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► **INSIDE**

FORENSIC MICROBIOLOGY

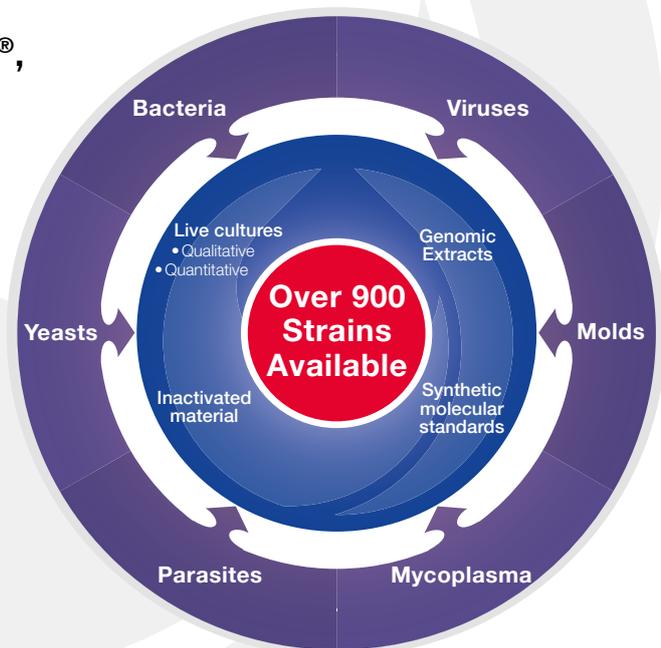
The necrobiome
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Paul Sainsbury reviews the content of this issue

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Early Career Scientists shine at SfAM Summer Conference

Wow! What a month. I write this shortly after returning from the Society for Applied Microbiology's Annual Summer Conference in Dublin. The Committee Members and staff of SfAM really deserve massive congratulations for coming up with such a great agenda of events and speakers. The Early Career Scientists (ECS) group in particular hosted an excellent number of meetings, satellite events and other networking opportunities at the Intercontinental Hotel in Dublin. These are always so well received and the success of their programme is reflected by the sheer number of national- and international-based attendees starting out on their microbiological journeys.

Typically, SfAM is looking for a new Meetings Secretary who will have the opportunity to shape the feel of the Society's conference programme as Chair of the Meetings Subcommittee. Further details can be found on page 57 of *Microbiologist* and expressions of interest in the role should be sent to lucy@sfam.org.uk by 14 September 2015.

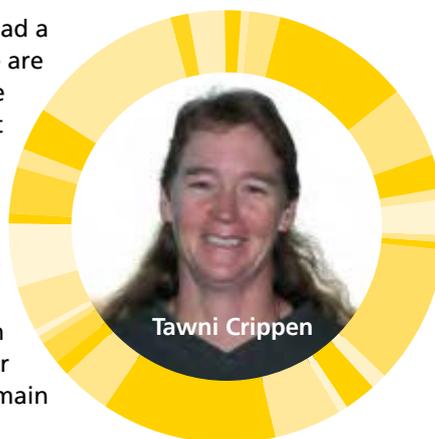
Now it has been a while since *Microbiologist* had a guest Features Editor, however in this issue we are delighted to have **Dr Tawni Crippen** from the Food & Feed Safety Unit at the US Department of Agriculture.

In her introduction to the main theme of this edition Tawni writes about the evolving and rapidly growing topic of forensic microbiology from discriminating distinct microbes to characterizing entire microbial communities on decomposing remains. Tawni has provided four fascinating features that explore some of the main areas within the subject.

These features reveal the role that forensic microbiology plays in the real world and the amount of time and effort involved in the most common processes. Some of the techniques described have revolutionized many aspects of criminology and have come to light through our continuing investigation into the relationships between medicine, man and microbe.

You will also find all the regular articles, features and news items in this issue including a historical perspective on the influenza pandemic by Stephen Winchester, and Karen Stanley reflects on her early career experiences and how she now draws on them in her current role in higher education.

This edition of *Microbiologist* contains graphic photos and descriptions of decomposing human corpses.



Tawni Crippen

NEWS IN BRIEF

Counterfeit drugs hasten spread of AMR

Cheaper treatments on the black market are driving the spread of antimicrobial resistance (AMR). <http://stanford.io/1eJHyHe>.

Phage treatment for Alzheimer's disease

Phage isolated from sewage may help treat Alzheimer's, Parkinson's and Creutzfeldt-Jakob diseases (CJD). <http://bit.ly/1fhzZli>.

Zombie bacteria

Research shows bacteria, killed with silver nitrate in solution, rose up like 'zombies' and killed surviving pathogens. <http://rsc.li/1EYdHF2>.



Paul Sainsbury, Editor



With the arrival of more accessible high-throughput sequencing techniques, forensic information used to interpret a crime scene is expanding to include microbial information

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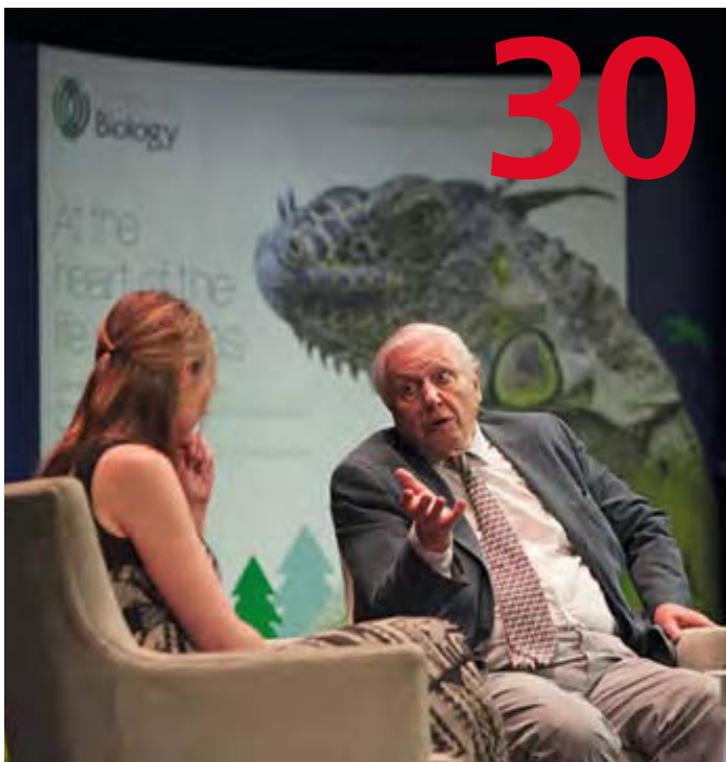
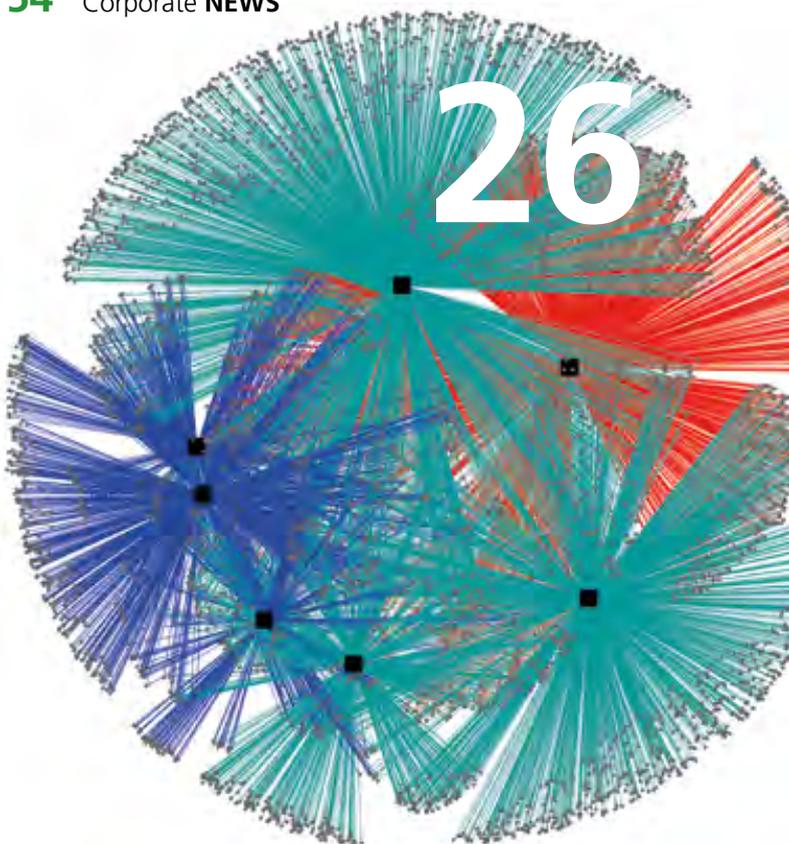


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President's column

As you will all be aware, antimicrobial resistance has been recognized nationally and internationally, as a global health threat and as one of the great scientific challenges we currently face. Many of you will be familiar with the Society's concerns over this from our position statement and you may also be pleased to hear we will be holding a special one-day meeting on *Antimicrobial Resistance (AMR)* in London on 7 December.

The Learned Society Partnership on Antimicrobial Resistance (LeSPAR) is a partnership of seven UK learned societies: Biochemical Society, British Society for Antimicrobial Chemotherapy, British Pharmacological Society, Royal Society of Biology, Royal Society of Chemistry, Society for Applied Microbiology and Society for General Microbiology which was formed to support actions that can begin to address this challenge*. Collectively, these seven Societies represent around 75,000 scientists. LeSPAR was established following the House of Commons Select Committee Enquiry on Antimicrobial Resistance, at which a number of learned societies gave evidence, and the publication of the UK Department of Health's five-year antimicrobial resistance strategy.

Like a number of SfAM Members, I recently attended one of the three LeSPAR workshops on *Antimicrobial Resistance: Environments, Evolution and Transmission*. The workshops were arranged as interdisciplinary networking opportunities to bring together researchers who have an interest in the evolution and transmission of AMR, from all career stages and across a range of disciplines and sectors. The workshops were held in London, Dundee and Nottingham; full reports on these will be available in due course. However, I thought I would share with you a couple of the issues which arose at the Nottingham workshop which I attended. One was a point made by Rachel Gomes from the University of Nottingham. Rachel is an Assistant Professor in Chemical and Environmental Engineering working on waste water disposal. She gave a talk on *AMR in the Outside Environment* which drew interesting parallels between the presence of antibiotics and their metabolic residues in waste waters, and the problem of other types of chemicals which are covered by EU regulations (Registration, Evaluation, Authorization and Restriction of Chemicals). She included an everyday example of failing to dispose of

antibiotics properly by flushing them down the toilet or putting them in the rubbish which goes to landfill. These practices expose environmental microorganisms to antibiotics unnecessarily whereas, simply returning the drugs for incineration would avoid this. This reminded me of a talk by Joakin Larsson at a recent EDAR3 conference. He described the levels of ciprofloxacin released per day in treated effluent from a pharmaceutical plant in India as ~ 5 times the level used each day nationally in Sweden. Whilst the practices Rachel talked about are not on this truly terrifying scale of environmental contamination, it served as a salutary reminder that very simple changes in our behaviour could be of benefit.

The next speaker was Jan-Ulrich Kreft a lecturer in Computational Biology from the University of Birmingham who described himself as a microbiologist turned mathematical modeller. Jan discussed *AMR in the Gut Environment* and why resistance persists. His talk illustrated the biological complexity of resistance control because of the multi-factorial influences of why a strain develops resistance and survives. He reminded us that whilst we talk about the 1,000s of species in our gut, the number of *strains* could be 100-fold higher and it is this development of diversity and its drivers which we need to understand. What both these talks demonstrated was the very great need for addressing the problem of AMR at different levels and applying interdisciplinary approaches to combat the problem of AMR. I look forward to further interactions of this kind.

And for those of you that asked – we had a fabulous Summer Conference and it was not just AC/DC who rocked Dublin!

**More information on LeSPAR, its remit and its activities to date can be found on the Society's website.*



Christine Dodd
President of the Society

Harper's Postulates

Notes from the Chief Executive

In January of this year, myself the Executive Committee and Chief Editors spent a busy and productive day in London establishing the future of the Society.

We looked at the activity of the Society from a number of different perspectives and came up with a solid set of aims, working principles, objectives and actions.

Our global aims are important, high-level and ambitious. For example, we aim to ensure that applied microbiology is a distinct and recognized field of scientific expertise with a high profile amongst all stakeholders. We aim to ensure scientists working in applied microbiology are trained to become excellent scientists and have the resources, facilities and legislative environment to flourish. And of course we aim for applied microbiology to remain a global activity unhindered by geographical, political, social and economic constraints.

As an organization, we aim to be the premier network for applied microbiologists in the UK and internationally. We also create best-in-class networking across disciplines, and support early career researchers through our Early Career Scientists Committee.

But with these ambitions, what we don't want to lose is our friendly, broad, inclusive, open and generous culture. We think it's our approachable and friendly ethos that makes us unique among similar organizations and is one of our important strengths.

Many of you will be familiar with our **Vision, Mission** and **Values**, but for those who need a reminder, they are:

Vision

SfAM envisages a future where applied microbiology research and development is strong in the UK and beyond, and the applications of microbiology contribute significantly to all global challenges facing humanity, including infectious diseases; the changing environment; sustainability of energy, food, water and land resources; and economic growth.

Mission

SfAM will achieve its vision by being the voice of microbiology and advancing, for the benefit of the public, the science of microbiology in its application to the environment, human and animal health, agriculture and industry. It will work in partnership with sister organizations and microbiological bodies to ensure that microbiology and microbiologists contribute to evidence-based policymaking within the UK, in Europe and worldwide. SfAM will build on a strong history of microbiology in the UK and will move forward in step with the next generation of microbiologists.

Values

SfAM is "The Friendly Society" and will always offer value for money. We are modern, innovative and progressive; we value integrity, honesty and respect; and we seek to promote excellence and professionalism, and to inspire the next generation of microbiologists.

The overarching message of our strategy is about support and promotion: support of applied microbiologists globally and at all stages of your career, and promotion of the importance of applied microbiology in solving global grand challenges.

A large part of our strategy is built around engagement. A membership organization is virtually meaningless without its Members, so engaging with you is a big priority for us. Having a strategic framework to underpin our work means that all our stakeholders, Members, Trustees and staff have a clear direction. So as the detail of our strategy is established, you'll hear more detail, and we want to engage with you to help shape that direction.



Lucy Harper
SfAM Chief Executive



Professor Tim Hunt's chauvinist remarks during his "trouble with girls" speech, made at a lunch for female scientists earlier this year prompted widespread condemnation and the loss of his job. Although Nobel Prize winner Professor Hunt surely still has a contribution to make to science and society, it is becoming more likely that his reputation, legacy and livelihood will be determined by the intense media and public interest in his recent ill-judged comments.

Having previously been a lone male in a lab with eight women, I can say his comments are ludicrous, however, they do provide a good opportunity to discuss some of the challenges facing women in academia.

Among STEM subjects, life sciences at an undergraduate level has always been fairly evenly split between males and females, certainly in comparison to civil engineering courses where as many as 85% of undergraduates are males. It is once we move beyond the undergraduate degrees that, even after decades of trying to narrow the gap, we are still seeing differences. A 2014 study looked at the male/female ratio in the top biological laboratories in the US and presented an illustration of the many challenges still faced by women in academia. What seemed instantly striking was that women made up less than 25% of professors – the figure is even lower in the UK. The research highlights that whilst the female/male PhD graduate workforce is nearly equal,

men are still being chosen to fill the most prestigious academic roles in the overwhelming majority of cases. So what causes this?

Is it a personal choice? A key time in a researcher's career is when they begin to establish their reputation is during a postdoctoral position. If you look at the statistics, this is where we observe the first reduced ratio of females to males in the workforce. Women make up less than 40% of postdoctoral researchers in life sciences.

Trying to manage a successful work/life balance may drive many women out of academia of their own accord. This may be due to the pressure to work long hours and weekends, as well as a chronic lack of infant day-care facilities at universities – waiting lists at big institutions can be as long as 2 years. A further issue in academia is the transition point between postdoctoral researchers and lecturers due to a lack of funding for faculty positions. Solutions to this particular problem warrant further in-depth discussion, however, it adds another competitive element and further pressure on women to delay starting families. It is at this stage which appears to translate into less than 30% of junior faculty positions in academic institutions being awarded to female colleagues.

What about sexism in science? Although overt sexism is unlikely to be the culprit there may still be an inherent, subconscious bias during the recruitment phase. If I told you "I heard a scientist on TV this morning discussing some really interesting facts about antimicrobial resistance" or that "I have just been to the doctor"; would you be guilty of replying "What did he say?"

Solutions to the problem of gender equality in science are something that global Governments have been working on for decades. One initiative in Britain is the Athena Swan Award, given to institutions showing a real commitment to driving equality among their research and teaching staff. At Kingston University we received an Athena Swan Bronze Award last year and toward this end have funded a number of postdoctoral positions supporting women returning to science from extended career breaks.

Hopefully over time we can begin to address these issues and this will result in a more balanced workplace.

FURTHER READING

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Tawni Crippen Guest Features Editor

Food & Feed Safety Unit, US Department of Agriculture

The body of an animal encompasses a multitude of compositionally and functionally unique microbial environments, from the skin to the gastrointestinal system. Each of these systems provides a distinctive genetic signature that may be useful in identifying its source, as the microbial communities have adapted over time in order to cohabit with a specific host. These adaptations occur at an accelerated rate in microbes in comparison to macroorganisms, such as vertebrates, due to short reproductive cycles, high mutation rates and the ease of gene exchange between microbes. The resulting community of microbes on and in animals is large and heterogeneous and related to such factors as anatomical location, gender and environment. There also appears to be a core set of bacterial phylotypes common to all individuals and transient rare taxa related to environmental influences (Gao *et al.*, 2007).

