Linking microbial ecology with **disease evolution**

In her role as an evolutionary biologist, **Dr Michele Nishiguchi** is deciphering the mechanisms behind bacterial infections. Here, she outlines why an experimentally tractable model has been so valuable to her work and highlights outreach to encourage Hispanic girls to work in marine science

DR MICHELE NISHIGUCHI

What are the aims of your latest research, and what applications do you hope your findings will have?

Some of the specific project objectives include determining how important environmental changes are in shaping the relationship between sepiolid squids and *Vibrio fischeri* bacteria, as well as tracking these changes to determine if there are correlations between abiotic factors – such as temperature, salinity and nutrient composition in the water column – and genetic changes – including population frequencies among certain genotypes and specific genes that are responsible for the symbiosis and specificity among different *Vibrio* strains. I am also interested in how quickly *Vibrio* bacteria can adapt to new host species, and whether the successful *Vibrio* bacteria that are capable of making that switch can do so because they can accommodate environmental changes better than other *Vibrio* genotypes.

Can you outline the association between bobtail squids and the luminescent bacterium *Vibrio*? What are the benefits of symbiosis for each organism?

The relationship between bobtail (or sepiolid) squids from the genus *Euprymna* (Cephalopoda: Sepiolidae) and *Vibrio fischeri* bacteria is one based on equal terms or mutualism; ie. a mutually beneficial relationship where each party gains by entering into the partnership with the other. The squids use the bacterial bioluminescence (or light production) for a behaviour called counter-illumination.

The squids are nocturnal, which means they come out at night to hunt and breed, but also need to avoid being eaten by predators. During the day, these squid bury themselves in the sand to hide from predators. During the night, they usually hover above the sand. If you imagine the down-welling moonlight through clear tropical waters shining on top of the squid, it will cast a shadow so that anything underneath will be able to see the squid. To counter this exposure the squid matches the down-welling moonlight with its own bacterial bioluminescence to reduce its silhouette and thus remains hidden from view.

The squid will essentially disappear, using a 'squid cloaking device' to avoid being seen. In turn, the *Vibrio* bacteria receive nutrients from the squid light organ and are capable of cloning themselves at a much higher rate than if they were living free in the water column. Thus being inside a squid light organ provides a nice safe place for *Vibrio* bacteria to stay in order to produce more of its own kind.

Why has this particular model proved to be such a successful tool for examining

the population biology of cospeciating organisms?

This mutualism is an ideal model because the individual partners can be studied separately, or in symbiosis. *Vibrio* bacteria can be isolated and cultured away from the squid light organ, and juvenile sepiolids are born without their *Vibrio* bacteria. As a result, establishing a symbiosis in the laboratory with any other type of *Vibrio* bacteria that may be foreign to that particular host is relatively easy. We can also create the same relationship with bacteria that have been mutated in the laboratory to enable testing of specific genes that may be responsible for the recognition and specificity of the symbiosis.

We can assess subtle differences among closely related *Vibrio* bacteria, as well as within and between host species, in order to determine whether the particular host species or the environment (since the *Vibrio* bacteria are obtained from the surrounding seawater at each generation) is important for shaping the symbiosis.

Can you highlight some of the outreach work that has accompanied your research?

My laboratory is quite involved in a number of outreach activities. This year we have volunteered our time to talk to 5th grade Hispanic girls and their mothers in a programme called 'Generaciones'. We have also given presentations to the Las Cruces High School Biology/Zoology class, and I have led a dissection on a squid to this same group after their visit to the university. We have been involved in the U.S. Science Education Alliance Science Share Fair, where we have a booth where the students can touch and look intimately at live marine invertebrates, which is normally difficult to do in a land-locked state. We also participated in an American Association of University Women's programme called 'Girls Can!' where we set up a touch tank with lots of cool invertebrates for them to see and feel.

Strength in **numbers**

A study on the **Significance of Adaptive Radiations in an Environmentally Transmitted Symbiosis** at New Mexico State University aims to build a better understanding of how mutually beneficial relationships contribute to the wellbeing of marine environments

MANY ECOSYSTEMS FACING the most intense pressure from human activity and climate change are found in the oceans. Ecologically sensitive marine areas, such as coral reefs, estuaries and seagrass beds, are struggling to survive as a result of severe overuse, pollution and, increasingly, global warming. Improving knowledge on the overall mechanisms that are driving bacterial speciation and adaption can help with predicting just how many of these marine ecosystems are beginning to respond to these threats.

Marine biologists at the New Mexico State University are delving into this field by studying both abiotic and biotic measurements using physiological and molecular approaches. The 'Significance of Adaptive Radiations in an Environmentally Transmitted Symbiosis' study is focussed on understanding how bacteria are able to adapt to different host species as well as different environments. The researchers hope that they can add to the growing body of knowledge on how bacterial infections are spread and in what ways this impacts adaptation in marine ecosystems.

A GLOBAL PERSPECTIVE OF ABIOTIC CHANGE

Dr Michele Nishiguchi, Principal Investigator for the project, has been working with colleagues from the Australian National Museum, The University of Newcastle, and The Centre of Marine Bio-innovation at New South Wales University in Sydney, Australia over the past 10 years. During this time, she has witnessed a number of transformations in the marine environment, particularly where changes in temperature gradients have been observed such as where the water is at the boundary between the tropical waters and the cooler water from the south: "I think this can all be linked to global climate change, where the world's oceans are experiencing a shift from cold to warm," Nishiguchi observes. The Maugean zone, for example, which was previously located very close to the border between Victoria and New South Wales, is now found much further south, meaning that warmer water species are starting to be seen moving into more southern coastal areas of South Eastern Australia.

