounds that are currently produced in edible crops may as well be produced through other means that are less prone to hostile use. Some small biotech companies that specialize in biopharming may face problems, but others that focus on different technologies well benefit from such a move.

Fertility Control

Currently, a variety of new methods for fertility control are under development, for use as contraceptives in humans but also for the biological control of pest animals. Some of them – such as the Australian mousepox experiment – pursue strategies that are based on vaccines, i.e. they try to direct an animal or human immune response against egg or sperm cells to prevent pregnancy and reproduction. It is too early to conclude that these experiments will be successful, but if so, "fertility vaccines" present opportunities for abuse. If live vaccines are used (as in the mousepox example) that can be transmitted from individual to individual, a large population (of animals or people) may easily be prevented from reproducing, with enormous long term social and economic consequences.

Applied to 'alien invasive' or introduced species, such vaccines pose serious ecological threats (if the vaccine spreads to the target's geographic origin); but also significant risk of abuse to cause deliberate harm. This is particularly the case if such vaccines are developed to eradicate species of food or economic importance – for example, a 'vaccine' to control feral pigs, goats, rabbits, or other mammals that pose an ecological problem where they have been introduced might be transported to deliberately damage agriculture in other areas.

Terminator Technology

So-called 'terminator technology' renders seed infertile to guarantee a seed corporation's yearly sales. It may eventually be abused for economic warfare. If terminator crops become widespread, it would be easy for a country or a company that controls the technique to stop sales to a specific country or region for political or economic purposes. After some years of planting such seeds, only limited quantities of other seed would be available, thus agriculture could be paralyzed, leading to serious economic crisis and/or famine.

Insect fighters

The idea to use insects to deliver biological warfare agents is not new. Insects were systematically explored as a mechanism to spread a variety of diseases (e.g. plague) in the World War II Japanese BW program and the postwar US program. In many cases, such insect vector BW was dismissed as too complicated and unreliable. But genetic engineering may open a new way to use insects as weapons. In the same way as genetically engineered plants may be misused as 'food weapons', insects may be engineered to produce toxic compounds and deliver them through their natural feeding habit – e.g. in the saliva of mosquitoes. Again, these compounds may exert a broad range of possible effect, from non-life-threatening illness to sterility to widespread fatal illness in a target population.

Techniques to use insects to deliver vaccines have already been developed and patented.[35] The idea to develop what one company calls 'flying syringes' is based on the hope of circumventing costly

[35] See European patent PCT/GB95/02639 and US patent application 20020124274 (September 5, 2002) by Imperial College of Science Technology and Medicine (London) for a 'delivery system".

vaccination programmes in which every individual must be inoculated by trained medical personnel. Genetically engineered mosquitoes or other biting insects could instead deliver minute quantities of vaccine through the saliva every time they bite. The relevant techniques are still in their infancy. In comparison to genetic engineering of crops, for example, insects lag behind; but within several years, development of insect combatants may become a real possibility.

It is, however, questionable, whether genetically engineered insects may really become a weapon of choice. It will be nearly impossible to control these insects and limit their activity to the target country. Even if insects are choosen that are thought to be restricted to certain climate conditions, natural evolution and/or global climate change may rapidly overcome this restriction. State sponsored biowarfare programs tend to be very concerned about restricting unintended distribution of the biowarfare agent – most typical bacterial biowarfare agents are not contagious – and will thus hardly engage in the flying syringe concept.

Current Projects in the US

The Sunshine Project has previously documented a series of recent offensive projects in the United States that draw on new developments in biotechnology. Military exploitation of new biotechnological possibilities, most notably with so-called "non-lethal" weapons, have fueled new weapons desires, even in countries that have renounced the use of biological weapons such as the US (and, in the case of "non-lethal" chemical weapons, Russia). The following three cases have been researched and previously published by the Sunshine Project, hence here we present only short summaries. Further reading is available on our website.