Pattern evidence is a common form of forensic information used to interpret a crime scene. Historically, pattern evidence has been limited to some obvious examples often seen in television programmes. These forms of evidence include items, such as fingerprints, hairs/fibres and ballistics. With the arrival of more accessible high-throughput sequencing techniques, pattern evidence is expanding to include microbial information. This form of evidence is reaching beyond classic point-source identification of individual pathogens, pollutants and weapons of bioterrorism.



They can now explore the possibility of identifying an individual's or animal's distinct microbial community before and after death, known as the microbiome and post-mortem microbial communities respectively, or where that person or animal has been, based on their unique microbial geo-signature (Benbow *et al.*, 2013).

Following Locard's Principle, contact between two objects results in material transfer. The same occurs with microbes being passed from an individual to an object simply through touch. In fact, the transfer of skin-associated pathogenic bacteria from an individual to an inanimate surface is not uncommon (Jeon *et al.*, 2013). Think how useful it would be forensically to identify if an individual had touched an object based on the specific residual bacterial profile left on a surface.

FORENSIC MICROBIOLOGY

Evolving from discriminating distinct microbes
to characterizing entire microbial communities
on decomposing remains

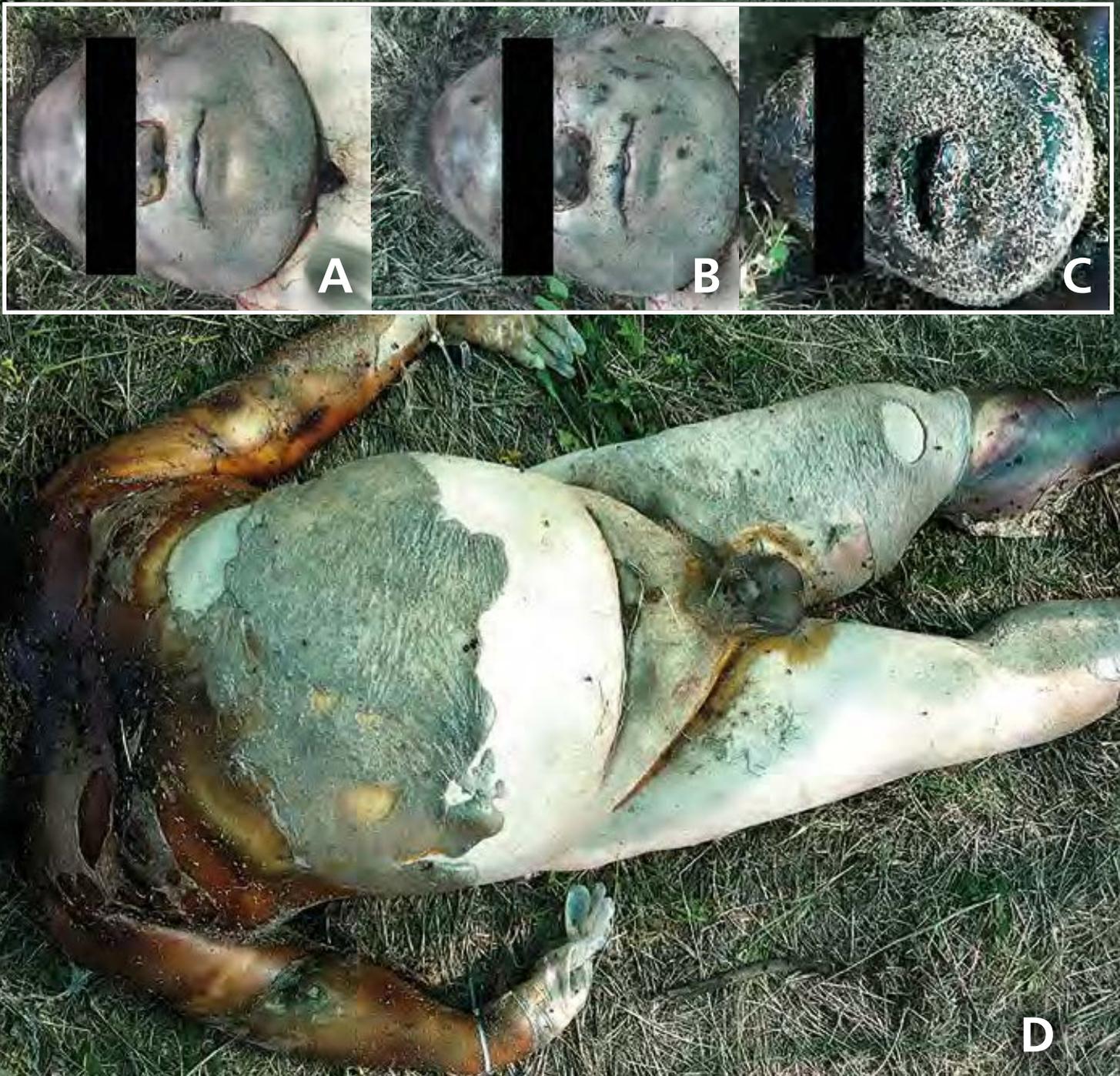


Figure Legend

The decomposition process of a cadaver can produce dramatic physical changes in a short period of time. For example, an individual placed in a field with an average temperature of 27°C progressed from: (A) fresh decomposition at 24 h, (B) to bloat with adult blow fly activity occurring at 48 h, (C) to active decomposition of the head with blow fly larval mass activity occurring at 108 h. However, decomposition is not a uniform process across all body parts: (D) the torso and extremities display marked differences in decomposition at 108 h.

(Courtesy of Dr Jen Pechal, Michigan State University, and the Forensic Anthropology Center at Texas State, San Marcos.)

Additionally, this application can be expanded from an individual to a much larger scale – the ecosystem in which the person is living. If the bacterial contents of flies or other arthropods utilizing remains of forensic interest can be linked to a geospatial and temporal event, then they can provide useful forensic evidence.

The microbiome is a complex Gordian Knot that is waiting to be unravelled. In some areas of the body, the transient and resident microflora may be too dynamic to be clear markers, whereas in other regions the microbiome may prove to be more static and unique (Li *et al.*, 2013). Only future studies will delineate this difference. What is clear is that the complexities defining the animal microbial ecosystem encounter a major bottleneck due to available data on a global scale. An international database founded on studies

FEATURES

designed with standardized sampling and analysis strategies is sorely needed to allow comparison across ecoregions and species. Such efforts have been initiated with the Earth Microbiome Project and the Human Microbiome Project, and efforts are now needed for an Animal Microbiome Project. Additionally, the possible dependence on rare taxa for differentiation requires that the number of studies/samples must be large to allow proper statistical separation and validation before the use of these samples in forensic case investigation. Such a database could assist in the epidemiological microbiology required for disease outbreaks, as well as forensic applications to wildlife trafficking and animal abuse cases.

The series of articles in this issue explore some of the more recent topics of research that have broad applications in areas including forensics.

- **So where did that *E. coli* in my hamburger come from?** In point-source investigations one must identify the microbial DNA of specific marker species or community profiles. These can show differential genetic diversity in the form of genotypes that are clustered in correlation with the geographic origin of the microbe. This is a type of forensic source tracking that can be used to determine the primary locations of microbial contamination. This is of most use in cases where contamination is causing disease or pollution important to public and animal health, as well as evidence in animal trafficking and abuse cases as discussed by Drs Foster, Pearson and Keim.
- **Ooh, what's that scum on my teeth?** Bacteria can exist in two states: as planktonic, free-floating, single cells or as sessile biofilm communities of multiple species encased in an extracellular matrix with a sophisticated communication system. In many environments such as the skin, biofilms are vital for survival and most bacteria exist in this communal arrangement. Dr Wood discusses the forensic aspects of bacteria living in the environment in a biofilm state.
- **Do bacteria have social media?** In a community, bacteria communicate through chemical messaging and quorum sensing. Some quorum sensing molecules, like indole, are known arthropod attractants. Interestingly this bacterial intracellular signalling molecule is also a precursor to tryptophan, which is used in the production of the neurotransmitter serotonin in other organisms. This makes one wonder about more direct effects of bacterial metabolic compounds on the behaviour of other organisms which encounter them. Janzen theorized that microbes manipulate the decomposition process to deter other necrophagous fauna in order to compete for utilization of the resource (Janzen, 1977). Drs Tomberlin and Crippen

discuss the interkingdom communications which occur between arthropods and bacteria during carrion decomposition.

- **Guess who's coming to decomp!** Sophisticated mechanisms of interkingdom communication have evolved over millennia. Both microbes and insects are interested in ascertaining the current occupants utilizing decomposing remains. Arthropods must locate these ephemeral resources and then decide if they represent an adequate environment to deposit and raise progeny (Tomberlin *et al.*, 2012). Microbes encounter challenges in getting to the resource, competing with other microbes and fauna that also want to exploit this rich nutrient supply and finally producing viable progeny. Drs Benbow and Pechal discuss the temporal and spatial aspects of the community of organisms associated with decomposing remains – the necrobiome.

FURTHER READING



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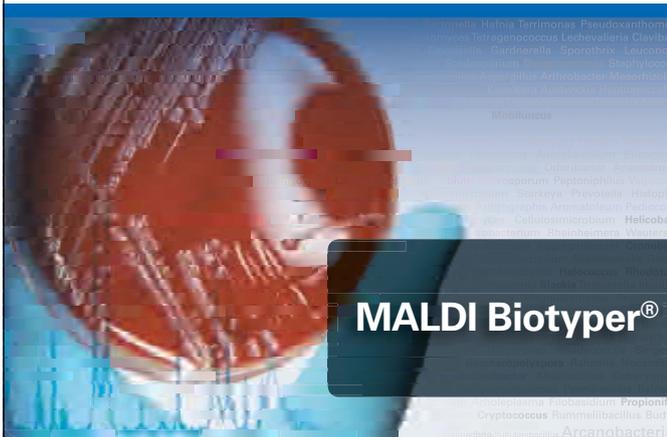
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GENOMIC MICROBIOLOGY

as applied to animal forensics

Figure 1. Tri-coloured bat (*Perimyotis subflavus*) covered in the fungus that causes white-nose syndrome. Genomic analyses are uncovering the origins and dispersal of the pathogenic fungus, *Pseudogymnoascus destructans*.
(Courtesy of Joe Hoyt.)

This approach has been applied to a fungal pathogen of bats that causes the disease white-nose syndrome

Stemming from research in a variety of fields, microbial forensics emerged as a discipline during the FBI assessment of the 2001 anthrax letter attacks (Budowle *et al.*, 2011). This prominent example of bioterrorism in the United States (US) occurred when spores of *Bacillus anthracis*, causative agent for anthrax, were mailed to various individuals in the media and Government. The result was a surge of microbial forensics research on pathogens that could potentially be used as bioterror agents, with a focus on analytical methods to determine the short-term, often epidemiological, and long-term evolutionary histories of these pathogens. Bacterial genomics arose as a key component of the investigation, as it was critical to determining which strain of *B. anthracis* was involved and how lineages that had been distributed to labs around the world were related to each other. Thus excluding the possibility of a natural outbreak and greatly narrowing the field of labs potentially containing this particular lineage.

The advancement of technology along with the increase in our understanding of bacterial population genetics and evolution opened the door for these methods to address other questions. In particular, advances in sequencing technologies enabled unprecedented discrimination among bacterial genomes by facilitating our ability to compare entire genomes and to assess the relationships among microbial samples. Many of the pathogens that could be involved in bioterrorist acts are zoonotic so, these advances can be applied to microbial forensics in wildlife and livestock, as well as human cases.

As a disease of livestock and other large ungulates, anthrax figures prominently in forensic microbiology in animals. For example, anthrax cases among heroin users in Europe were linked to drug contamination during transport, possibly in animal skins used along the trafficking route (Price *et al.*, 2012). Phylogenetic

analyses involving a worldwide collection, linked clinical samples to those obtained in Turkey. This suggested that, the heroin may not have been contaminated at its origin in Afghanistan, but that it may have passed through, and been contaminated, in Turkey when en route to Europe. In addition, no evidence for a bioterrorist event was identified (Price *et al.*, 2012).

A more comprehensive sample collection from this part of the world would increase confidence and geographic resolution to confirm these conclusions. This molecular epidemiological approach has also been used in other anthrax cases involving contaminated animal products such as hides and wool. Bacterial genomics also allows investigation into the more ancient spread of this disease, such as the introduction of *B. anthracis* into North America, via humans, and the large game animals they hunted, across the Bering land bridge (Kenefic *et al.*, 2009).

The genomic approach and analysis framework used in anthrax cases can be applied to the study of other wildlife and livestock pathogens such as plague, tularaemia, brucellosis and glanders. Microbial genomics has traditionally been expensive; research on human pathogens and/or bioterror agents has received the lion's share of funding. The decline in sequencing costs and more widespread availability have enabled these novel genomic epidemiological approaches to be applied to studying wildlife and livestock pathogens; often in diseases where there are significant ecological and/or economic impacts. Rosenblum *et al.* (2013) employed genomics to document the worldwide spread of the chytrid fungus that has been responsible for the global decimation of amphibians. At the Department of Molecular, Cellular, and Biomedical Sciences in the University of New Hampshire, this approach has been applied to a fungal pathogen of bats that causes the disease white-nose syndrome, where genomics has provided exceptional resolution for assessing likely



Figure 2 Plague is a bacterial disease carried by rodents. Genomic epidemiology on the bacterium *Yersinia pestis* can be used to track the spread of the pathogen. Above: collecting potentially infected fleas in Arizona from prairie dog burrows. (Courtesy of Katy Parise.) Below: Gunnison's prairie dogs from a colony in Arizona.

disease sources and transmission. Biek *et al.* (2012) used whole genomes to infer transmission patterns of bovine tuberculosis among badgers and cattle in the UK and Ireland. Genomic epidemiology has recently been applied to studying outbreaks of brucellosis in cattle, elk, and bison in the Greater Yellowstone Ecosystem in the western US, allowing understanding of the timing of pathogen introductions and spread. In each of these cases, genomic approaches allow large numbers of genomes to be compared against one another to assess pathogen dispersal, transmission and evolutionary history which provide critically needed context for microbial forensics.

A primary need for forensic investigations of pathogens is a comprehensive database of genomic data from isolates collected worldwide for each species of interest. While such a reference collection only rarely allows for precise source attribution – determining with certainty where an isolate originated – it does help focus the investigation and rule out other potential sources. The next step is to develop phylogenetic trees that detail the evolutionary relationships among strains. These relationships may be deep within the tree, showing more ancient connections, or may represent shallower branches indicative of more recent

A primary need for forensic investigations of pathogens is a comprehensive database of genomic data



Malicious disease outbreaks have been identified by the genomic linkage to laboratory strains

differentiation. The easiest material to obtain is from historical archival collections, which include laboratory reference strains. Malicious disease outbreaks have been identified by the genomic linkage to laboratory strains. For clonal bacteria, a category in which many of these zoonotic pathogens belong, mutations that are diagnostic for specific lineages can be identified and linked to features such as geography. As detailed in the publications cited above, phylogenetic data can be related to geographic data to develop insights into how these pathogens have been disseminated either by humans, livestock or wild animals.

The transition from using genomics for molecular epidemiology and determining evolutionary relationships, to microbial forensics is conceptually simple but can be difficult in practice. Standard veterinary and public health investigations lack many of the material and methodological controls that judiciary systems require. Crime scene and evidence control systems that law enforcement investigations use would not be common for a public or veterinary health investigation. Without these, prosecution of perpetrators could be compromised. It is unreasonable for public health efforts to take on these additional forensic burdens and the best solution is for rapid engagement of law enforcement when an outbreak appears suspicious. This will require coordination between Government agencies prior to the need for their cooperation. Wildlife, veterinary, public health and law enforcement officials need to work as cooperating units to identify and then investigate disease outbreaks that result from criminal acts.

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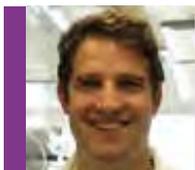
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The decomposition process is driven by bacteria

Almost in the fashion of the legendary phoenix, microbial life springs from decaying matter such as the carcass of an animal. The decomposition process is driven by bacteria, and these microbes not only spread across the carrion, but gather together forming biofilms. In both cases, cell signalling between bacteria plays an important role as does interkingdom signalling between bacteria and insects. By understanding the temporal events related to the decomposition of an animal resource, the time of death may be approximated.

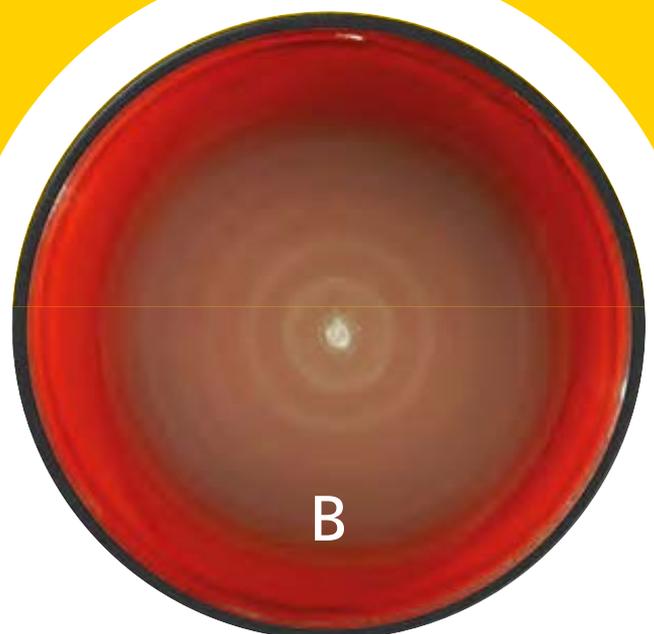
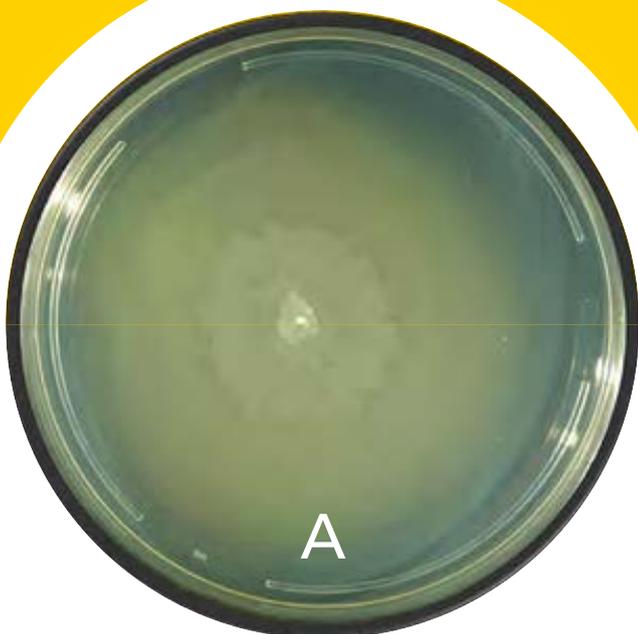
Cell signalling

Many bacteria communicate by constantly secreting small molecules, as the number of bacteria increases, the concentration of these compounds increases until a threshold concentration is reached. The signal is then internalized or activates a membrane receptor that leads to changes in gene expression and increased production of the original signal. This method of communication is known as quorum sensing (QS) and using QS, bacteria sense their numbers and begin to act in concert. This leads to a change from single cell

behaviour to group behaviour and the cells resemble primitive tissues. QS is particularly important for the expression of virulence traits for many pathogens, and it is important for the rapid movement across surfaces known as swarming. Other traits such as competence, conjugation, antibiotic production, sporulation and biofilm formation are also influenced by QS. Interestingly, often the ability of the bacterium to respond to environmental stress is also linked to the QS ability of the cells. In addition to bacterial cell signalling, bacteria also communicate with their surroundings across kingdoms using chemical cues, influencing animals such as insects during their utilization of an animal resource.

