**Predicting the future: A fish story.**

Prokaryotic genetics has an illustrious past. In its early days, it helped us learn how genetic information is stored, replicated, repaired and expressed. These questions were of importance to science and philosophy even beyond their importance to the general field of biology. "Could man understand his own inheritance?" The lessons learned from bacteria were applied everywhere and the tools that emerged were used to investigate of virtually every aspect of biology. Since those early days, work on prokaryotes became less central to biology as a whole. I think this situation is changing and a very exciting time is on the horizon for those working on the biology of prokaryotes. But first the fish story……

**A fish story -** Once upon a time, some very bright and ambitious fishermen set out to catch the very biggest fish of their age – *the hereditary material, mutation, replication, repair, transcription, translation, genetic code, gene regulation*. These fish were extremely fat since together they defined the – *the chemical underpinnings of heredity*. The fish were clearly visible to everyone, flopping around not far from the shore. To catch these fish, the ambitious fishermen constructed a boat – *phage and bacteria* – and set forth into the lake. The questions were so important and the tools so powerful, that a horde of students, post-docs and professionals from a variety of disciplines got into the "prokaryotic" boat and joined the fishermen in their quest. The crowds on the shore watched and cheered them on, eager to share in the catch.

The fish were caught – one by one – *the biggest questions were answered*. The crowds cheered, ate the catch and went to look for fatter fish in other ponds. The tools devised by the fisherman – *partial diploids, selective detection of rare events, cloning, sequencing* -- were applied to catching other sorts of fish –*eukaryotic cell biology, development, neurobiology*. While the prokaryotic fishing expedition paid big dividends, the crowds went to other ponds. The fisherman found that their pond was yielding fewer big fish.

As fish became rarer and smaller, the fishermen got restive. Although their tools were powerful and their skills were great, the crowds on the shore had dwindled. They

came to an interesting decision, "Never mind the fat fish", they said, **"LET'S STUDY THE BOAT".**

In "studying the boat", they addressed the biology of bacteria and phage (per se) without worrying about whether or not they were getting at obvious big-ticket questions with mass scientific appeal. This was a perfectly respectable decision. In doing this, they joined forces with an army of microbiologists who had long been studying intricacies of microbial metabolism, virulence and ecology without benefit of genetics. This pre-genetic army was also skilled but had no debilitating history of longing wistfully for the era of "fat fish". Work on the biology of prokaryotes has progressed very well – even though the large crowds on the shore moved on. We have learned a fabulous amount about microbial structure, behavior, and metabolism and even population structure. Microbiology is a respectable sub-discipline, like many other biological sub-disciplines. It moves forward in a business-like way doing excellent work with no pretense of trying to answer all of the world's problems. Sure, those previously involved with "fat fish" get a little wistful sometimes. It surely bothered Gunther Stent who described his malaise ("The Coming of the Golden Age") in terms that were disturbing to me when I first read them as a beginning assistant professor. Stent may have overstated the case. One must "get real" after all -- scientific revolutions don't come along every day and lightning may not strike twice in the same discipline. While there are lots of wonderful questions to investigate using prokaryotes, as a late-comer to the boat, I shared some of Stent's concern and kept hoping for more. Lately I am getting the feeling that microbiology's peaceful period may be coming to an end.

**The fish are back and they look fat to me.** At the risk of wearing out the fish story, I think some things are happening that may bring prokaryotes and their genetics back to center stage of biology. We may be in a position to play a key role in answering some global biological questions that qualify as "fat fish" in the minds of merciless public opinion. I apologize if this sounds like a sermon (which it is), but here is a list of a few areas that seem most promising to me.

1. <u>Working the interface between population biology and cellular function.</u>

For reasons that escape me there is a traditional fracture line in biology that separates people working on population biology from those working on mechanistic cell biology. I think that the crux may be what each group means when they say they're trying to figure out "how something works". Population people mean "what is the selective value of the mechanism, what benefit does it provide, how did it evolve. Mechanistic people mean "how do the nuts and bolts fit together and function in a proximal sense". Ham Smith once told me that he didn't care about why something evolved, he just wanted to find a puzzle and figure out how to put the parts together. A population biologist I know says that molecular biologists are just interested in assembling the "parts list" that evolutionists can use to figure out the interesting aspects of biology. I see merit in both views (they surely simplify one's life), but I always imagined that there was a lot in between. Are there sequences, proteins, mechanisms, whose "function" can only be understood in the light of population biology? I think the list of candidates is getting longer and may finally rise above skyline so someone notices.

Mechanists and populationists have different ways of explaining population-based structures and functions. Transposable elements are usually discussed by populationists as independent entities that are costly to the host, but persists by virtue of their "selfish" ability to move horizontally – they make extra copies faster than copies can be eliminated by selection. Mechanists often propose physiological values of these elements; perhaps they serve as a variable aspect of cellular mutability or as mediators of host chromosome rearrangements or as movable promoter elements. Ultimately the truth may unify the two viewpoints information.