Material degrading microorganisms[36]: Natural microorganisms are capable of degrading nearly every kind of material. These organisms are sometimes used for environmental cleanup purposes ('bioremediation'); but are generally too slow and unreliable for weapons purposes. Genetic engineering, however, is enabling development of organisms effective enough for use as biological weapons. The British government recently warned: *"Bioremediation technologies clearly have the potential for development of a means of warfare or for hostile use against materiel crucial for normal civilian life or military operations, such as oils, rubbers and plastic."*[37] This potential has raised the interest of several US government research institutions, including the US Naval Research Laboratory, where microorganisms that degrade a variety of materials (plastics, rubber, metals, etc..) were genetically engineered to make them more powerful and focused for bioweapons purposes.

Fungi against drug producing plants[38]: About a decade ago, the United States increased efforts to identify microorganisms that kill drug-producing crops. In the late 1990s, this research focused largely on two fungi. Testing of *Pleospora papaveracea* to kill opium poppy, conducted in Tashkent, Uzbekistan with US financing and scientific support, was completed in 2001. Pathogenic *Fusarium oxysporum* strains developed in the United States to kill coca plants were scheduled for field testing in Colombia in 2000, but international protests led to a halt to this project.

Military use of psychoactive substances: So called "non-lethal" chemical weapons were developed by the US military in the 1950s, especially a hallucinogenic substance called "BZ" . But BZ was considered to be unreliable, leading to its removal from the US chemical arsenal in the late 1960s. Today, modern neurobiology is developing an increasingly comprehensive knowledge of a broad range of specific neuroreceptors and psychoactive substances that trigger (or inhibit) them. Military temptation to exploit these discoveries have made "non-lethal" chemical weapons again attractive for the military. A case in point was the use of a gas in the Moscow theatre hostage situation in 2002. Projects at the US Army's Aberdeen Proving Ground and at the US Marine Corps Research University have recently investigated the military utility of a variety of incapacitating agents, including calmatives, seizure inducing agents and other psychoactive substances. The US and Russia are also developing delivery devices for chemicals with a range of more than 2.5 kilometers – a distance that makes only sense for warfare scenarios, and not for domestic law enforcement purposes.

[36] See Sunshine Project Backgrounder #9 (http://www.sunshine-project.org/publications/bk/bk9en.html) for further reading.
[37] See footnote 14
[38] For extensive reading on Agent Green see the Sunshine Project Backgrounder No. 4 and additional materials at www.sunshine-project.org.

6. Ethnic specific biological weapons

Current wisdom holds that population specific biological weapons are practically and theoretically impossible. Practically, many consider it impossibly difficult to use genetic variability to kill or otherwise affect populations. Others, including geneticists, argue that no suitable ethnic specific genes exist in the first place. Both notions are wrong. New technologies are indeed available to translate specific genetic sequences into markers or triggers for biological activity. And a recent analysis of human genome data in public databases revealed that hundreds, possibly thousands, of target sequences for ethnic specific weapons do exist. It appears that ethnic specific biological weapons may indeed become possible in the near future.

Weapons targeting specific population groups do not need to be deadly. They could cause temporary incapacitation, illness, sterility, permanent fatigue, or any other condition that may not be fatal but desirable from an aggressors perspective. They may be used in an all out war, in the battlefield or against civilian population, or they may be used in covert operations in conflict situations and with long-term effects, in order to destabilise, harm economically or weaken an enemy society.

Techniques to translate genetic sequence into a weapons effect

The development of ethnic weapons with very specific effects would be easiest with techniques that use a genomic marker as a trigger for an activity that is unrelated to the location of the marker, i.e. the effect would be triggered even if the sequence is in a non-coding or non-translated region of the genome. As far as we are aware, this kind of technology does not yet exist.