Swarming

One manifestation of group behaviour based on cell signalling is the spreading of bacteria over the animal resource via swarming; the cells move to take complete advantage of the resources presented by the decaying animal. In swarming, cells become hyper-flagellated and walk on the surface of the carrion in pulsed rounds of rapid movement that depend on bacterial signalling



Forensics through **BIOFILM** MICROBIOLOGY

(Figure 1). To facilitate swarming, cells frequently secrete glycolipid or lipopeptide biosurfactants as wetting agents to reduce surface tension. In this way, bacteria like *Proteus mirabilis* rapidly cover the surface of the decaying animal.

Biofilm formation and dispersal

Not all the cells choose to travel. As the bacteria spread, they also form biofilms, vibrant communities of cells (Figure 2) where cells are cemented together by protein and sugar polymers and even by DNA (a fine example of nucleotides serving a structural rather than a genetic role). By living in biofilms, cells are protected from changes in the environment and they can devour the decaying animal.

To commence biofilm formation, bacteria attach to surfaces via their appendages, such as fimbriae and flagella, and microcolonies begin to form as cells cement themselves together in what has been termed the biofilm matrix. For some strains, pioneering cells leave trails of matrix sugar polymers and DNA which attract other cells to the growing biofilm and create

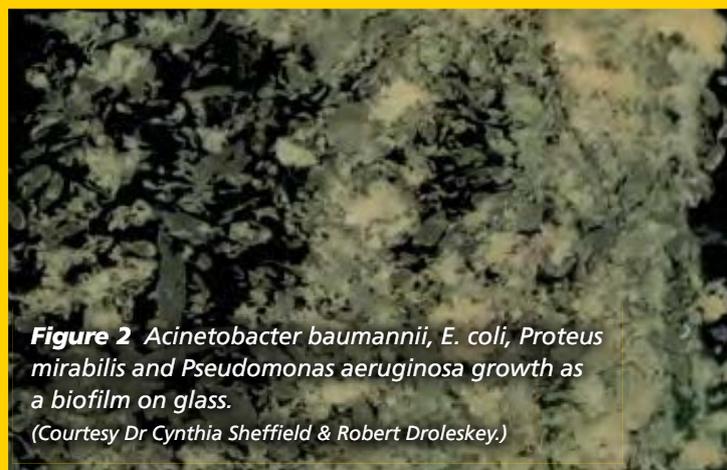


Figure 2 *Acinetobacter baumannii*, *E. coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa* growth as a biofilm on glass.
(Courtesy Dr Cynthia Sheffield & Robert Droleskey.)

connecting channels. Eventually, cells may build architectural marvels that allow nutrients to enter and waste to be removed.

The key internal switch that converts motile bacteria to sessile ones (and back to motile ones) is cyclic diguanylate (c-di-GMP): in all bacteria examined to date, biofilm formation increases as c-di-GMP levels increase. In an elegant regulatory balance, often involving many forms of each of these types of enzymes, cells use diguanylate cyclases to form c-di-GMP and phosphodiesterases to reduce c-di-GMP levels. Overall, c-di-GMP levels are first low to increase surface motility, high to form biofilms and reduce motility, then low again to allow cells to become motile again. In effect, a form of guanosine, the building block of RNA, is converted into an internal signal that is used by the cells to control biofilm formation (another example of cells using nucleotides in a role not directly related to genes).

Figure 1 The bacteria *Proteus mirabilis* swarming using peritrichous flagella on:

- A** tryptic soy agar (TSA),
 - B** TSA + 5% sheep's blood and
 - C** swarming impeded by growth on charcoal agar.
- (Courtesy Dr Tawni Crippen & Robert Droleskey.)



FEATURES

As with us, cells in biofilms often leave their homes, this behaviour is termed biofilm dispersal. Dispersal allows cells to leave in search of more nutrients as well as to colonize more locations when nutrients are plentiful. For example, the opportunistic pathogen *Pseudomonas aeruginosa* will disperse in response to both a sudden decrease and a sudden increase in nutrients. Dispersal requires cells to remove the cement that tethers them together and often to become motile. Internally, the signal for biofilm dispersal is a reduction in c-di-GMP levels brought on by the activation of phosphodiesterases.

Interkingdom signalling on carcasses

Cell signalling, swarming and biofilm formation/dispersal are all utilized to allow bacteria to recycle carrion. Some of the volatile signals of decay that are produced by the bacteria are perceived by animals such as blow flies. These signals are derived from the non-odorous proteins, fats and oils of the animal resource. Ammonia is the most common nitrogenous product and is formed as a secondary product of urea decomposition. Skatole, indole, mercaptans and sulfides make the most penetrating odours of putrefaction. Another large group of attractants are fatty acids, which are usually bacterial fermentation products and decomposition components.

A connection has been found between the signals used by bacteria for swarming over the animal resource and for attracting flies that help transport the bacteria to new resources; hence, signalling between bacterial cells and between bacteria and flies are interrelated. By using a bacterium, *Proteus mirabilis*, derived from the salivary glands of a blow fly, *Lucilla sericata*, transposon knockouts were created and the mutants were screened for reduced swarming. It was found that fly attractants such as putrescine, lactic acid, phenol, NaOH, KOH and ammonia also restore swarming for cells with the swarming mutations. In addition, cells deficient in swarming attracted fewer blow flies and reduced their egg laying. Hence, well-known fly attractants also facilitate bacterial swarming. Therefore, as the bacteria swarm they attract flies, with their volatile swarming signals, which then transport them to new resources, and they alert flies to lay eggs to help the decay process.

Indole plays a prominent role in interkingdom signalling between bacteria and insects that facilitate carrion decay. Indole is a QS compound for *E. coli* (this bacterium secretes mM quantities of this compound), and in the gastrointestinal tract, indole from *E. coli* both tightens the host epithelial cell junctions as well as

reduces the virulence of pathogens such as enterohaemorrhagic *E. coli* and *Ps. aeruginosa*. For insects, indole has been isolated from human skin as a bacterial metabolite that elicits a strong response by mosquitoes, it also attracts blow flies.

Biofilm forensics

Since bacteria initiate the decay of the animal resource and their volatile degradation products attract insects, the timing of death should be related to the extent of bacterial swarming, the extent of biofilm formation, and the extent and type of fly egg laying. Since different flies have different olfactory preferences, it should be possible to relate the timing of the death to the volatiles produced as well as to the type of insect that is attracted. Therefore, understanding the progression of carrion decay in terms of the degree of bacterial swarming, the volatile signals produced by the bacteria, and the extent and type of insect colonization should lead to more accurate predictions of the demise of the animal.

Acknowledgements

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Figure 1 Maggot blow fly, *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) (forefront), and secondary screwworm, *Cochliomyia macellaria* (Fabricius) (background), on carrion. (Courtesy of Chin Heo.)

FORENSIC MICROBIOLOGY

from an entomological perspective

Entomologists have long applied insect-based information in criminal investigations. Such evidence can be used to help estimate the time of death, if a deceased individual had consumed drugs, if abuse occurred prior to their death or the remains were moved from one location to another. In examining insect evidence associated with decomposing human or other animal remains, the forensic entomologist is determining the time of insect colonization to infer a potential time of death.

Historically, this was accomplished by estimating the time needed for insects to develop to the stage (e.g., larva, pupa or adult) at which they were found on the remains. But it turns out that this requires a broader understanding than previously anticipated of the relationship between insect community structure, arthropod development and the decomposition process. What was first thought to be a rather direct relationship, we now know to be much more complicated. A tremendous amount of research is currently being conducted in a variety of settings and

locations to explain the biological mechanisms regulating insect attraction, colonization and utilization of human remains. It turns out that microbes play vital roles in this process. For example, microbes serve as symbionts of fly larvae by producing antimicrobial agents that suppress pathogens on vertebrate carrion (Erdmann & Khalil, 1986); and – as mentioned in the previous article – through signalling methods such as quorum sensing to alert flies of the availability of quality resources for their offspring.

Time of colonization of human remains by insects, such as necrophagous flies (Figure 1), can vary. In some instances colonization can occur prior to, or immediately after death (Anderson & Huitson, 2004). While in other cases, colonization could be delayed by hours or even days (Goff, 1992). We know that there are a number of environmental conditions including rain, temperature and wind that can affect colonization (Byrd & Castner, 2010). We understand that many biotic factors, such as the classic vertebrate scavenger, the vulture (Figure 2), contribute to this colonization and

decomposition process, but are only now learning that some of the “less visible” biotic factors, e.g., microbes, more specifically bacteria, are key factors in the process.

The utilization of next-generation genetics has led to novel discoveries associated with microbial diversity, particularly bacteria, associated with decomposing vertebrate remains. For example, research from the Department of Entomology, Texas A&M University has determined the importance of bacterial succession on pig carrion – within the first five days of the animal's demise. It is estimated that in 62% of cases, the time since death can be explained by the bacterial communities that are present (Pechal *et al.*, 2013).

Microbes release many of the volatiles associated with the decomposition process, such as indole, dimethyl disulfide, cadaverine and putrescine, at the time of death or soon thereafter (Vass *et al.*, 2002). A number of insects have evolved highly sensitive olfaction systems that allow them to detect, locate and evaluate the remains as potential food for their offspring (Easton & Feir, 1991). Such sensitivity gives them survival advantages, as availability of carrion is unpredictable, usually present for only a short period of time (depending on the time of year and location) and highly competed for by other fauna. So if not located quickly, insects miss the opportunity to deposit their offspring in this rich environment.

Furthermore, research is demonstrating that volatiles from microbes could be playing a major role in regulating insect succession (shifts in insect community structure, e.g., the number of species present) on vertebrate carrion. More simply put, the odours emitted from microbes on decomposing remains are regulating which insects colonize human remains at different time points after death of the individual. Additionally, necrophagous flies competing for the same remains are attracted or repelled by volatiles released by microbes associated with other species of flies. So the temporal appearance of fly species and their possible deposition of bacterial species onto decomposing remains may ultimately influence the bacterial community structure present. This is significant because such an influence may also affect insect succession during decomposition.

An understanding of the microbial community structure during decomposition could enhance our ability to determine time of death. This information has led to new techniques for estimating the time of death of a person based simply on what insects are arriving at remains at given time points after death. Such approaches are revolutionizing the field of forensic entomology, as practitioners are no longer limited to those arthropods feeding on the remains, but can now utilize those insects attracted to the human body and the microbes they interact with to determine time of death. Determining if human remains have been moved from one location to another is also critical for forensic investigations and microbes could prove to be a critical tool for such purposes (pages 26-29).

Presently, we are witnessing the bridging of multiple disciplines within the forensic sciences to better understand how human remains decompose. Entomology and microbiology serve as a model of how such collaborations result in advancements, and applications, vital to solving crime globally; however,

Figure 2 Black vulture, *Coragyps atratus*, scavenging a white-tailed deer, *Odocoileus virginianus*, carcass. (Courtesy of Dr Tawni Crippen.)



The utilization of next-generation genetics has led to novel discoveries associated with microbial diversity

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many challenges still remain partially due to the breakneck speed at which high-throughput technologies are improving. In particular, the validity of these techniques for determining forensically relevant information, database limitations (e.g., many microbial species have yet to be classified), experts able to bridge computer science, microbiology and entomology within an ecological and forensic context, and most importantly Government or private support for continued research. With continued support from granting agencies throughout the world, such limitations can be addressed resulting in scientifically validated techniques and associated analysis of forensic evidence for use in a court of law.

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Blow fly (Diptera: Calliphoridae) larvae feeding on decomposing vertebrate animal.

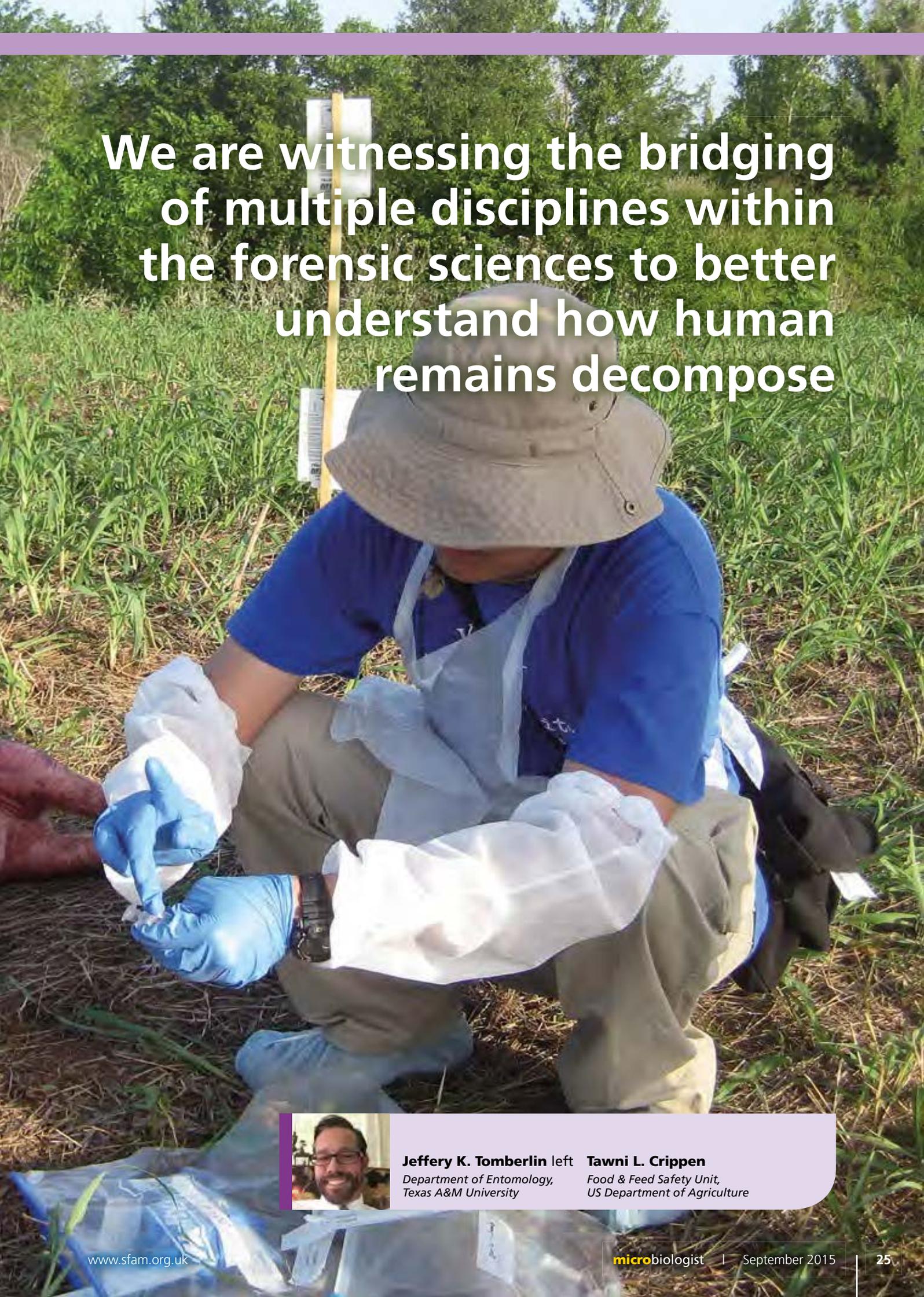
(Courtesy of Chin Heo.)



Entomologist collecting insect evidence from decomposing swine carcass, *Sus scrofa*.

(Courtesy of Chin Heo.)

We are witnessing the bridging of multiple disciplines within the forensic sciences to better understand how human remains decompose



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MICROBIAL INTERACTIONS OF THE NECROBIOME

Basic research and forensic applications

Over the last several years there has been an increasing recognition of the importance of establishing a strong basic science foundation in forensics. With the development, reliability and increasingly widespread use of high-throughput metagenomic sequencing techniques, the forensic sciences are poised to harness ‘-omics’ technology to bridge basic science with medicolegal applications and applied microbiology. Previous studies have demonstrated the ability to detect and identify unique host-associated microbial communities of living humans, and more recently the potential use for these human-associated microbial communities in forensics, e.g., tracing a keyboard to its user via hand microbial communities (Fierer *et al.*, 2010). These communities have been described as the post-mortem microbial communities, the post-mortem microbiome and the carrion microbiome, along with several other terms and descriptions. However, for this article, we use the human post-mortem microbiome (HPMM) to describe this community and argue that the

characterization and assessment of intra-individual variation of the HPMM remains understudied.