Perhaps the most striking example is genetic recombination. Modern mechanists describe recombination as a set of functions that help restart chromosome replication forks following DNA damage. In contrast, population biologists describe recombination as a system for reassorting alleles so that optimal combinations appear. It is shocking that the same functions are said to do very different things in two areas of research. There must be a unified way to discuss this body of information that makes coherent sense of the contributions to short-term repair and long-term genetic variation.

In our own work, the interminable discussion of "adaptive" or "stress-induced" mutation falls in this area. One side of the debate (which appeals to molecular biologists)

finds it attractive to suppose that stress is mutagenic. They assume that Cairns' genetic system resembles a classical bacterial selection experiment in which rare large-effect mutations generate colonies that appear above a non-growing lawn of cells that are stressed by the conditions on the selection plate. The colonies are attributed to regulated mutation. The other side (which appeals more to population people) assumes that the mutant colonies appearing under selection are initiated by extremely common small-effect mutants, which normally escape detection in stringent laboratory selection. Because the Cairns experiment uses relaxed selection conditions and a prolonged period of growth under selection, these common small-effect mutations adapt and improve until they are counted as full revertants. Stress enhances revertant yield by serving as an agent of natural selection – not as a mutagen. Resolution of this controversy will require critical examination of both models, both of which involve complex series of events. We think this examination will reveal new and important insights into mechanisms of selective adaptation and mutation formation.

2. Making falsifying experimentation a more integral aspect of evolutionary biology.

I've always been envious of evolutionists whose discipline seems to have proceeded rather impressively by a process that depends only weakly on experimentation. Theory demonstrates feasibility and models are pitted against each other on the basis of mathematics. (Just think of the saving in Tris buffer alone.) This is of course not strictly true -- natural observations (rather than designed experiments) provided reality checks. The experiments designed to test theory are often, however, ones in which the outcome is planned. An experiment that disproved genetic drift would not be treated as a serious threat to the body of theoretic work. This situation may reflect the difficulty of planned experiments given the traditional focus of evolution on multi-celled organisms with long life spans and small populations, compared to those of bacteria.

One demonstrable "fat fish" for bacterial work is in the application of serious experimentation to evolution. By "serious", I mean experiments that provoke theory or can be used to falsify theory. Experimentation of the sort that drives the rest of science. The cadre of people in microbial experimental evolution is expanding revealing new aspects of population biology that would be difficult to infer by first principles. Ideas

can be falsified. It seems to me that evolution has been hampered by too much focus on centromeres and diploidy. The most fundamental aspects of evolution can be well-addressed using microbes, where the connection between mutation and phenotype is not delayed by diploidy and sexual exchanges. Notable examples are the long-term cell culture experiments and the analysis of intracellular insect symbionts. While all aspects of molecular evolution are benefiting from new ways of determining and analyzing sequence information, bacteria are positioned best for experimental work due to their small genomes and large populations.

3. Genome sequence analysis – a fusion of physiology and population genetics. This area is in its infancy. Initially it served to assign functions to new genes and reveal metabolic patterns in new organisms. Increasingly sophisticated approaches are allowing it to provide details of microbial population structure, the nature and formation of species. The small size of microbial genomes allows this approach can be applied to more species and to more individuals in a single species.

4. Some of the fat fish appearing in the prokaryotic pond are perennial questions that have never been landed by the field of population biology. Evolution as a discipline has followed Darwin's lead in concentrating on multi-celled organisms with sexual reproduction. While evolution concentrated on bigger critters, the prokaryotes and their phages have slowly become the elephant in the room of evolution. Aided by the revolution in sequence information a growing cadre of microbial evolutionist have taken their place on the stage and threaten to take over the business (my not-so-humble opinion here). A few of the fish that are present are the following.

Definition of a species had remained one of the most elusive problems of biology. It is becoming clear that the "biological species concept" is embarrassingly out of date when one recognizes that it applies to a minority of the organisms on earth and the actually tests of mating are seldom applied in stringent ways in assigning organisms to one species or another. It seems that a consideration of all organisms (sexy and not so) can we say that we understand the nature of species.

Evolutionary origins of sexual reproduction has been explained in a variety of ways, but it remains difficult to account for the appearance of a form of reproduction that is two-fold less efficient than simple binary fission, or asexual reproduction. While interesting explanations have been offered, they don't really consider asexual prokaryotes as part of the process. Why did almost all of life remain asexual? Consideration of bacteria in thinking about this problem is (I suspect) going to reveal more satisfactory ways of thinking about the problem.