There are, however, techniques available that can inhibit genes with a specific sequence. They target mRNA, the molecule that transmits information from the DNA to the place of protein synthesis within a cell. One of these techniques, called RNA interference (RNAi), uses a mechanism by which a specific RNA sequence is degraded by the cell if an externally applied RNA molecule of the same sequence is entering the cell (for review, see Cerutti 2003). A similar approach called antisense technology inhibits further mRNA processing by binding endogenously produced mRNA to an externally applied DNA molecule with the corresponding sequence. The latter technology is currently under development by the US company Ibis Therapeutics.[39]

Both technologies lead to the inhibition of a specific target gene with a specific sequence. If the sequence of the target gene varies from one population to another, this can be used to interrupt the gene in one population and not in the other. Military abuse of this technology would require the identification of population specific sequences in genes that are active and vital for the body function.

Ethnic specific genetic markers

Do such genetic markers exist? Markers that are present in one population (at least to a certain percentage) but not in another? Many human geneticists are eager to emphasize that genetic diversity within a population is far greater than between populations. This view is also reflected in a 2001 background paper prepared by the British Government for the last Review Conference of the BWC. It states that "*there is as yet no indication of differences that could be used as the basis for 'genetic weapons' which would target particular ethnic groups".*[40]

99.9% of the genetic sequence of any two human individuals is said to be identical – but the remaining 0.1% accounts for a total of 3 million "letters" of the human genome. There are thought to be several tens of thousands coding genes in the human genome, thus it is possible that every single gene between one individual and another could be slightly (or greatly) different, even if there is 99.9% homology in overall genetic sequence. Some of this huge genetic diversity breaks out in differences between populations. These genetic populations (using the term in its biological sense) appear to often

[39] www.ibisrna.com
[40] See footnote 14

correspond with (culturally-determined) ethnic groups (for a detailed discussion on human genetics and the pitfalls of racial genetic profiling in general see Sankar & Cho 2002, Aldhous 2002, Schwartz 2001, Wood 2001).

From a biological weapons perspective, population specificity would mean more than just a small variation in allele frequencies in different ethnic groups – no effective weapon could be designed that targets a genetic constitution that is also present to any significant extent in the population of the aggressor. From a military perspective, population specificity would mean that these genetic sequences are not or only to a very limited extent present in one (the aggressor's) population while the same sequences are present in a significant percentage of an opposing population.[41]

While it would certainly be desirable to have a very high percentage – up to 100% – of the target population bearing the target genetic marker, this is by no means a prerequisite for a militarily useful weapon. Even if only some 10% or 20% of a target population would be affected, this would wreak havoc among enemy soldiers on a battlefield or in an enemy society as a whole. Thus, if discussing genetic markers for ethnic specific weapons, sequences would be needed that have a frequency close to 0% in one population while having a significant frequency in another one. For the purpose of this paper, we assume that a frequency of 20% or higher may be enough from a military perspective.

A systematic search in two databases revealed that genetic sequences fulfilling these specifications not only exist, but they do so in unexpectedly high numbers. Our analysis focussed on so called single nucleotide polymorphisms – SNPs – that are by far the most common source of genetic variation. SNPs are basically single-letter variations in the human DNA sequence. In the past years, several million SNPs have been identified by private and public entities. The SNP Consortium (TSC), representing a group of big pharmaceutical companies and not-for-profit organisations, keeps a public database on a large number of SNPs. Another SNP-database, the SNP500Cancer database, is kept by the Cancer Genome Anatomy Project of the US National Institutes of Health.[42]

Cytochrome P450 genes

The many genes in the cytochrome P450 system have been suggested as possible targets for ethnic specific weapons, for two reasons. They show high ethnic diversity, and they are involved in the detoxification of toxic substances. The notion is that ethnic groups with specific polymorphisms in a cytochrome P450 gene may be less able to detoxify a specifically designed biological or chemical weapon and thus be more susceptible to its action.

From our view, these genes are probably useless as a basis for ethnic weapons, as diversity in most of these cases relates to different percentages of certain alleles in different population, not situations in which one population has a certain allele while the other does not. Hence, a significant part of the aggressor's population would be potentially vulnerable. In addition, the P450 system comprises many dozens of enzymes with overlapping activities. Targeting a chemical or biological compound to one specific P450 enzyme would be very challenging.