An increased understanding of the HPMM and microbiomes of other organisms associated with cadavers (e.g., insects) could lead to identification of novel biological markers useful in forensics. Trace evidence is typically physical in nature, such as fibres, hairs or pollen, that link a perpetrator to a victim or crime. However, as technology continues to advance and the computation power increases to efficiently analyse large data sets (e.g., high-throughput metagenomics), scientists and practitioners within applied microbiology will be positioned to explore alternative and underexplored research avenues linking widely distributed microorganisms (e.g., bacteria, fungi, protists and algae) to human cadavers or other objects at a death scene investigation. To do so, a more comprehensive understanding of the ecological factors that affect these linkages is necessary.



Figure 1 Two components of the necrobiome: necrophagous insect larvae (maggots) and microbial communities of the cadaver and soil beneath the carrion resource. Complex interactions between insects and microbes are hypothesized to occur during this process of decomposition.

To better appreciate the biotic interactions of microbial taxa during vertebrate carrion decomposition, it is necessary to place this community within the larger network of cadaver-associated organisms (e.g., invertebrates and vertebrate scavengers) – the network of species that has been defined as the necrobiome (Figure 1). The necrobiome is composed of the community of organisms associated with decomposing animals that includes members of all three domains of life (Bacteria, Archaea and Eukarya). The primary eukaryotes of the necrobiome are often considered the insects, particularly blow flies (Diptera: Calliphoridae); these flies thrive in decomposing vertebrate remains,

organic materials (e.g., garbage) and vertebrate wastes (e.g., manure). Blow fly adults are primary colonizers of decomposing vertebrate remains, and are commonly the first forensically important insect taxa to arrive at human remains in response to odours given off by the metabolism of microbial communities associated with the carcass. The microbial members of the necrobiome are considered the epinecrotic microbial communities. These microorganisms are associated with carrion, including human cadavers, throughout the decomposition process, and research into these communities has seen an increase in studies and publications over the past several years.

The necrobiome is composed of the **community of organisms** associated with decomposing animals

FEATURES

As an example of this research, the Department of Entomology at Michigan State University has determined there are certain microbial taxa characterized from the internal microbiome of two cohorts of adult blow flies collected at two time points (blue = initial day; green = 10 days later) and shared taxa that were similar to some taxa of the epinecrotic communities of a decomposing carcass (salmon carcass; red). These microbiome associations are visualized in a co-occurrence network (Figure 2). The nodes (black boxes) represent the individual samples from the blow flies or the carcass with the edges (coloured lines) connecting to the identified operational taxonomic units (OTUs; grey circles). The variability of microbial communities is apparent amongst each node but there are community similarities based on adult cohort and the carrion resource. Thus, there is evidence that the microbiome of necrophagous blow flies and that of the carcass (or corpse) potentially interact during the decomposition process.

An increase in the basic knowledge of interactions amongst microbial communities during decomposition is proving to be important for developing hypothesis-driven questions that could be applied in future forensic work and microbiology. One of the more provocative and applicable questions related to epinecrotic communities is: how do these complex assemblages of microorganisms interact with primary consumers, such as the necrophagous insects commonly used in forensic investigations (Figure 1), of a carcass or human corpse?

The interactions between microbes and necrophagous insects can begin within hours after death when the first insects are attracted to, evaluate and consider colonizing a cadaver (Figure 3); this period of decomposition time with extensive interactions occurring is also where both insect and microbial evidence can be easily collected. During this time, epinecrotic communities are hypothesized to be altering the quality of the resource and thus mediating insect

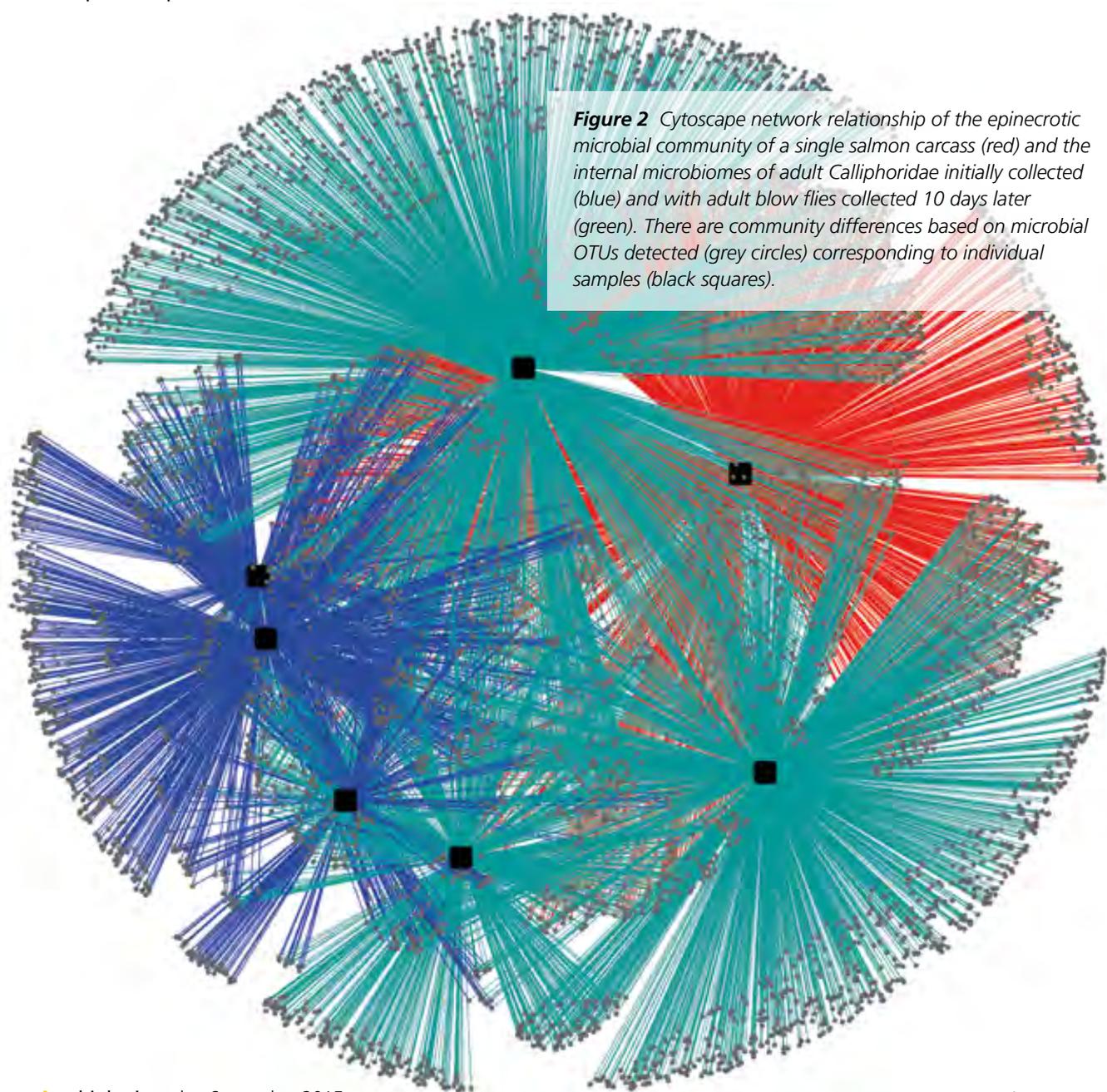


Figure 2 Cytoscape network relationship of the epinecrotic microbial community of a single salmon carcass (red) and the internal microbiomes of adult *Calliphoridae* initially collected (blue) and with adult blow flies collected 10 days later (green). There are community differences based on microbial OTUs detected (grey circles) corresponding to individual samples (black squares).

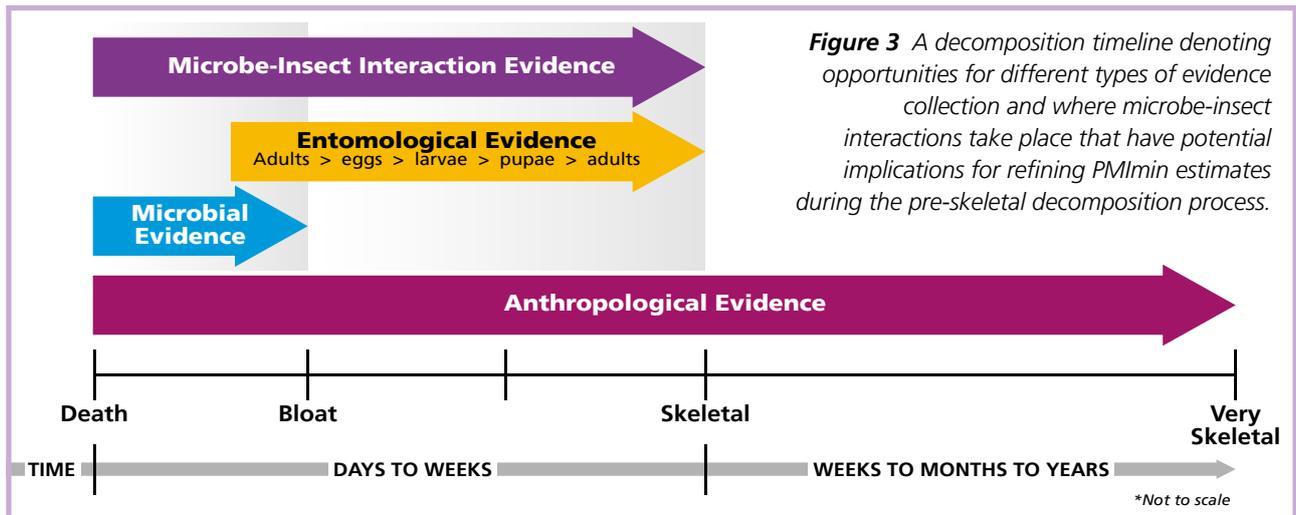


Figure 3 A decomposition timeline denoting opportunities for different types of evidence collection and where microbe-insect interactions take place that have potential implications for refining PMImin estimates during the pre-skeletal decomposition process.

community colonization. Support for this hypothesis has been demonstrated in studies where the production of metabolically derived volatile organic compound signatures from microbes were shown to influence necrophagous fly behaviour (Tomberlin *et al.*, 2011; Tomberlin *et al.*, 2012). A better understanding of mechanisms controlling community assembly patterns that influence carrion/corpse necrobiome diversity and interactions is key since decomposition is a consumer-driven process, and changes in the organisms utilizing the remains ultimately dictates the decomposition process and rates. These mechanisms can influence how potential microbial and entomological evidence could be used in legal investigations. One common way that biological evidence is used in forensics is for estimating the minimum range of time between death and the discovery of human or animal remains, or the minimum post-mortem interval (PMImin).

Estimating a PMImin range with strong statistical inference continues to be a challenge for forensic scientists. In 2009, the US National Research Council (NRC) called for a need to provide quantitative error rates in inferences derived from most forensic evidence. Many studies concerning community assembly for either the insect or microbial taxa do not employ validations to define error rates. Consequently, regardless of their admittance into a court of law, these techniques fail to meet the Daubert guidelines which govern the admissibility of evidence into the courtroom in the USA (Saks & Faigman, 2008) or the defined parameters outlined by the NRC. There is a timely need for increased hypothesis-driven forensic microbiological studies that can facilitate defining and refining error rates related to PMImin estimates and other ways that

microbes can be potentially used in forensics. However, as it has been shown in other emerging disciplines, a strong foundation in the basic biology and ecology of a system is necessary for identifying applications for improving the human condition. The necrobiome and interactions of microbes and insects on decomposing humans is no exception within the broader field of microbiology.

FURTHER READING

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M. Eric Benbow left
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Department of Osteopathic Medical Specialties
Michigan State University

Jennifer L. Pechal right
Department of Entomology
Michigan State University

BIOFocus

We are in an exciting phase for the organization as we begin the transition to becoming the Royal Society of Biology. Granted by the Sovereign, acting on the advice of Ministers, this is a greatly welcomed recognition for the discipline of biology and the contribution of biologists. This change recognizes the collective efforts of many individual Members and Member Organizations to reflect and communicate priorities for the bioscience community through the Society, and provides clear acknowledgement of this collective impact.

We were able to announce this news at a recent Society event, where Sir David Attenborough Hon. FRSB captivated an audience of nearly 500 guests at the Science Museum in London. In conversation with TV paleopathologist Professor Alice Roberts FRSB, Sir David spoke of the importance of natural history film-making in connecting people to the plight of the world's biodiversity and climate. The event raised nearly £20000 to support our charitable aims.

"Over half the world's population is urbanized and the thought that some children may grow up not looking at a pond or knowing how plants grow is a terrible thing," he told guests at the event.



At the heart of the life sciences



Sir David Attenborough Hon. FRSB in conversation with Professor Alice Roberts FRSB

We are in an exciting phase for the organization as we begin the transition to becoming the Royal Society of Biology

Sir David described how science broadcasting had changed since his career at the BBC began in the 1950s, when television was in its infancy. He said that natural history film-makers benefited vastly from collaborating with scientists, improving the medium to the point where it had, on occasion, been able to inform biology itself.

After a wide-ranging interview including questions from the audience, Sir David spoke of the urgency of various environmental concerns, especially ocean acidification.

“The thing that worries me almost more than anything is what is happening to the oceans. It is on the way to cause major, major problems of depopulation of fish stocks – and if you even just take the selfish attitude of what that does to human beings, it robs a vast number of people of their livelihoods and food.”

But Sir David did say that broadcasting had helped foster greater public understanding of such issues.

Alongside public understanding, comes political understanding, and another recent event worth highlighting is this year's Parliamentary Links Day, an annual event that we organize on behalf of the science and engineering community, aiming to strengthen dialogue between scientists and politicians.

The newly appointed Minister of State for Universities and Science, Jo Johnson MP, addressed over 200 MPs and representatives from the science community. In one of his first appearances since being appointed to the role in May, Johnson reiterated the Government's commitment to investing £6.9bn in science infrastructure capital by 2020/21. Mr Johnson set out three 'themes' that he highlighted as priorities for his department – the acceleration of collaboration between universities and businesses, 'making the most' of the UK's scientific expertise and output, and ensuring the UK nurtures the best scientific talent in the world and continues to inspire others into science and engineering careers.



Jo Johnson MP
Minister of State
for Universities
and Science

Also at the event shadow minister, Rt Hon. Liam Byrne MP, called for cross party consensus on the need to spend 3% of GDP on science, a level of funding achieved by countries including Germany and Korea. He said that ring-fencing the science budget was 'helpful but meaningless political jargon' and that in real terms funding for science in the UK was falling.

At the Society of Biology we're looking forward to working with colleagues and politicians to try to ensure that future funding does not place the UK at a disadvantage in the international arena.



Mark Downs FSB
Chief Executive, Society of Biology

ECCMID 2015

The Society for Applied Microbiology attended the 25th European Congress of Clinical Microbiology and Infectious Disease (ECCMID) in Copenhagen, Denmark, on 25–28 April. This congress is the largest of its kind, bringing together leading microbial experts, companies and scientists from all over the world.

Organized by the European Society of Clinical Microbiology and Infectious Disease (ESCMID), the event ran for four days and welcomed over 10,697 attendees from 112 countries. Over 200 workshops, sessions and lectures were delivered, all geared towards participants with different levels of experience and individual learning requirements.

Fighting Antimicrobial Resistance

A topic that generated incredible in-depth discussions and one that remains at the forefront of SfAM's agenda is antimicrobial resistance (AMR).

Over the last two decades, global communities have gradually acknowledged the importance of tackling this issue – for example, the World Health Organization (WHO) has recently defined AMR as a “major global threat” to public health. As a result of changing political agendas, university discoveries and national news, interest in finding solutions to this problem has increased dramatically.

Clinicians and other specialists attending ECCMID agree there is a general consensus that it is not the world of big pharma that will provide all the answers to AMR.

SfAM will hope to discuss many of the issues raised in the discussions at a specialist meeting on AMR, to be held at the Royal Society of Medicine, 1 Wimpole Street, London, on 7 December 2015. The meeting will bring together researchers and experts involved in AMR prevention and control. The meeting will host speakers specializing in AMR from human and animal health perspectives, covering AMR as a threat to global public health.

Tackling Ebola

In addition to fighting AMR, the prominence of Ebola in the news over the past few years led to increasing discussions on how to best tackle such infectious diseases. Médecins Sans Frontières (MSF) were honoured with the special excellence award in recognition of

their recent work as well as decades of fighting infectious diseases.

Sylvie Briand from the WHO who presented the seminar “**Ebola Outbreak Response: Shift in Paradigm**” – emphasized the importance of scientists closely monitoring the disease outbreak and symptoms.

Showing that efforts to contain the outbreak had so far proved successful Marta Lada, from Connaught Hospital, reported that in Sierra Leone, two new cases are currently being detected per day compared to the 60 per week, which were occurring in October last year.

Pipeline Corner

The pipeline corner session, new for ECCMID in 2015, allowed SMEs to showcase their products in early development with new mechanisms and approaches. The 12 companies presenting at pipeline corner discussed novel and innovative approaches of fighting bacterial diseases by focusing on tackling the most resistant bacteria.

These included a wide variety of approaches ranging from chemically synthesized and natural products to tackling specific resistance mechanisms. One interesting recurrent theme was the search for very narrow spectrum and pathogen-specific antibiotics.

In addition to the pipeline corner session, over 200 sessions were delivered at ECCMID including keynote lectures, symposia, oral sessions, educational workshops, meet-the-experts sessions and around 2,500 poster presentations. More than 3,000 abstracts were presented. One of the key themes to emerge from the ECCMID conference is the importance of innovation and new discoveries.

Lucy Harper, SfAM Chief Executive added:

“The ECCMID conference is an extremely productive networking opportunity for SfAM to attend and the scientific excellence presented in the talks and workshops was inspiring. We will certainly be attending the next event in Istanbul, 2016.”