The role of purifying selection as a force for conservation of genetic information seems like one that may become more important. Traditional evolutionists (including Darwin) have focused on the ability of natural selection to drive adaptation, create novelties and drive divergence of lineages into distinct species. However while this is interesting, the predominant role of selection has always been to remove deleterious mutations (which means almost all new mutations) from the population. That is, the vast majority of mutations that form are deleterious (the silent majority) and are quietly removed from a population by selection. A very tiny fraction of mutations prove beneficial, but their effects are more obvious since the cause observable measurable changes. While the idea of purifying selection is an old one, it is one that Darwin seems to have missed and most of his followers have under-appreciated. (Perhaps the big book should have called Origin and Maintenance of Species.)

5. Thinking about metazoan somatic tissue as a microbial population. If one considers a metazoan organism as a population on single replicating cells, it is clear what a miraculous feat has been achieved. Some have estimated that a human body hosts the order of $10^{14}$ acts of cell division per year (even though at any moment the total population is less than $10^{14}$. Cells in this population are under continuous selection to grow – since natural selection rewards improved reproductive success even when the reward are not realized long-term. We worry that perhaps 2/3 of humans experience some form of cancer during their life span and wonder how this is possible. I suspect that Cairns to develop his system for study mutation in non-growing cells in hopes of discovering a stress-induced mutagenesis that would explain cancer. However when one

considers the number of somatic cell divisions, the more poignant question is probably, "How does 1/3 of the population remain cancer free?" Taking a bacterial viewpoint of this problem may help reveal aspects of stem-cell biology that will explain how somatic cells manage to defeat natural selection so successfully. Treating somatic tissues as microbial populations growing under selection may reveal new aspects of chronic disease and malignancy.

6. Origins of life is becoming a more and more important area of biological investigation. This field has been the domain of geologists and chemists for a long time, but the influences of prokaryotic biology are changing this situation. The body of information on prokaryotic metabolism and cell biology are suggesting new ideas of thinking about origins. Probably many biology students (and maybe even some professors) will live to see published claims that life has been "created" in the laboratory. Work on the genetic code suggests that it evolved before the appearance of the last universal common ancestor of modern life. It seems likely that mutation, natural selection, informational exchange all occurred well before there was even an RNA-based form of life. Questions of how life arose is one that should attract the interest of many with a background in prokaryotic biology. Origins of life is, after all, the granddaddy of all biological fish.

**But this isn't bacterial genetics….** You may be dismayed that the proposed "fat fish" concern population biology rather than metabolism and cellular mechanisms. The mass of detailed work on microbes (and recent work on their genomes) has made it possible for us to visualize their livestyles, perhaps better than one can visualize the biochemical underpinnings of life for a fly or a mouse. Mutations generate phenotypes more directly in microbes than in big organisms. Somatic cell populations (and sexual reproduction) complicate the connection between genotype and phenotype, which is central to how selection operates. We can measure mutation rates better in microbes and know a huge amount about relative rates at which various mutation types form. All this simplifies thinking about how selection affects populations.

This is not meant to disparage the "fat fish that" developmental/cell biologists have caught and are still catching. Perhaps the fattest and most elusive fish on the scene are the epigenetic mechanisms that control expression of genes and (perhaps) affect local mutation rates. These mechanisms make it possible for multi-celled organisms to persist by preventing natural selection from operating on somatic cells. The defeat of natural selections effect on somatic populations shifts the level of selection to whole multi-celled organism. The epigenetic information (acquired during development) resembles in some ways the genetically-encoded information revealed by work on microbes. Like DNA sequence information, somatically acquired epigenetic information may show redundancy. The base sequence of one strand of DNA (plus a few simple rules) allows you to predict absolutely the sequence of the other strand – a redundancy that allows, replication, editing, repair, recombination and transcription. Similarly the epigenetic mechanisms that control somatic cell gene expression may show redundant information content. The information inherent in DNA methyl groups may dictate (and be in turn dictated) by patterns of histone modification – just as the sequence of one DNA strand dictates (or is dictated by) the sequence of the other. A whole new sort of encoded information may be on the horizon. Prokaryotes show only vestiges of this sort of epigenetic inheritance (methylation patterns, reversibly invertible segments, addiction modules, contingency loci, gene amplification), but some of these may help sort out epigenetic mechanisms in big critters. While all this is great fun to think about, the complexity of somatic inheritance may complicate thinking about selection-mutation interactions in populations of big organisms and make bacteria more tractable systems in which to study conventional evolutionary processes.

**What does it all mean?**

Prokaryotic genetics has had one great run and seems poised to have another. Some new areas that seem particularly promising are ones in which traditional microbiology interfaces with very different areas of biology such as evolution and somatic cell biology. We can bring our detailed understanding of microbial mechanisms to bear on problems that are common to all living things. The biggest fish always lurk at

the interfaces between disciplines. We should steer our boat toward sites where currents converge and keep fishing.