Both databases provide data on allelic frequencies in different populations for at least part of the SNPs. We analysed a total of nearly 300 SNPs, all in coding regions or genes[43], from both databases.

[41] It must, however, be questioned how good the 'zero' frequency of the target allele on the aggressor's side has to be. This may depend heavily on the effect of the ethnic weapon and on the political system of the aggressor. Dictatorships may well accept more 'collateral damage' in their own society than others. And if the effects are non-lethal and long-term – such as sterility – it may be more acceptable for an aggressor to have some victims on its own side. If used in a battlefield, an aggressor could also screen and select its soldiers according to this specific sequence, or could apply specific countermeasures.

[42] http://snp500cancer.nci.nih.gov/snplist.cfm. This program studied the genome of 102 individuals of self-described heritage: 24 of African/African American heritage, 31 of Caucasian heritage, 23 of Hispanic heritage, and 24 of Pacific Rim heritage. In this database, we analysed 193 randomly selected SNPs (all validated SNPs in chromosomes 6 and 10). A total of 24 SNPs (12%) showed an allelic freqency of ≥ 10% in at least one population with a 0% frequency in at least one other population. 3 of these (1.6%) had a frequency of 20% or higher in one population.

[43] As discussed above, it appears to be a prerequisite for militarily useful genetic markers to have them appear in coding sequences or genes that are active in the human body, rather than in apparently silent parts of the human genome. If new technologies are developed that can use even apparently inactive genomic sequences as a trigger for the desired effect, this would make it easier to translate these genetic differences into weapons.

An unexpectedly high number of these SNPs are indeed population specific: 6.7% of the SNPs in one database (see table 1 below) and 1.6% of the SNPs in the other include one allele that is not present at all in one population while it has a significant frequency of more than 20% in another population.

Chrom. #	No. of SNPs with TSC-ID and frequency data on 2 or more populations	0 : ≥ 1% (n)	0 : ≥ 10% (n)	0 : ≥ 20% (n) (pop:pop)	TSC-ID
1	17	5	2	1 (A:C)	1166809
2	18	4	2	1 (A:AA) 1 (A:AA)	0493622 0231219
3	8	1	1	1 (A:AA)	0207612
4	12	1	1		
5	9	1	1		
6	9	3	3	1 (C:AA)	1104025
7	8	2	1		
8	11	2	2	1 (C,A:AA)	0668661
9	7	2	1	1 (A:AA)	0815601
10	6	0			
Total (n) (%)	**105 (100%)**	**21 (20%)**	**14 (13,3%)**	**7 (6,7%)**	

Table 1: Ethnic specific SNPs in the TSC-database

From the database of The SNP Consortium (TSC)[44], SNPs were analysed for an ethnic specific allele distribution. The TSC database distinguishes between Caucasian, Asian and African-American samples[45]. From 105 randomly selected SNPs[46] in coding regions of the human genome, 21 had an allele frequency of 0% in one population but were present in at least one other population, 14 of these with a frequency ≥ 10% and 7 of these with a frequency ≥ 20%.
pop – population; A – Asian; C – Caucasian, AA – African American (e.g. A:C means that the minor allele is not present in the Asian population and has its highest frequency in Caucasians).

This finding is consistent with results from Stephens et al. (2001) who identified a total of 1,452 SNPs out of 3899 SNPs (37.2%) to be population specific, although the majority of these were rare SNPs. However, Stephens et al. (2001) also noted that "*not all population-specific alleles were observed at a low frequency. In the African-American and Asian samples, some population-specific alleles were found at frequencies >25%.*"

In some cases, the frequency differences can be very high. For example, in our analysis of 105 SNPs from the TSC database, one SNP (TSC0493622) has a 0% : 94% ratio between major populations (see diagram 1 below). The G-allele of this SNP was present in 94% of the African-Americans and in 0% of the Asians sampled. The nature and function of the gene encoded by this genetic region is still unknown. Another example for a relatively high frequency difference is a polymorphism at the human melanocortin 1 receptor locus (MC1R), an enzyme involved in skin color formation. In a study by Rana et al. (1999) one allele was not identifiable in any Africans, but showed a frequency of 70% in East and Southeast Asians.