Paul Sainsbury

MEMBERSHIP Benefits & Options

Benefits

The Society for Applied Microbiology is the voice of applied microbiology within the UK and was founded in 1931. Society Members play a leading role in shaping the future of applied microbiology, and enjoy many benefits, including:

- The opportunity to apply for one of our many grants or funds.
- Access to our five peer-reviewed journals: *Journal of Applied Microbiology* (JAM), *Letters in Applied Microbiology* (LAM), *Environmental Microbiology*, *Environmental Microbiology Reports* and *Microbial Biotechnology*.
- Free access to the entire collection of digitized back files for JAM and LAM dating back to 1938.
- A topical quarterly magazine, *Microbiologist*.
- Substantially reduced rates for attendance at SfAM meetings and conferences.
- Networking with worldwide professionals in over 80 countries
- Access to private Members' area of the SfAM website.
- Monthly email bulletins with the latest news from SfAM.
- Invitation to the annual *Environmental Microbiology* and *Journal of Applied Microbiology* lectures.
- Fostering cross disciplinary research.
- A 35% discount on the extensive Wiley-Blackwell collection of titles.

Detailed information about all these benefits and more can be found on the Society website at: www.sfam.org.uk/membership.

GRANTS & AWARDS

Many grants, awards and prizes are available to Members including the W H Pierce Memorial Prize and prizes for student oral presentations and posters at the Summer Conference. In addition to these substantial awards, the Society has funds to assist Members in their careers as microbiologists. These include the President's Fund, Conference Studentships, Sponsored Lecture Grants and the popular Students into Work Scheme.

Full details of all the Society's grants and awards, together with application forms, can be found on the website at www.sfam.org.uk/grants.

JOURNALS

The Society publishes two monthly journals: *Journal of Applied Microbiology* and *Letters in Applied Microbiology*. We also produce this quarterly colour magazine, *Microbiologist*, which contains features, topical news stories and full details of our meetings. The Society is also a partner with Wiley-Blackwell in the monthly journals: *Environmental Microbiology*, *Environmental Microbiology Reports* and *Microbial Biotechnology*. See more at www.sfam.org.uk/journals.

All Full and Student Members receive free access to the online versions of the Society's journals, and can also submit papers to our journals via an online submission service.

MEETINGS

We hold three annual meetings: the Winter Meeting is a one-day meeting with parallel sessions on topical subjects; the Spring Meeting is a one-day meeting tailored for personnel in clinical microbiology; and the Summer Conference is held every June/July and comprises a main symposium, a poster session, the AGM and a lively social programme. All Members are invited to our prestigious annual lectures held to commemorate the success of two of our journals: *Environmental Microbiology* and the *Journal of Applied Microbiology*. We also hold *ad hoc* meetings on topical subjects and enter into joint ventures with other organizations on topics of mutual interest.

WEBSITE

www.sfam.org.uk is the best source of detailed information on the Society and its many activities. It has a fully interactive Members-only area (www.sfam.org.uk/membersonly) where you can find archive issues of *Microbiologist*, exclusive SfAM documentation and much more.

Membership OPTIONS

- > **Full Ordinary** gives access to our many grants and awards, online access to the *Journal of Applied Microbiology*, *Letters in Applied Microbiology*, *Environmental Microbiology*, *Environmental Microbiology Reports* and *Microbial Biotechnology*, copies of *Microbiologist*, preferential registration rates at Society meetings, and access to the Members-only area of the website.
- > **Full Student** confers the same benefits as Full Membership at a specially reduced rate for full-time students not in receipt of a taxable salary.
- > **Associate** is only open to those with an interest in applied microbiology without it being a prime aspect of their job. For example, school teachers and those taking a career break, on maternity leave, or working temporarily in other areas. It does not provide access to any journals or Society grants and awards.
- > **Honorary** membership of the Society is by election only and this honour is conferred on persons of distinction in the field of applied microbiology. Honorary Members have access to our online journals.
- > **Retired** is available to Full Members once they have retired from their employment. Retired Members are entitled to all the benefits of Full Membership except grants and access to the Society's journals.
- > **eAffiliate:** this category of membership is open to microbiologists residing in Band I developing countries and is free of charge. It is an online only membership and provides access to the eAffiliate bursary only.
- > **eStudent:** this category of membership is open to undergraduate students only. It is an online only membership and is free of charge. This category of membership does not provide access to the Society's grants or journals.
- > **Corporate** is open to all companies with an interest in microbiology. Corporate Members benefits include:

 - Quarter page advertisement in each issue of *Microbiologist* (which can be upgraded to a larger size at discounted rates).
 - The opportunity to publish press releases, company news, etc., in each issue of *Microbiologist*.
 - FREE banner advert on the Society website with a direct link to your company site.
 - Up to three Members of company staff attending Society meetings at Members' rate (this means a 50% discount on non-Member registration rate).

Join us!

You can apply for membership online (www.sfam.org.uk/join) or offline. To apply offline, please contact the Membership & Finance Co-ordinator, Julie Wright on +44 (0)1234 326846, or email julie@sfam.org.uk.

Membership CHANGES

We would like to warmly **welcome** the following new Members to the Society.

BELGIUM

J. Steensels
S. Wuyts

CANADA

S. Chaganti

CYPRUS

D. Evripidou

DENMARK

M. Z. Islam

DUBAI

A. M. Kader

ESTONIA

J. Stsepetova

FINLAND

P. I. Kuusela

FRANCE

M. Chevalier
S. Lortal

GHANA

A. Parry-Hanson Kunadu

GREECE

T. Papadopoulou

INDIA

A. K. Prajapati

IRAN

L. Mokhtarejad

IRELAND

C. Campion
S. De Paor
G. Halley
E. Joyce
T. Russell
H. Tanner

ITALY

A. Levante
A. Saldan

IVORY COAST

L. Ban Koffi

KENYA

G. Nattoh Indinda

LIBERIA

B. I. Shobayo

MEXICO

F. Estrada Velazquez
S. Garcia Cerna
J. Garcia Maldonado
M. J. Granados-Baeza
E. A. San Juan Juarez

NIGERIA

T. Adebote
S. A. Ajani
J. A. Amao
V. N. Anakwenze
C. G. Anaukwu
H. Audu Jimoh
A. Ayilara
K. Banwo
S. K. Dike
C. R. Falegan
C. D. Iwu
C. M. Obiekwe
D. A. Odabo
N. J. Odimba
S. A. Odunfa
C. M. Ogbukagu
O. E. Olakanmi
A. Olanbiwoninu
O. E. Olasinde
R. Onu
C. H. Ozonyiri
L. Salam
G. A. Ugwuanya
C. Uwabor

PUERTO RICO

Y. Bernier-Casillas
M. De La Rosa
J. Liquez y Gonzalez
J. Manero-Perea
Y. Rivera-Cuevas

SAUDI ARABIA

A. M. Ali

TURKEY

M. Karamese

UGANDA

C. Kamugisha

UK

A. Abdulameer
D. Al Harbi
Z. Alkudmani
C. Anyanwu
D. Apostolou
C. Arenas
C. Bell
N. Bench
E. Brookes
P. Carillo-Barragan
S. Challa
A. Coffey
J. Collier
H. Cruickshank
A. Dabrowska
M. Dale
C. Danquah
R. Dawson
S. Despoina
C. Earl
M. Enright
R. Gangappa
A. Ghareeb
N. Gizzie

L. Grist

A. Hameed
B. Hammed
K. L. Hillitt
S. Hiscott
M. Hodgkinson
L. Hoyles
N. Ikpe
J. Iremonger
R. Janes
T. Jenkins
F. Jorgensen
T. Jousmi Tagne
A. Krishnankutty
C. Lapworth
M. Lee
R. Lo
C. Marshall
N. Mejia
S. Micklewright
C. Moore
W. Mullen
J. L. Murray
D. O'Connor

O. Osayagbon

O. Oyeniji
S. Purton
J. Raven
C. Richards
A. Riseley
S. Scott
N. Sejjic
E. Siegwart
P. R. Vitola
M. Watts
M. Welch
P. Wigley
W. L. Wong

UAE

S. Muralidharan

USA

J. Corley
R. Crowther
K. Duncan
M. Ereemeeva
S. H. Geiger

C. Hoang

R. Horne
H. Jackson
A. Johnson
S. Joseph
J. McIntosh
S. O'Leary
W. Pecher
B. Potter
C. Sinigalliano
A. Srivastava
M. Sullivan
K. Turner

CORPORATE

Luminex B.V.

DEATH

We were saddened to learn of the death of the following Member of the Society:

G. Macfarlane

OBITUARY

George Macfarlane

George (on the right side of the picture) did his PhD in Dundee on nitrate reduction by bacteria in sediments. This meant wading out at low tide in Kingoodie Bay to get his samples – not a pleasant task. His interest in anaerobic microbial ecology stemmed from that.

He moved to an MRC unit in Cambridge in the early 1980s where he became a leader in gut microbiology, an extremely popular research area today. He was one of the first microbiologists to use mixed microbial inocula to understand interactions (realizing that bacteria mainly grow in pure culture when on plates or in broths). It is ironic that today the gut microbiome is often cited to be an emerging discipline. George's massive contribution over almost 35 years disproves that! He designed continuous culture models of the gut, validated them, and used them alongside clinical studies over several decades of pioneering research into gut microbiology, including carbohydrate metabolism, proteolysis, pro/prebiotics, *Clostridium septicum*, biofilms in the gut, immunology, ulcerative colitis, Barrett's oesophagus and the effect of ageing on the gut microbiota (and its mitigation).

George paved the way for gut microbiology as an important research discipline that can actually help people. He was an innovative thinker, always ready for a challenge. He published hundreds of papers and is one of the most cited authors in UK science. We have lost an international leader who had so much more to contribute.

George had very high professional standards and high expectations of himself and his colleagues but he was also the most kind and generous person you could ever wish to meet. He was always willing to help his colleagues and was particularly keen on supporting his graduate students. George had an excellent sense of humour and made science fun as well as educational.

George was always a strong supporter of SfAM and served on the Executive Committee. George was also one of the longest serving Senior Editors for SfAM journals, JAB/JAM/LAM.

George's untimely passing is a shock to us all. He will be badly missed by his friends, his colleagues and of course, his family. He inspired many and the scientific legacy he has left is substantial.

Glenn Gibson and Andrew McBain



PRESIDENT'S FUND

Protein-RNA toxin-antitoxin systems in bacterial resistance to phage

Bacteria and their viral parasites, the bacteriophage, have been engaged for millennia in their co-evolutionary battle of resistance and counter-resistance. Bacteriophages are the world's most abundant biological entities, and as such place an immense selective pressure on their hosts – phages outnumber bacteria by 10:1, and are estimated to kill ~30% of the standing stock of bacteria each day in some environments (Suttle, 2007). Unsurprisingly, multiple anti-phage defence systems have evolved over the course of this ancient predator-prey relationship.

Bacterial phage resistance systems include the prevention of phage attachment by masking or altering surface receptors, inhibition of phage genome injection and cleavage of incoming nucleic acids by restriction-modification or CRISPR-Cas systems (Labrie *et al.*, 2010). With these defence systems bacteria can hide from, or fight off, their phage parasites. However, another general mechanism exists where infected cells do not directly interfere with any action by the incoming phage, but instead commit "altruistic suicide" before the phage can replicate, thereby protecting the clonal population. This process, known as abortive infection, is highly effective, but also risky – cells must carry the means of suicide at all times, to be deployed rapidly in response to infecting phage.

My work focuses on one particular abortive infection system, ToxIN_{Pa} of the plant pathogen *Pectobacterium atrosepticum* (Fineran *et al.*, 2009). ToxIN_{Pa} comprises a toxic ribonuclease protein (ToxN_{Pa}) coupled with an RNA inhibitor (ToxI_{Pa}). ToxN_{Pa} causes cell death when

overexpressed; coexpression of ToxI_{Pa} rescues the cell. ToxIN_{Pa} was the first described member of the prevalent Type III toxin-antitoxin systems, where an RNA antitoxin acts directly on the toxin protein to mitigate its harmful effects. The presence of ToxIN_{Pa} in a cell prevents phage replication without changing the survival outcome for infected cells, confirming that the system acts through abortive infection. The model is that phage infection causes ToxI_{Pa} to be released or degraded, resulting in the release of ToxN_{Pa} and the premature death of the infected cell, along with the attacking phage (Figure 1).

ToxI_{Pa} is a small RNA that directly inhibits an enzyme, ToxN_{Pa} – an extremely rare activity for a naturally occurring RNA molecule. The crystal structure of the ToxIN_{Pa} complex revealed a great deal about its mechanism (Blower *et al.*, 2011). Three ToxI_{Pa} pseudoknots, which are themselves generated by ToxN_{Pa} cleaving the repetitive ToxI_{Pa} precursor, bind three ToxN_{Pa} proteins in a striking triangular assembly, in which the active site of ToxN_{Pa} is shielded. This structure raised more questions: how does ToxN_{Pa} specifically recognize ToxI_{Pa} and assemble the triangular complex? And how is this complex activated to produce a lethal, altruistic response to incoming phage?

The activity of the antitoxin ToxI_{Pa} was explored through *in vitro* inhibition and complex assembly experiments which showed that, unexpectedly, inhibition by ToxI_{Pa} is completely self-contained, requiring no cellular factors or energy input. The ToxIN_{Pa} complex can also self-assemble *in vitro* from ToxN_{Pa} mixed with processed or unprocessed forms of ToxI_{Pa}. The ToxI_{Pa} RNA therefore

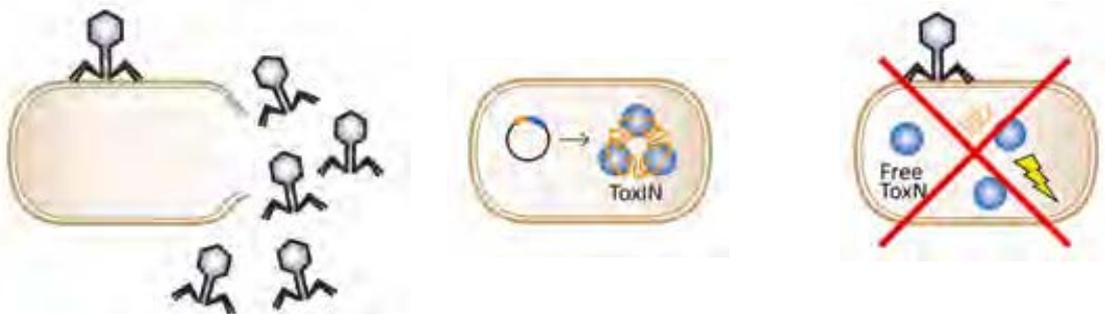
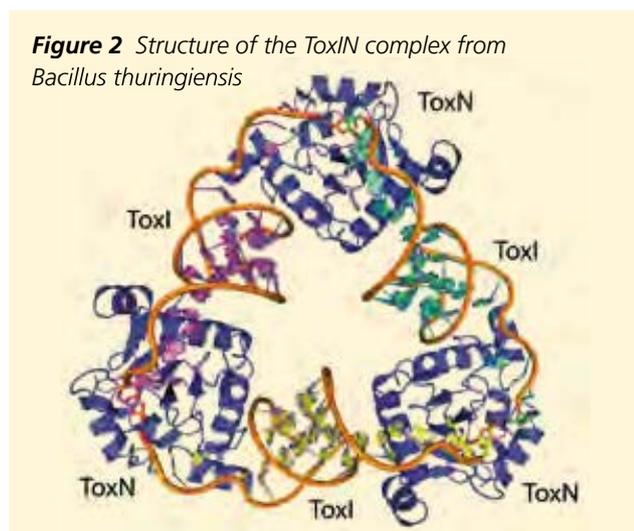


Figure 1
Model for abortive infection by ToxIN

inhibits its toxin partner by driving assembly of the trimeric ToxIN_{Pa} complex – a process dictated entirely by the RNA and its interactions with ToxN_{Pa} (Short *et al.*, 2013).

In addition to being self-contained, inhibition by ToxI_{Pa} is selective. This was shown through cross-talk experiments with ToxIN_{Pa} and the components of a closely related system, ToxIN_{Bt} of *Bacillus thuringiensis*. Both ToxI antitoxins could inhibit their own, but not their counterpart's, ToxN toxin *in vivo*. This specificity arises through very subtle structural variations in the ToxI and ToxN molecules, revealed by the crystal structure of ToxIN_{Bt} (Figure 2). Overall, the structure of the *Bacillus* ToxIN_{Bt} complex is similar to that of ToxIN_{Pa}, forming a triangular assembly of 3ToxI:3ToxN. However, subtle differences in both the ToxI and ToxN components result in considerable changes to the protein-RNA interfaces which maintain the inactive complex. This second structure shows how antitoxin recognition can retain extraordinary specificity, within a shared Type III structural framework.



These experiments revealed the entirely self-contained nature of ToxI RNA antitoxins and the specificity with which they target their cognate toxins. Still unknown is what triggers release of the toxin in response to incoming phage. Work in our group and others has explored this issue by isolating phage “escape” mutants – phage that spontaneously become insensitive to the resistance activity of ToxIN_{Pa}. One bacteriophage, ΦTE, can replicate in *toxIN*_{Pa}-containing cells by expressing its own copy of the ToxI_{Pa} antitoxin (Blower *et al.*, 2012). This phage can also transduce *toxIN*_{Pa} between cells, thus promoting its survival by maintaining its own specialized host population in which many other phage cannot replicate. Studies of escape phages of a homologous system, *antiQ-abiQ*, have shown that diverse mutations can enable phage that would normally be aborted to survive, although their precise mechanism is unknown (Samson *et al.*, 2013). Further work will be needed to determine the molecular events

during phage infection that release ToxN from its inactive, ToxI-associated state to the free protein that kills its own host cell.

In summary, our work has shown how a small, processed RNA can act as an extremely specific and strong inhibitor of a toxic enzyme, through assembling a trimeric protein-RNA complex. The mechanism behind the reverse activity – the release of the toxin in response to incoming phage – remains to be elucidated in detail. A grant from the SfAM President’s Fund enabled me to attend the 2nd International Meeting on Regulating with RNA in Bacteria in Germany this year, where I presented some of this work.