The laboratory is primarily funded by the U.S. National Institutes of Health and the National Science Foundation. In addition, some of the students completing research here are supported by the New Mexico Space Consortium (through NASA) and the Howard Hughes Medical Institute. The biggest challenge to running a research laboratory of this scale is being able to effectively manage a large working group of scientists, graduate, undergraduate students and technicians. Together this group has tackled a number of strategies that have been developed to address specific research questions, and Nishiguchi is now observing many successful findings that are emerging from their research.

DYNAMICS OF SQUID/BACTERIA RELATIONSHIPS

A key component of the work at the Nishiguchi Laboratory is their study of the sepiolid squid-*Vibrio* light organ association. They are investigating the mechanisms that drive bacterial colonisation of animal tissues and the population and community dynamics between squid hosts and their *Vibrio* bacterial symbionts.

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INTELLIGENCE

SIGNIFICANCE OF ADAPTIVE RADIATIONS IN AN ENVIRONMENTALLY TRANSMITTED SYMBIOSIS

OBJECTIVES

To examine both free-living and symbiotic *Vibrios* under a variety of conditions in the laboratory (salinity, temperature) to determine if abiotic factors contribute to the distribution of *Vibrio* among wide ranging host populations.

KEY COLLABORATORS

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Dr Natalie Moltschaniwskyj, University of Newcastle

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PROFESSOR MICHELE NISHIGUCHI is an evolutionary biologist whose work spans the bridge between microbial ecology and disease evolution. She is best known for deciphering the mechanisms for bacterial infections that occur in animal hosts.

Nishiguchi is well recognised both nationally and internationally, and has a reputation not only among microbial ecologists, but also molluscan biologists as well.

One of the most valuable factors of this particular symbiotic relationship is the association can be manipulated in the laboratory between the squid host and the bacteria. Within a matter of a few hours of juvenile squid being hatched the bacteria establish this relationship through an environmental route. Because the light organs of the juvenile squid are essentially sterile, the bacteria are able to quickly 'infect' the host squid by recognition and then colonisation. These relationships are complex and whilst the bacterial specificity is continued throughout the whole of the squid's life, other strains of *Vibrio* bacteria are able to line up in a 'hierarchy' to cross-infect related species. The working group at the Nishiguchi Laboratory are adding to this area of research by further investigating the differences between related strains of bacteria at a molecular level and how this impacts the speciation of *Vibrio* bacteria.

A number of standard methods are used to learn about the effects of environmental changes on species. The techniques that have been chosen by the researchers at the Laboratory to study the relationship between sepiolid squids and their *Vibrio* symbionts are mainly molecular based, although they have also employed other methods to work out how genetic mutations are linked to phenotypic changes, including carbon source utilisation, phenotypic assays and biofilm production. They have a preference for using *Vibrio* bacteria for the molecular genetic studies because these bacteria appear to like mutation, meaning the scientists can relatively easily identify genes that are responsible for different behaviours by knocking out targeted genes: "Bacteria are generally very good at accommodating change without having a mutation occur, so it is important to find out whether there is a genetic link to adaptation in many of the cases that we investigate," Nishiguchi outlines.

One of the most significant findings to emerge from their research is that the researchers have been able to demonstrate that *Vibrio* bacteria take 400 generations to evolve into a new host squid, a finding that they recently published in the scientific journal *Evolution*: "What is even more astounding," Nishiguchi notes, "is that the phenotypic changes that are observed in the evolving new lines mimic those that are present in wild-type strains of the *Vibrio* bacterium that they are evolving to". Such major changes are not coming entirely from genome mutations, but from the genes that are up or down-regulated (so increases/decreases in proteins responsible for adaptation) in response to a different host. One of the strands of research on this particular topic has involved completing a genome analysis for six geographically isolated *V. fischeri* genomes coming from different sepiolid squids. It is hoped that this information will feed into understanding how genes are adapting under pressure for selection from the host or the environment. In addition, the group have sequenced, and are presently analysing, the transcriptomes of four of these *V. fischeri* strains to determine how the regulation of either the mRNA or protein is important for establishing new host relationships," she highlights.

TACKLING THE BIG QUESTIONS

There are two key goals that the researchers working at Nishiguchi Laboratory are actively pursuing. Firstly, they are keen to understand how changes in the environment are shaping symbiotic relationships so that more information on how population frequencies of bacteria are being impacted, this in turn will allow a deeper awareness of how environmentally transmitted symbiosis functions.

This knowledge is an important step towards improving human health by recognising how closely related pathogenic relationships operate, such as those that cause cholera. Secondly, they hope to be able to record how major changes impact populations: "By monitoring environmentally sensitive organisms, such as the ones in the mutually-beneficial relationship between squid and *Vibrio* bacteria, we can begin to determine how healthy our ecosystems really are" comments Nishiguchi. Her hopes for the future of their research lies in being able to correlate genetically-based population structures with both dynamic changes in time and space and changes in environmental factors. This kind of task, whilst not impossible, is complex and multifaceted, so the team will be looking to their collaborators and partners to combine expertise among scientists from a number of diverse fields for mutually beneficial outcomes.

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