[44] http://snp.cshl.org/ as of June 24, 2003
[45] See http://snp.cshl.org/allele_frequency_project/panels.shtml for a description of the panels.
[46] All SNPs in coding (both synonymous and non-synonymous) regions with a TSC-ID number and with allele frequencies provided for at least two different populations from the first 100MB of chromosomes 1-10 were included in the analysis.

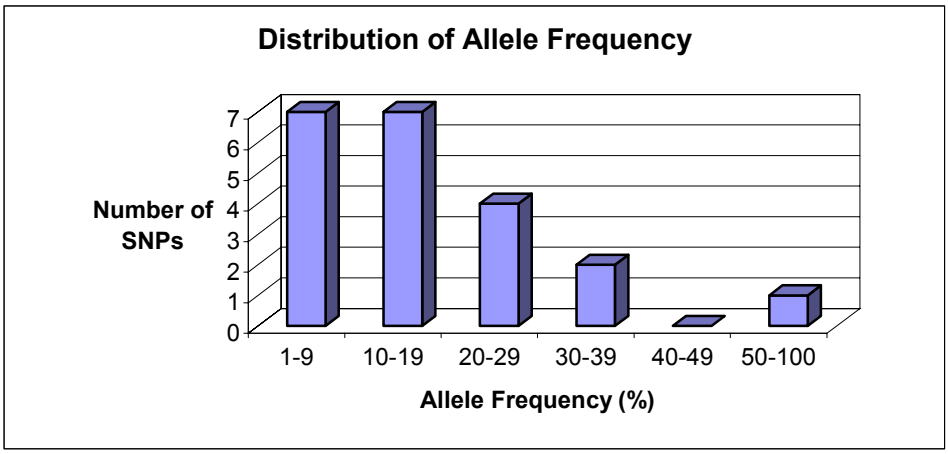

Diagram 1: Allele frequency of the 21 ethnic specific SNPs in the TSC database

The majority of the population specific SNPs had a rather low frequency for one allele of less than 20%, but some SNPs with higher frequencies were also identified. 14 SNPs had an allele frequency of 19% and less, while only 7 SNPs had an allele frequency of 20% and higher. For SNPs with ethnic specific alleles in 2 populations, the higher frequency value was choosen for this diagram.

Some caution should be applied not to overestimate or interpolate our results. Both datasets as well as the work of Stephens et al. (2001) are based on a limited number of individuals for each population group[47]. Hence, alleles with a very low frequency in any one population may have been missed. Therefore it is possible and likely that some of the alleles that where not identified in one population group may well be present at low frequencies in these groups, so that many of the SNPs that were included in our analysis as they showed a 0% frequency for the minor allele would have to be excluded as their real frequency may be higher than 0%.

On the other side, it is safe to assume that a certain percentage of the SNPs included in our analysis will prove to be population specific even if larger numbers of individuals were screened. There are examples of unsuccessful searches for alleles in large populations: The gene for thiopurine methyl transferase (TPMT) is an enzyme involved in metabolism of certain pharmaceuticals. Allele *3A, which is the predominant mutant TPMT allele in individuals of European heritage, has not been identified in East Asian populations despite the analysis of a total of 1068 individuals in 5 independent studies (see van Aken et al. 2003 for review).

To summarize, in can be estimated that a considerable number of ethnic specific SNPs do exist. Recent numbers suggest that SNPs occur with a frequency of about every 200 base pairs in the coding sequences of human genes (Schneider et al. 2003). Given the total number of about 3 billion base pairs, some 15 million SNPs may exist in the human genome. If in a conservative estimate only 0.1% (as compared to the 6.7% and 1.6% determined in our analysis of the two datasets) of these do occur population specific frequencies (here defined as 0% in one population and > 20% in another), some 15,000 possible target sequences may exist for future bioweaponeers.