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Francesca Short
University of Dundee

SfAM Spring Meeting 2015

Morning session

This year's *Spring Meeting* was held at the Sheffield Hilton Hotel on 16 April. It focused on 'Hot Topics in Mycology and Mycobacteria' and 'Case Studies'. The meeting introduced us to new developments in antimicrobial resistance (AMR) and current issues in medical mycology. There was also an opportunity to hear a number of case studies aimed at delegates with an interest in biomedical science.

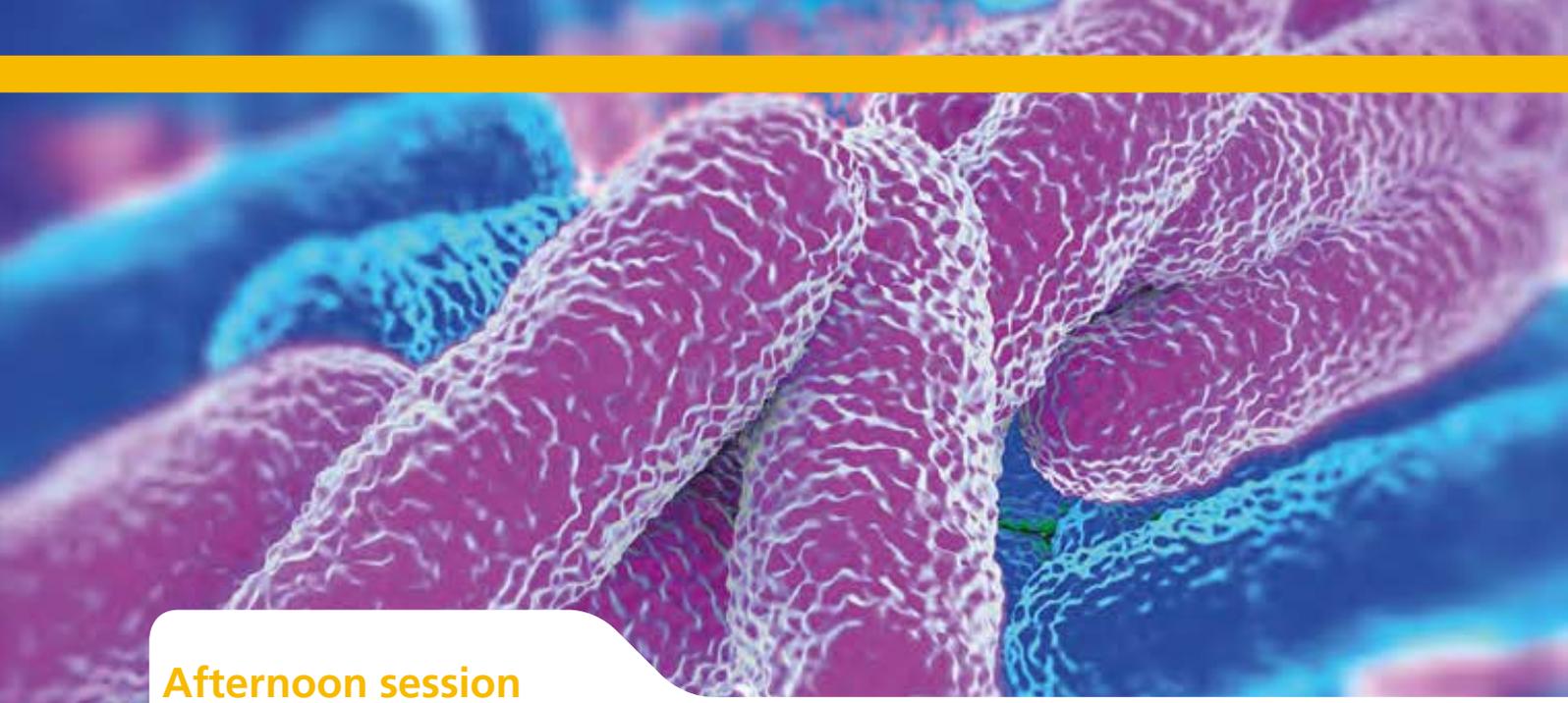
Neil Woodford (AMRHAI Reference Unit, PHE) opened the morning session with some fascinating insights into the gathering momentum of the global fight against the spread of AMR. Over the last few years, AMR has risen to the forefront of political agendas across the globe. Both the UK and the US now have AMR action plans built around reducing the transmission of infectious diseases, targeting antibiotic prescribing, educating professionals and improving surveillance. The World Health Organization has also identified surveillance as a high priority and is calling for routine monitoring of key organisms in all countries around the world. Tackling AMR will require new therapeutics and there is an urgent need for a change in the economic landscape to incentivize the development of novel antibiotic classes. At present the costs for developing new classes of antibiotic compounds are prohibitive, particularly in light of the risks that the lifetime of successful compounds may be limited by the development of resistance.

An overview of the key fungal pathogens and techniques for their diagnosis was given by Elizabeth Johnson (PHE Mycology Reference Laboratory). The galactomannan test is useful for invasive *Aspergillus* infections, which constitute around 80% of invasive moulds. Improved sensitivity can be gained by using bronchoalveolar lavage fluid compared with serum.

Detection of β -glucans, a common component of the cell wall of many different fungi, is achieved using a biological assay based on the primitive immune response of the horseshoe crab. This approach needs to be carried out and monitored very carefully, but can be useful for the general detection of fungi. Only mucoraceous moulds and *Cryptococcus* are not well detected by the β -glucan assay as they have minimal β -glucan in their cell walls. *Candida* spp. are responsible for the majority of fungal invasive infections and these are best analysed by MALDI-TOF. It is already possible to distinguish between most of the major *Candida* spp. using this technique and the databases are being refined to include the rarer species. Drug resistance is also an issue with fungi and problems are emerging with azole-resistant *Aspergillus* strains. In the Netherlands, some of these strains appear to be present in the environment, possibly due to the use of azoles in agriculture.

Tim Brown (PHE National Mycobacterium Reference Laboratory) provided an overview of the strategies employed to identify mycobacterial infections. *Mycobacterium tuberculosis* can only be handled in a category 3 laboratory, and this places a limitation on the amount of phenotypic typing that is practicable. Nevertheless, culture is still required for pathogen detection as the PCR-based assays are not yet sufficiently sensitive to be used alone. Phenotypic assays are used for antibiotic profiling, although the PCR detection of markers for some antibiotic resistance traits have high sensitivity and specificity. Programmes are underway to type mycobacteria using variable number tandem repeat (VNTR) analysis or whole genome sequencing. The level of detail obtained enables transmission routes to be traced, even when the samples from different patients have been cultured several years apart.

Nick Jakubovics
Newcastle University



Afternoon session

Having digested both our stimulating morning of hot topics together with our lunch, the audience were treated to a fascinating “pot-pourri” of microbiological case studies. The first of these presented by Dave Partridge, described a male Jamaican patient with persisting gastrointestinal signs. After extensive diagnostic workup and treatment with steroids, a small bowel obstruction was noted and surgical intervention planned. Diagnosis was further complicated by the presence of *E. coli* in his blood cultures, however clues revealed from histology that reported large numbers of plasma cells and eosinophils paved the way for a diagnosis of parasitic infestation with *Strongyloides stercoralis*, confirmed by demonstration of large numbers of larvae in his stool microscopy. It was hypothesized that the prior steroid treatment predisposed this individual to hyper infection syndrome. He recovered well following ivermectin. This was followed by an illuminating presentation by Joanne Bullivant providing all with an insight into diagnostic microbiology in Mekele, Ethiopia. Here the daily challenges included power supply and working out the time of day (Ethiopians work on a 6-hour day with a different calendar making conversions complicated). Despite their lack of equipment and training, the laboratory staff had a huge thirst for knowledge and a twinning venture between Sheffield and Mekele was bridging the gap by providing training opportunities.

We were treated to a fascinating “pot-pourri” of microbiological case studies

The audience then learned of a case of *Stachybotrys* sp. given by Stephen Wilson. He described a case of a man with a passion for eating dates who had indulged eating a date covered with “black dust”. With a history of asthma, he presented with pyrexia, dry cough and confusion. Further analysis of the data revealed causal agent *Stachybotrys chartarum*, a mould often associated with sick building syndrome and capable of producing trichothecene mycotoxin. The respiratory tract theme was then continued with a case of neonatal pneumonia presented by Julie Samuel. Despite best efforts, the neonate sadly succumbed to their infection. Microbiological cultures yielded *Legionella pneumophila*. Subsequent investigations revealed the likely source to be a birthing pool used for delivery. This had been filled some 14 days prior to delivery with water maintained at ambient temperature enabling *Leg. pneumophila* to flourish.

Laura Prtak went on to describe a case of a farmworker who sustained a crush injury to his hand. The injury was slow to heal despite antimicrobials and grafts. Upon further investigation, “jelly-like” clots were noted. Cultivation revealed a Zygomycete fungus later identified as *Lichtheimia corymbifera*, which responded to treatment with amphotericin B. Such infections are unusual in immunocompetent individuals, consequently this case reminded the audience to consider mycoses in non-immunocompromised patients. The session was closed with the description of an intercerebral abscess associated with *Streptococcus milleri* following cosmetic dentistry performed in Poland.

These presentations reminded all delegates of the need to remain vigilant and to think out of the box to find effective solutions. Certainly the audience had plenty of “food for thought” for their journey home.

Sally Cutler
University of East London

SfAM Winter Meeting

2016

19 January 2016 | 09:00 – 17:00

One Great George Street, Westminster, London

PSYCHROPHILES AND EXTREMOPHILES

We would like to invite extreme microbiologists to join us for the SfAM Winter Meeting on Psychrophilic and Extremophile Microbiology.

This one-day conference will explore the reservoirs of undiscovered biodiversity represented by psychrophilic bacteria. These bacteria have evolved to live only in the presence of extremely cold, harsh conditions. Understanding these chilly bugs is of great interest to microbiologists as they may hold a number of undiscovered biological secrets. How do they keep their stability? How do they synthesize enzymes capable of catalysing specific biochemical reactions under sub-zero conditions? The applications of psychrophilic and extremophile organisms for the applied microbiologist are vast and many of these will be discussed at the event.

The meeting will be structured around a series of lectures and case studies by international and national guest speakers and researchers, followed by a number of opportunities to discuss and reflect on the ways in which the study of psychrophilic and extremophile microbiology is applied, experienced and understood.

Early Bird		Regular Rate
£50	Member	£80
£30	Student Member	£60
£100	Non-Member	£130
£60	Student Non-Member	£90

Denver Russell Memorial Lecture

Charles Cockell (*right*)
from the University of
Edinburgh, UK
Psychrophiles and Astrobiology.



Speakers

Rosa Margesin
University of Innsbruck, Austria
Psychrophilic Microorganisms (biodiversity,
taxonomy, ecology).

Tony Collins
University of Minho, Portugal
Cold Adapted Enzymes, their Characteristics
and Applications.

Tom Curtis
Newcastle University, UK
Low Temperature Anaerobic Digestion.

Vincent O'Flaherty
National University of Ireland, Galway,
Low Temperature, Full-flow Anaerobic Digestion
using a Granular Sludge Fluidized Bed.

Tim Sandle
Bio Products Laboratory Ltd, Elstree, UK
Is there Life in my Cold Room? A Pharmaceutical
Industry Case Study.

Alan Dobson
University College Cork, Ireland
Mining our Oceans for Novel Bioactive Molecules
with Biotechnological Applications.

Antimicrobial Resistance 2015

PREVENTION › CONTAINMENT › CONTROL

7 December 2015 | 9:15 – 17:00

The Royal Society of Medicine, 1 Wimpole Street, London

The Society for Applied Microbiology's Antimicrobial Resistance meeting gathers prominent stakeholders from Government, funding agencies, pharma and academia to discuss the current efforts in tackling AMR.

David Cameron, UK Prime Minister, echoed the thoughts of many world leaders and scientists when he said that a failure of action now will lead to an "almost unthinkable scenario where antibiotics no longer work and we are cast back into the dark ages of medicine".

This one-day conference presents solutions and updates on AMR through keynote speakers, case studies, presentations, expert opinion and a panel discussion.

	Rate
Member	£35
Student Member	£20
Non-Member	£50

Speakers include

Anthony McDonnell

Review on Antimicrobial Resistance

Will Gaze

University of Exeter

Jon Otter

Imperial College Healthcare NHS Trust

Vanya Gant

UCLH

Tim Potter / Matt Dobbs

Westpoint Farm vets

Nicola Williams

University of Liverpool

Katie Hopkins

PHE

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BOOK**

The bookings page and programmes for all SfAM events can be accessed through www.sfam.org.uk

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by email: sally@sfam.org.uk or phone: **+44 (0)1933 382191**

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2015 SfAM AGM

84th Annual General Meeting of the
Society for Applied Microbiology
1 July 2015, 5.45 pm Intercontinental Dublin.



Present:

47 Members attended the AGM. This included:

President **Christine Dodd** (CD)
General Secretary **Mark Fielder** (MF)
Treasurer **Steve Davies** (SD)
Meetings Secretary **Andy Sails** (AS)

In attendance:

Lucy Harper (LH)
Paul Sainsbury (PS)
Clare Satchell (CS)

1 Apologies for absence

None.

2 83rd Annual General Meeting

The minutes of the 83rd Annual General Meeting held in Brighton in 2014 were published in the September 2014 issue of *Microbiologist*. They were approved and accepted by those present.

Proposed: **John Rigalsford**
Seconded: **Sally Cutler**

3 Matters arising from the previous minutes

None.

4 Report of the Trustees of the Society 2014

The President noted the success of the Society during the previous year, particularly with respect to funding and events attendance. She also announced the retirement of Phil Wheat and the appointment of Lucy Harper as the Society's Chief Executive.

CD announced a new process for electing officers to the Society which would bring increased

transparency. A search committee would be formed to identify potential individuals for election. The committee would include the President and Chief Executive of the Society, one other trustee and an external representative, such as a Member of the Subcommittee which the Officer chairs. They would be asked to identify 2–4 individuals and Members of the Society would also be asked for nominations.

LH gave a summary of the Society's strategy until 2018 and indicated a strategy document would be published in due course with feedback being very welcome.

The General Secretary noted the increased uptake of grants during the past year and this had been reflected in the attendance of so many international early career researchers at the Summer Conference.

MF gave a summary of the Society's communications and public engagement activities.

MF acknowledged the contribution of the ECS Committee and the continued value of their yearly programme of events to the Society.

The Meetings Secretary gave a summary of activities and gave special thanks to the Events Organizer, Sally Hawkes. AS also thanked Mike Dempsey for his contribution to the Winter Meeting programme and also to James Prosser for giving the Environmental Microbiology Lecture.

The Treasurer reported that the Society's value has continued to rise and that net assets now stand at £7952212. SD noted that the voice of the Society has increased during 2014. SD was proud to report that £245K worth of individual grants has been awarded to Members and that Members had also benefited from a large subsidy of £110K provided for the Society's meetings.

5 Adoption of the Annual Report 2014

Copies of the Annual Report of the Society for 2014 had been distributed previously.

Proposed: **Sally Cutler**
Seconded: **Linda Thomas**

6 Election of new Members (including honorary Members), deaths and resignations

A list of the names of applicants for membership and a list of deaths has appeared in the *Microbiologist* throughout the previous year. The Society also holds a summary list of new Members and resignations throughout the previous year.

7 Nomination and election of the General Secretary, Clare Taylor

CD announced MF would be stepping down from his role as General Secretary this term and on behalf of the Society's Members, staff and officers thanked him for his hard work.

Clare Taylor was nominated as the next General Secretary and the meeting agreed unanimously with this decision.

Proposed: **Andy Sails**
Seconded: **Samantha Law**

8 Nomination and election of the Meetings Secretary, Andy Sails

AS had come to the end of his term and was eligible to stand for a further year.

Proposed: **Sally Cutler**
Seconded: **Mark Fielder**

Andy Sails was duly elected.

9 Nomination and election of the Treasurer, Steve Davies

SD had come to the end of his term and was eligible to stand for a further year.

Proposed: **Samantha Law**
Seconded: **Linda Thomas**

Steve Davies was duly elected.

10 Nomination and election of new Ordinary Committee Members

Sally Cutler, Samantha Law, Mark Reed and Nick Jakubovics had all come to the end of their terms and this created four vacancies for new Ordinary Committee Members. CD thanked all of them for their contributions to the continued success of the Society.

There were four nominations for the four vacancies:

Charlotte Duncan – proposed by **Mark Reed** and seconded by **Clare Taylor**. **Claire Hill** – proposed by **Samantha Law** and seconded by **Sally Cutler**. **Mike Dempsey** – proposed by **Andy Sails** and seconded by **Christine Dodd**. **Phillip Wheat** – proposed by **Steve Davies** and seconded by **Andy Sails**.

All were unanimously elected.

11 Any other business

None.

The meeting concluded at 18.15.

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CAREERS

Beyond the bench: an academic journey



Higher Education is going through a period of rapid change, not only in the UK but around the world. Karen Stanley reflects on her early career experiences and how she now draws on them in her current higher education role.

A potential career in the higher education (HE) sector must cross through the minds of many an undergraduate or postgraduate student. The university atmosphere is exciting and challenging, and the culture can be intense and stimulating. If you work in a research post for a life science department in a UK university you begin to see another dimension of university life: the mysterious workings of a dynamic research community where fundamental and applied microbiology research may be taking place in various guises.

I never took it for granted that I would get a permanent position in higher education, i.e., a lectureship. As I continued through a series of postdoctoral positions I watched highly talented and successful research fellowship holders fail to be offered permanent posts at various institutes around the world. As I look back now, I realize that as well as building up my microbiology interests and research experiences, I had started gathering other experiences on my CV that have helped me develop my HE career. These have included being prepared to move city or country for what I considered 'a job with my name on it'. For me this included Aberdeen, Mexico City and Kumasi, Ghana.