It should be noted that some of the ethnic specific SNPs we identified in our analysis have a known function and are indeed readily expressed in human tissue. For example, the SNP rs2894804 from the SNP500Cancer database is located in a gene called GSTA1, coding for glutathione S-transferase. This enzyme functions in the detoxification of xenobiotics, including carcinogens, therapeutic drugs and

[47]The SNP500Cancer Database is based on 23-31 individuals per population group; the TSC-database is based on different panels, most of which included 12-42 individuals per population group; Stephens et al. included 18-21 individuals per population group.

environmental toxins. It was present in the African-American population with a frequency of 23% while it was not identified in any of the other three populations.

Conclusions

It must be stressed that ethnic or population specific weapons are still a future threat and may not be accomplished within the coming decade. However, the notion that they are impossible and would violate the laws of nature is wrong and outdated. Practical steps can and must be undertaken today to prevent the future development of these kind of weapons. A key step would be to restrict the amount of ethnic specific genomic data to an absolute minimum. We are, however, currently witnessing a scientific development that is actually doing the opposite: creating vast amount of genetic data for different populations and ethnic groups. This happens in a variety of contextes:

- **Pharmacogenetics and pharmacogenomics**: In order to elucidate genetic influence on drug safety and efficacy, an increasing number of studies on pharmacogenetically relevant genes are being undertaken. These include studies on genes for enzymes involved in drug metabolism such as the cytochrome P450 system and many others, but also genes coding for drug transporters or drug target proteins. For the safe implementation of pharmacogenetics on a global basis or in multicultural societies, reliable data on allele frequencies relevant to all populations is needed. Hence many pharmacogenetic studies investigate ethnic specific genetic differences relevant to drug action and are thereby generating large data sets that genetically profile on an ethnic basis. This problem may be circumvented by using pooled samples from a representative cross-section of all relevant population for the analysis of SNPs. Techniques are available today to calculate allele frequencies in pooled samples from up to several 100 individuals. Through this method, all relevant alleles in a pooled sample of all relevant populations could be determined without generating ethnic specific genetic data. The field of pharmacogenetics is specifically risk-prone, as the relevant genes are directly involved in drug metabolism or drug action and may thus be much more easily converted into triggers/markers for the action of biological or chemical agents than other genetic markers.
- **The HapMap Project**: In October 2002, an international project to create a map of haplotypes[48] in the human genome was launched.[49] In this US$ 100 million public-private undertaking, genetic variations in four populations will be investigated: US residents with European ancestry, Han Chinese, Japanese and Yorubas in Nigeria. The HapMap project will provide vast amounts of genetic markers specific for any of the four populations. In the light of the possibility of hostile abuse of these genetic markers the HapMap project should be reconsidered.
- **Forensic genetics**: Genetic fingerprinting enables to match a suspect's DNA with that found at a crime scene. However, law enforcement is striving to get more information out of crime scene DNA, including the "race" or ethnicity of the culprit. First steps have been taken in the direction of ethnic affiliation estimation by use of population-specific DNA markers (Shriver et al. 1997). The US National Institute of Justice recently issued a $496,000 grant to the University of Arizona to predict skin colour from DNA samples,[50] and the US-based company DNAPrint Genomics Inc. is offering to determine "race proportions" from crime scene DNA, although the technique is still prone with difficulties (Brenner 1998). It appears that these applications – if successful at all – could be of less concern from a bioweapons perspective, as they do not necessarily rely on markers that show a 0 : x percent distribution in different populations. In the course of the development of more sophisticated approaches for forensic ethnic affiliation estimation, however.

[48] Haplotypes are blocks of closely linked SNPs in a genome and are currently viewed as the best tool to study human genetic variation.
[49] see http://hapmap.cshl.org/ for details.
[50] NIJ grant number 2002IJCXK010.

if a systematic search for ethnic specific markers is undertaken it may reveal markers abusable for bioweapons purposes.