I started thinking of myself as a microbiologist during my time as an undergraduate. I studied Applied Biology at Bath University and was on a thin-sandwich course which offered two different 6-month placements; the first at an Agricultural and Food Research Council (AFRC) crop research station where I joined a research project to investigate the effects of a yield-limiting

fungal rust disease of coppiced willows, grown for biomass. On leaving school I had worked temporarily in the mortgage department of a large finance house and there identified my first 'career criterion' – to find a career path that took me outdoors, or away from the desk, whenever possible. The AFRC placement fitted the bill and more – outdoor microbiology research!

My second placement was in the food microbiology department of the research and development headquarters of a multinational corporation. I had a project of my own, I regularly reported results at meetings with managers who generously nurtured me, and indeed all placement students, and developed my confidence in a number of ways (an issue I'll return to). The microbiology laboratory bench was also an acceptable alternative to the desk. I reckon these placements remained among my most formative experiences for possibly as long as 10 years after I graduated, even compared with my PhD.

Placements focused my thinking in several ways – they gave me an idea of the areas of microbiology I really wanted to be involved in, and a passion later referred to as 'a fire in the belly'. I have always kept this phrase in mind. It is more visceral and energetic than '...passionate about...' but conjures an image of a flame that might go out if starved of oxygen. I found a PhD project that I really wanted. It involved field work on rural farms and trapping of small mammals to test for *Campylobacter*. It spanned diagnostics, veterinary public health, environmental microbiology and microbial ecology. I loved it. Like many who contribute to this



section of the *Microbiologist*, I can identify people who have influenced me at this important time – my PhD supervisor, Keith Jones, encouraged me to always *enjoy* my work. It became another important ‘career criterion’.

There are several prerequisites for a lecturing post in UK HE and these include a PhD as well as a period of postdoctoral research ‘training’, during which you need to develop the expertise in theoretical and practical aspects of your subject area to underpin your first teaching role. These are exciting times to be involved in HE teaching. Universities are beginning to discuss the development of career progression pathways based on good teaching. Developing confidence in our students is an important aspect of good teaching.

On the other hand, most HE roles, even in ‘post-92’ or ‘teaching led’ universities, require experience and skills in areas that might broadly be termed ‘income generation, business development and innovation’. It has always been an important part of the job to deliver on this once in post, but anyone who reads the *Times Higher Education* cannot miss the current discussions about the pressures that these place on new and established lecturers alike. I have been lucky, in drawing on my previous overseas experiences, to be involved in income generation linked to ‘curriculum innovation’. Some five years into my post I undertook a secondment in international development that involved developing innovative approaches to collaborative training of young scientists and healthcare workforces, between UK and overseas institutions and Governments. I used

my experience in writing research grants to bid for overseas funding to support these projects and draw on my experience teaching and supporting part-time NHS-based MSc students in promoting the concept of continuing professional development to healthcare workforces in developing countries.

Through such projects, my HE role has offered me the opportunity to develop and use soft skills that I never imagined a science career would exercise. SfAM must also take credit for this aspect of my own career development as I served as an Ordinary Committee Member about eight years ago. I am ever grateful to senior Members of the SfAM Committee who mentored me on how to contribute effectively to a committee format of negotiation and decision-making.

So, perhaps unsurprisingly, in addition to microbiology lectures and labs I teach on our modules that embed career development. I encourage students to ask themselves where (in career terms and, literally, where in the world) they want to be in one, five and ten years’ time and to identify their own ‘career criteria’. When the department needed to meet various benchmarking criteria I was delighted to develop an ecology module. This includes, amongst other topics, my favourite areas of microbial ecology and I have recently introduced a field trip...

Karen Stanley
Sheffield Hallam University

JournalWATCH

2015 highlights and featured articles from SfAM journals

Journal of Applied Microbiology

www.journalappliedmicro.com

Microbial signature lipid biomarker analysis – an approach that is still preferred, even amid various method modifications

C. Willers, P. J. Jansen van Rensburg and S. Claassens



The lipid composition of microbial communities can indicate their response to changes in the surrounding environment induced by anthropogenic practices, chemical contamination or climatic conditions. A considerable number of analytical techniques exist for the examination of microbial lipids. This article reviews a selection of methods available for environmental samples as

applied for lipid extraction, fractionation, derivatization and quantification. The discussion focuses on the origin of the standard methods, the different modified versions developed for investigation of microbial lipids, as well as the advantages and limitations of each. The advantages and disadvantages of lipid analysis compared with other popular techniques are clarified. It is clear from recent literature that this technique stays relevant – mainly for the variety of microbial properties that can be determined in a single analysis.

<http://onlinelibrary.wiley.com/doi/10.1111/jam.12798/abstract>

Effects of sublethal doses of silver nanoparticles on *Bacillus subtilis* planktonic and sessile cells

M. Gambino, V. Marzano, F. Villa, A. Vitali, C. Vannini, P. Landini and F. Cappitelli

Due to their antimicrobial activity, silver nanoparticles (Ag-NPs) are being increasingly used in a number of industrial products. This suggested accumulation of Ag-NPs in the soil might affect plant growth-promoting rhizobacteria and, in turn, the plants. This article describes

the effects of Ag-NPs on the soil bacteria *Azotobacter vinelandii* and *Bacillus subtilis*. In growth-inhibition studies, *A. vinelandii* showed extreme sensitivity to Ag-NPs, compared with *B. subtilis*. We investigated the effects of Ag-NPs at subinhibitory concentrations, both on planktonic and sessile *B. subtilis* cells. At low concentrations, Ag-NPs killed *A. vinelandii* and affected cellular processes in planktonic and sessile *B. subtilis* cells. In summary, re-direction of gene expression, linked to selective toxicity, suggests a strong impact of Ag-NPs on soil bacterial communities.

<http://onlinelibrary.wiley.com/doi/10.1111/jam.12779/abstract>

Letters in Applied Microbiology

www.lettersappliedmicro.com

Synergy among thymol, eugenol, berberine, cinnamaldehyde and streptomycin against planktonic and biofilm-associated foodborne pathogens

Q. Liu, H. Niu, W. Zhang, H. Mu, C. Sun and J. Duan

In this study, the antibacterial activities of four main essential oils' components (thymol (Thy), eugenol (Eug), berberine (Ber) and cinnamaldehyde (Cin)) were evaluated against two foodborne pathogens, *Listeria monocytogenes* and *Salmonella* Typhimurium, either alone or in combination with



streptomycin. Our data highlighted that the combinations of specific components from essential oils and streptomycin were useful for the treatment of foodborne pathogens, which might help prevent the spread of antibiotic resistance through improving antibiotic effectiveness. These findings indicate that the combination of specific components of essential oils with streptomycin may provide alternative methods to overcome the problem of foodborne bacteria both in suspension and in biofilm.

<http://onlinelibrary.wiley.com/doi/10.1111/lam.12401/abstract>

Comparison of eleven commercially available rapid tests for the detection of *Bacillus anthracis*, *Francisella tularensis* and *Yersinia pestis*

A. A. Zasada, K. Formińska, K. Zacharczuk, D. Jacob and R. Grunow

Rapid detection of highly pathogenic bacteria causing anthrax, plague and tularaemia is crucial for the limitation of negative effects of a potential release (natural, accidental or deliberate). In the study, commercially available rapid tests for the detection of *Bacillus anthracis*, *Yersinia pestis* and *Francisella tularensis* were investigated in terms of sensitivity, specificity and ease-to-perform. The study showed problems which could be faced during testing and results interpretation. Conclusions from this study should be helpful not only in selection of the most appropriate test for a particular group of First Responders but also in undertaking decisions in situations of a suspicious contamination which have high social and economic impacts.

<http://onlinelibrary.wiley.com/doi/10.1111/lam.12392/abstract>

Microbial Biotechnology

www.microbialbiotech.com

Extraction and purification of high-value metabolites from microalgae: essential lipids, astaxanthin and phycobiliproteins

S. P. Cuellar-Bermudez, I. Aguilar-Hernandez, D. L. Cardenas-Chavez, N. Ornelas-Soto, M. A. Romero-Ogawa and R. Parra-Saldivar.



The market trend and consumers growing interest in natural and healthy products have forced researches and industry to develop novel products with functional ingredients. Microalgae have been recognized as a source of functional ingredients with positive health effects since these microorganisms produce polyunsaturated fatty acids, polysaccharides, natural pigments, essential

minerals, vitamins, enzymes and bioactive peptides. For this reason, the manuscript reviews two of the main high-value metabolites which can be obtained from microalgae: pigments and essential lipids. Therefore, the extraction and purification methods for polyunsaturated fatty acids, astaxanthin, phycoerythrin and phycocyanin are described. In addition, the effect that environmental growth conditions have in the production of these metabolites is described. This review summarizes the existing methods to extract and purify such metabolites in order to develop a feasible and sustainable algae industry.

<http://onlinelibrary.wiley.com/doi/10.1111/1751-7915.12167/abstract>

Genetic characterization of caffeine degradation by bacteria and its potential applications

R. M. Summers, S. K. Mohanty, S. Gopishetty and M. Subramanian

The ability of bacteria to grow on caffeine as a sole carbon and nitrogen source has been known for over 40 years. Extensive research into this subject has revealed two distinct pathways, *N*-demethylation and C-8 oxidation, for bacterial caffeine degradation. However, the enzymological and genetic basis for bacterial caffeine degradation has only recently been discovered. This review article discusses the recent discoveries of the genes responsible for both *N*-demethylation and C-8 oxidation. All of the genes for the *N*-demethylation pathway, encoding enzymes in the Rieske oxygenase family, reside on a 13.2 kb genomic DNA fragment found in *Pseudomonas putida* CBB5. A nearly identical DNA fragment, with homologous genes in similar orientation, is found in *Pseudomonas* sp. CES. Similarly, genes for the C-8 oxidation of caffeine have been located on a 25.2 kb genomic DNA fragment of *Pseudomonas* sp. CBB1. The C-8 oxidation genes encode enzymes similar to those found in the uric acid metabolic pathway of *Klebsiella pneumoniae*. Various biotechnological applications of the genes responsible for bacterial caffeine degradation, including bio-decaffeination, remediation of caffeine-contaminated environments, production of chemical and fuels, and the development of diagnostic tests have also been demonstrated.

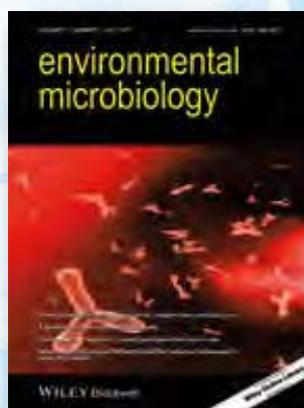
<http://onlinelibrary.wiley.com/doi/10.1111/1751-7915.12262/abstract>

Environmental Microbiology

www.env-micro.com

The soil resistome: a critical review on antibiotic resistance origins, ecology and dissemination potential in telluric bacteria

J. Nesme and P. Simonet



The Darwinian hypothesis of an 'arms-shields race' between antibiotic producers and resistant strains is often cited to explain antibiotic resistance gene determinants (ARGD) origins and diversity. ARGD abundance and antibiotic molecule exposure are, however, not systematically linked, and many other factors can contribute to resistance gene emergence, selection and dissemination

in the environment. Soil is a heterogeneous habitat and represents a broad spectrum of different ecological niches. Soil harbours a large genetic diversity at a small spatial scale, favouring the exchange of genetic materials by

PUBLICATIONS

means of horizontal gene transfer (HGT) that will contribute to ARGD dissemination between bacteria and eventually acquisition by pathogen genomes, therefore threatening antibiotic therapies. Our current knowledge on the extent of the soil resistome abundance and diversity has been greatly enhanced since the metagenomic revolution and help of high-throughput sequencing technologies. Different ecological hypotheses explaining their high prevalence in soil and questioning their transfer rate to pathogens, in respect to these recent experimental results, will be discussed in the present review.

<http://onlinelibrary.wiley.com/doi/10.1111/1462-2920.12631/abstract>

Bees under stress: sublethal doses of a neonicotinoid pesticide and pathogens interact to elevate honey bee mortality across the life cycle

V. Doublet, M. Labarussias, J. R. de Miranda, R. F. A. Moritz and R. J. Paxton

Microbial pathogens are thought to have a profound impact on insect populations. Honey bees are suffering from elevated colony losses in the northern hemisphere possibly because of a variety of emergent microbial pathogens, with which pesticides may interact to exacerbate their impact. To reveal such potential interactions, we administered at sublethal and field realistic doses one neonicotinoid pesticide (thiacloprid) and two common microbial pathogens, the invasive microsporidian *Nosema ceranae* and black queen cell virus (BQCV), individually to larval and adult honey bees in the laboratory. Fully crossed experiments were carried out in which treatments were administered singly or in combination. Common microbial pathogens appear to be major threats to honey bees, while sublethal doses of pesticide may enhance their deleterious effects on honey bee larvae and adults. It remains an open question as to whether these interactions can affect colony survival.

<http://onlinelibrary.wiley.com/doi/10.1111/1462-2920.12426/abstract>

Environmental Microbiology Reports

www.env-micro-reports.com

Novel basal, fungal lineages from freshwater phytoplankton and lake samples



S. Ishida, D. Nozaki, H.-P. Grossart and M. Kagami

Zoosporic fungal parasites are known to control the extent and development of blooms of numerous phytoplankton species. Despite the obvious importance of ecological interactions between parasitic fungi and their phytoplanktonic hosts, their diversity remains largely

unknown due to methodological limitations. Here, a method to genetically analyse fungi directly from single, infected colonies of the phytoplanktonic host was applied to field samples of large diatom species from mesotrophic Lake Biwa and eutrophic Lake Inba, Japan. Although previous research on the interaction between lacustrine fungi and large phytoplankton has mainly focused on the role of parasitic Chytridiomycota, our results revealed that fungi attached to large diatoms including not only members of Chytridiomycota, but also members of Aphelida, Cryptomycota and yeast. The fungi belonging to Chytridiomycota and Aphelida form novel, basal lineages. Environmental clone libraries also support the occurrence of these lineages in Japanese lakes. The presented method enables us to better characterize individual fungal specimens on phytoplankton, and thus facilitate and improve the investigation of ecological relationships between fungi and phytoplankton in aquatic ecosystems.

<http://onlinelibrary.wiley.com/doi/10.1111/1758-2229.12268/abstract>

Seagrass biofilm communities at a naturally CO₂-rich vent

C. Hassenrück, L. C. Hofmann, K. Bischof and A. Ramette

Seagrass meadows are a crucial component of tropical marine reef ecosystems. Seagrass plants are colonized by a multitude of epiphytic organisms that contribute to broadening the ecological role of seagrasses. To better understand how environmental changes like ocean acidification might affect epiphytic assemblages, the microbial community composition of the epiphytic biofilm of *Enhalus acroides* was investigated at a natural CO₂ vent in Papua New Guinea using molecular fingerprinting and next-generation sequencing of 16S and 18S rRNA genes. Both bacterial and eukaryotic epiphytes formed distinct communities at the CO₂-impacted site compared with the control site. This site-related CO₂ effect was also visible in the succession pattern of microbial epiphytes. We further found an increased relative sequence abundance of bacterial types associated with coral diseases at the CO₂-impacted site (*Fusobacteria*, *Thalassomonas*), whereas eukaryotes such as certain crustose coralline algae commonly related to healthy reefs were less diverse. These trends in the epiphytic community of *E. acroides* suggest a potential role of seagrasses as vectors of coral pathogens and may support previous predictions of a decrease in reef health and prevalence of diseases under future ocean acidification scenarios.

<http://onlinelibrary.wiley.com/doi/10.1111/1758-2229.12282/abstract>



Melissa McCulloch
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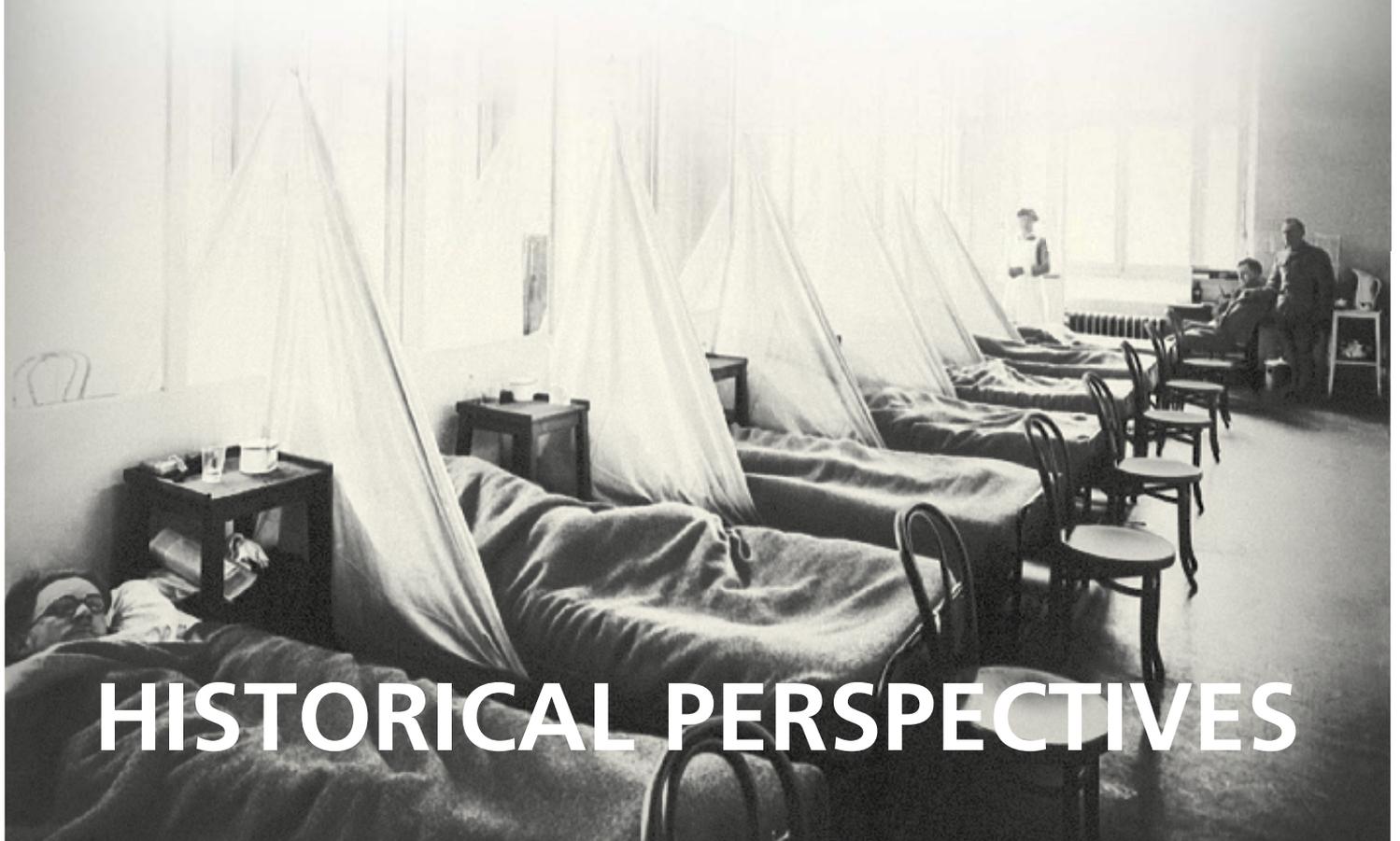
The INFLUENZA PANDEMIC of 1918-1919

Introduction

One of the more startling statistics of the influenza pandemic 1918–1919 is that evidence suggests up to 500 million individuals became infected (one third of the world's population). This is estimated to have led to 50–100 million deaths and dwarfs the approximated 16 million military deaths due to World War I from 1914 to 1918. This comparison does not tell the full story as it is understood that a proportion of those deaths during the 'Great War' were in fact attributable to influenza virus as opposed to direct conflict.