- **Others**: Some human genetic studies touch on critical genetic data in politically tense areas, such as work on ethnic (Bhattacharyya et al. 1999) or even caste (Bamshad et al. 2001) associated genes in India, or genetic differences between the Basque and non-Basque population in Spain (Arrieta et al. 1997). A thorough assessment of benefits – if any – of this research and the associated risks of abuse appears to be necessary.

7. Conclusions and recommendations

To summarize, genetic engineering can clearly contribute to make classical biowarfare agents more effective, it can ease access to them, enable the construction of novel BW agents and opens the avenue for a broad array of new types of weapons. It is of crucial importance for scientists and policymakers around the world to address the increasing threat and redouble efforts to strengthen the ban on biological weapons and to control critical technologies.

While the science behind the examples given in this paper is a reality, in most cases the hostile utilization of it (hopefully) has not occurred, so far. For example, terminator technology or fertility control technology do not appear to have been exploited for hostile applications, but it is obvious that once such technologies are more broadly exploited (particularly in commerce), they may become easily acquired and used with malign intentions.

Molecular biology and genetic engineering are still in their infancy. More technical possibilities will arise in the years to come that can be abused for hostile purposes. More efficient classical biowarfare agents will most likely play only a marginal role, even if the genetically engineered superbug is still routinely featured in newspaper reports. More likely and more alarming are the new types of weapons for newly-prevalent types of conflicts and warfare scenarios, for example, low intensity warfare and covert operations, for economic warfare or for sabotage. To prevent the hostile exploitation of biology now and in the future, a bundle of measures must be taken. First and foremost, the Biological Weapons Convention needs to be strengthened through multilaterally agreed, legally binding verification measures. In addition, three immediate steps are of specific importance:

- **All projects that violate the Chemical and Biological Weapons Conventions must be immediately abandoned**. In the United States, such projects include the development of material degrading microbes, development of so-called "non-lethal" (bio)chemical weapons (including delivery devices), and continued development of biological agents to eradicate narcotic crops. Other countries that are engaged in similar projects – such as Russia, which maintains stockpiles of incapacitating chemical weapons and, likely, an R & D program on them – must also halt such research. These agents undermine the Chemical and Biological Weapons Conventions, are lowering the political threshold for use of biological weapons, and are likely to have tremendous environmental and health impacts. Pursuit of these agents as weapons would be a step down a slippery slope, that, following the same logic, could easily lead to the use of other biochemical and biological warfare agents in conflict. Failure to stop these projects will encourage other countries to follow suit with R & D projects on biotechnological weapons, leading to an unravelling of key disarmament treaties.
- There is an urgent need to ensure that **governments restrict themselves and ensure maximum transparency in their biodefense programs**, to prevent a race for offensive capabilities under cover of defense. We call on all governments to adopt the 'Government Undertaking on Biodefense Program', which has recently been brought forward by the Sunshine Project. It contains, among others, a provision that "*biodefense programs will not, for any purpose, utilize or construct, including single-gene changes, novel biological agents with an enhanced offensive potential"* such as treatment resistance, environmental stability, or enhanced pathogenicity.

- **Research restrictions** are necessary in certain situations, for example, in cases where a military abuse appears to be imminent, where no effective multilateral arms control or non-proliferation efforts are presently feasible, and where other technical avenues to reach the same scientific goal are (potentially) available. These criteria apply specifically to the production of bioactive compounds (pharmaceuticals, vaccines) in edible crops, but may also be relevant for some aspects of pharmacogenetics, were the generation of huge amounts of ethnic specific genetic data may be avoided by choosing other techniques that serve the same research purpose. The current 'bioterrorism' discussion in the scientific community focuses entirely on restricting the *publication* of certain research results. This is shortsighted, and may easily be abused to conceal illicit research, particularly since it may be better not to generate dangerous information in the first place. Full transparency in all aspects of biomedical research and development should be guaranteed.

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