Morbidity and mortality

Estimations of influenza pandemic morbidity and mortality possess their own history that is punctuated by repeated upward revision. Indeed, the perception of the geographical impact and timing of the pandemic is not assisted by the nickname 'Spanish Flu'. This tag may reflect the press freedom at the time to report the severity in neutral Spain. By comparison, there was strict wartime censorship in many European and North American countries that are likely to have been experiencing comparable outbreaks.



HISTORICAL PERSPECTIVES

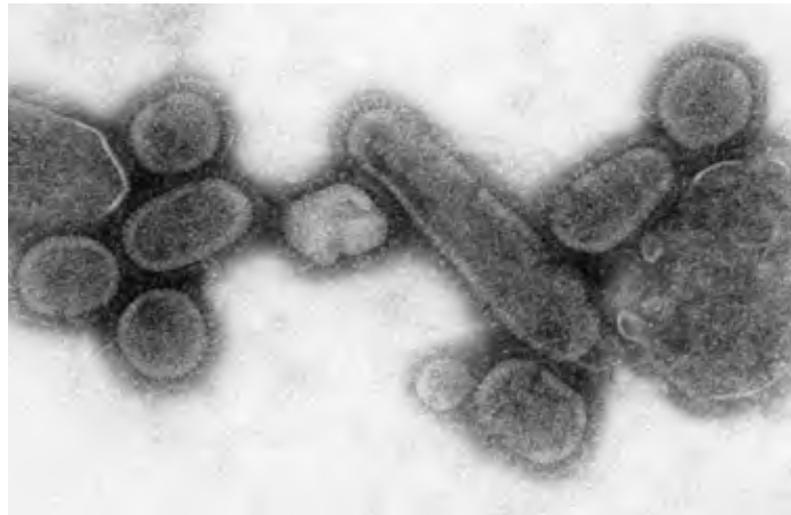
Sir Frank Macfarlane Burnet was a physician, immunologist and virologist who secured a Nobel Prize in 1960 for the *discovery of acquired immunological tolerance*. Prior to his death in 1985 at the age of 85, his research proposed that 50–100 million deaths were due to the influenza pandemic, surpassing the estimates of Edwin Oakes Jordan during the 1920s of 21.5 million deaths. This was subsequently supported by further epidemiological study in 2002 by Johnson and Mueller who also challenged the previously accepted pattern of three waves of the influenza pandemic occurring in early 1918, late 1918 and early 1919. They described the limitations of preceding data analysis including misdiagnosis, loss of medical records, and absence of registration and certification.

Strikingly, the severity of the influenza pandemic of 1918–1919 was uniquely observed in healthy adults aged 15–34 years of age. This is in stark contrast to other pandemics that tend to have greatest impact upon more seemingly vulnerable individuals including the elderly and immunocompromised. The pandemic of 1918 persists as a threat to public health as virtually all influenza A virus infections in humans since then have been attributed to descendants of the 1918 virus. The 5 million cases of severe influenza and 500,000 deaths occurring annually in regional epidemics across the world, serve to emphasize the significance of this legacy.

Epidemics and pandemics

Nevertheless, the 1918 influenza pandemic was not the first recognized influenza pandemic. In 1510, Portugal had secured a novel trading monopoly within the Indian Ocean through the conquest of Goa following a defining triumph against a Muslim Armada in 1509. At this time, an influenza virus pandemic is considered to have arisen in Asia prior to quickly spreading through trade routes to Africa and Europe. The notion of transmissible *seminaria* causing epidemic diseases was proposed by an Italian physician Girolamo Fracastoro in 1546. Therefore, it was not likely to have been immediately apparent in 1510 that the pandemic actually marked the return in Europe of an epidemic illness associated with intense coughing from 100 years prior in 1410. This condition was referred to as *horion* or *le taq* and also associated with cases of miscarriage. It is further understood that subsequent influenza pandemics occurred in 1557 and 1580.

'Sweate' or English sweating sickness (Latin: *sudor anglicus*) was described as an acute disease attributed to epidemics, initially in England in 1485 and subsequently throughout Europe until 1551. The origin of Sweate remains an unsolved mystery though and has more recently been attributed to hantavirus infections due to characteristic resemblances with the clinical features of hantavirus pulmonary syndrome (HPS). Earlier still, there were numerous European epidemics between 1173 and 1387, two of them referred to as



A negative stained transmission electron micrograph (TEM) of reconstructed 1918 influenza virions.

(Photo: Cynthia Goldsmith. Content: CDC/ Dr Terrence Tumpey/Cynthia Goldsmith.)

'influenza', a popular Italian term that was ascribed to respiratory illness centuries on. These were all preceded by the respiratory illness *febris Italica* (Italian fever) that affected the Carolingian Empire in 876–877.

However, this was not the first described potent combination of a devastating epidemic occurring concurrently with human strife. The plague of Athens struck during the second year of the Peloponnesian War in 430 BC. Scientists have long debated the actual cause of the plague, however a number of proposed pathogens have included *Yersinia pestis*, influenza virus and Ebola virus. Recent evidence now implicates typhoid fever, as DNA sequences of *Salmonella enterica* have been identified in the dental pulp of teeth from a mass burial pit in the Kerameikos ancient cemetery of Athens.

Influenza viruses

Influenza infections in humans are predominantly caused by influenza A or B virus, though C viruses can also be the aetiological pathogen. Influenza A virus can be categorized into numerous subtypes by the specific combination of the two surface molecules, haemagglutinin (HA, H1–H18) and neuraminidase (NA, N1–11). The latest novel influenza A virus to be discovered is H18N11 following detection and serological evidence in the Peruvian flat-faced fruit bat, *Artibeus planirostris*.

The great diversity of influenza A viruses is due to the process of reassortment, that is, essentially the swapping of genes between influenza viruses. This is facilitated by the segmented viral genome and can occur during co-infection of the same cell by two different influenza A viruses. Contrastingly, the influenza B virus possesses only one subtype as gene reassortment is rare and consequently all recorded influenza B virus epidemics have occurred regionally.

The resulting influenza viruses that were produced were significantly more virulent in mice than the wild-type strain

Influenza A virus H1N1

Influenza A virus of the H1N1 subtype was the aetiological pathogen for the 1918–1919 pandemic. It has been proposed that the 1918 H1N1 virus existed in an environmental niche prior to evolution by adaptation in a novel human host. It is likely that the 1918 virus was an avian-like virus that adapted to humans as opposed to the human/avian reassortment virus in the 1957 and 1968 pandemics. Sequence analysis demonstrates all eight gene segments resemble the avian virus though with more than the anticipated number of silent nucleotide mutations. There was one circulating strain in 1918 that binds exclusively to $\alpha(2-6)$ sialic acid receptors in human/mammalian cells due to mutations E190D and D225G in HA.

It may be that widespread exposure to a closely related virus had previously occurred in humans as there was another H1N1 influenza strain in 1918 with dual

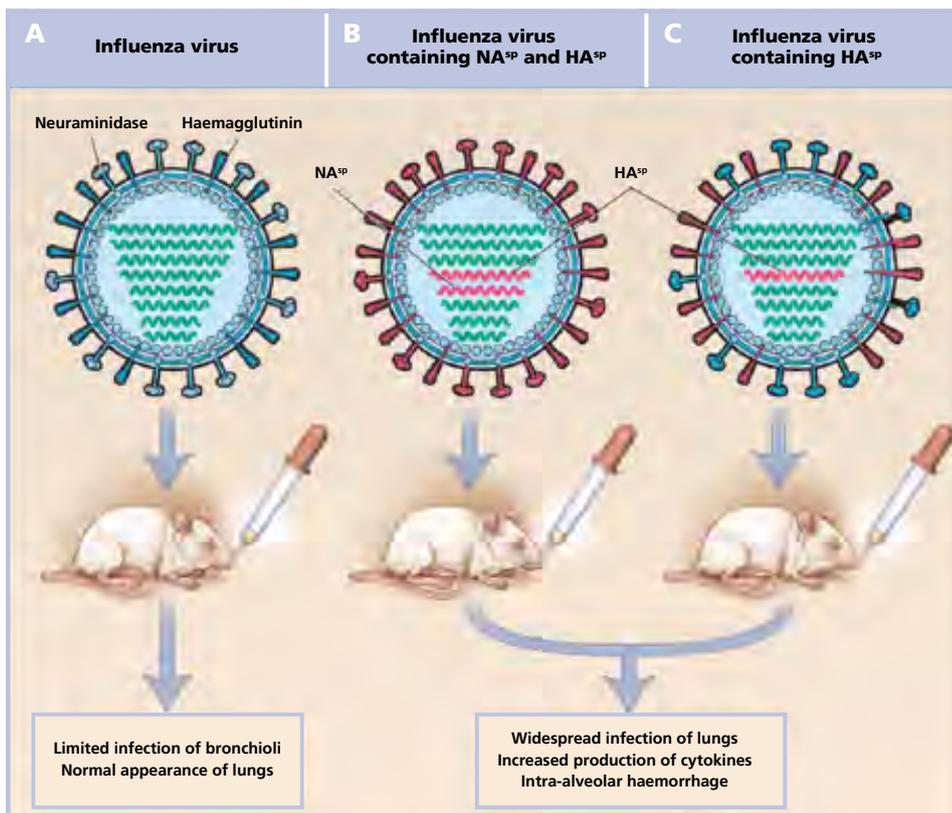
binding to mammalian $\alpha(2-6)$ and avian $\alpha(2-3)$ sialic acid receptors. In contrast to H5N1, there is no evidence to suggest high pathogenicity in avian hosts of 1918 H1N1.

Pathogenesis

In 1918–1919, the mortality was observed to be in excess of the background level for influenza and this may be due to two related clinical syndromes. Predominantly, bronchopneumonia was present with variable pathology and distribution throughout the lungs. Pathogenic bacteria were cultured almost routinely by autopsy and these included *Streptococcus pneumoniae* and *Streptococcus pyogenes*. In up to 15% of fatal cases, an acute respiratory distress-like syndrome occurred with characteristic ‘heliotrope cyanosis’.

Though these syndromes were not unique to the 1918 pandemic, there was a significant increase in

occurrence. Moreover, Kobasa *et al.* synthesized the haemagglutinin (HA^{SP}) and neuraminidase (NA^{SP}) genes based upon the 1918–1919 strain through reverse genetics. The resulting influenza viruses that were produced were significantly more virulent in mice than the wild-type strain if they expressed HA^{SP} with or without NA^{SP}. This could lead to inflammatory cell infiltration and haemorrhage that are synonymous with the 1918 pandemic. Additionally, Tumpey *et al.* constructed an influenza virus with all eight segments of the



Haemagglutinin as a Driver of Influenza Virulence.
(Reproduced from Hoft and Belshe, 2004)



The British Army Hospital, Etaples, with damage caused by German Aircraft. (Imperial War Museum (Q27492).)

1918 pandemic virus exhibiting a high-growth phenotype in human bronchial epithelial cells.

As one half of the 1918–1919 pandemic deaths were noted to have occurred in young adults who were aged 20–40 years old, it has been proposed that those born before 1889 had prior exposure to a precursor virus that elicited a degree of immunity. The theory of an enhanced immunological response within this age group contributing to the severe pathogenesis has also been suggested.

Host versus Environment

However, it may be that an environmental exposure that was unique to young adults led to the observed severity. It has been put forward that the living conditions of soldiers and civilians at the end of the First World War may have contributed. Certainly, this was an era of stress, overcrowding, partial starvation and an atmosphere contaminated with respiratory irritants including chlorine. In contrast, it has been postulated that the emergence of the pandemic in 1918 may have been prolonged due to the travel restrictions imposed in World War I.

Nevertheless, there were documented outbreaks of respiratory disease in France and the UK from 1915 to 1917 with high mortality in 25–35 year olds. Notably, the outbreak in a British army base at Etaples (northern France), in the winter of 1916 where soldiers predominantly resided in tents and temporary wooden barracks. This occurred almost identically at Aldershot Barracks (UK) in the spring of 1917, even though clinical samples have not been obtained for either of these outbreaks. The role of other pathogens can also not be excluded and the impact of host and environmental factors remains uncertain.

A host-specific molecular clock method has been utilized by Worobey *et al.* to unravel this mystery. This demonstrated that those born prior to ~1880 and after 1900 would likely have a degree of protection against 1918 H1N1. Conversely, the high mortality of individuals who were born between ~1880 and 1900 may have mainly been as a result of exposure to a doubly heterosubtypic putative (antigenically distinct) H3N8 virus in their childhood.

Global Threat

The unprecedented morbidity and mortality that occurred in 1918 would likely be more limited in present times with modern medical and public healthcare in the developed world. However, population growth and increases in global travel would enhance the rate of spread and the threat to the developing world of a repeat pandemic remains as potent. The historical evidence would suggest no pattern or cycle to these pandemics, ensuring a fundamental lack of predictability. Moreover, the hypothetical threat posed by the potential for human-to-human transmission as a result of mutations to the highly pathogenic avian H5N1 influenza virus is considerably more devastating.

Final Thoughts

It is interesting to consider that despite the continuing progress in the scientific understanding and medical management of influenza, there are still many elements of its history that remain a mystery. It is almost a century on and the 1918–1919 influenza pandemic serves as a stark reminder of the capacity for infectious diseases to wreak havoc on human civilization. Moving forward, the hope is to get ahead of the curve by containing the threat posed by influenza H1N1, H5N1 or any other known or unidentified species of influenza virus.

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Stephen Winchester
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The latest news, view and microbiological developments from our Corporate Members

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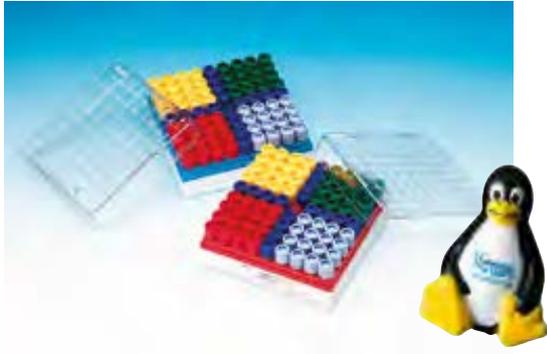
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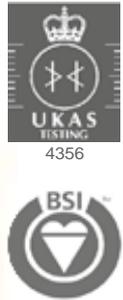
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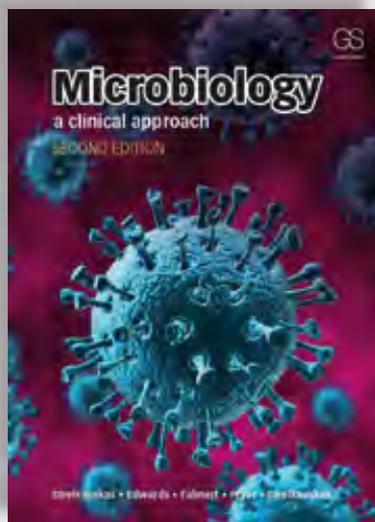
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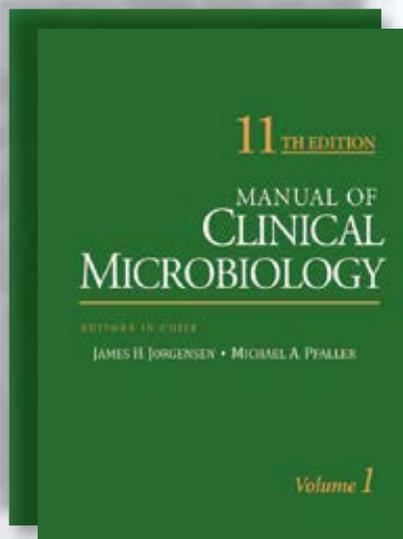
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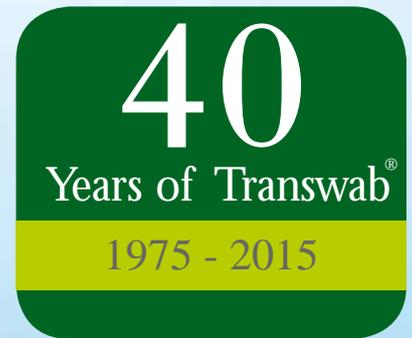
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