Deadly bacteria pop up in fruit flies

Talk about a killer headache.

Bacteria that are normally content just to meddle in the sex lives of insects may also erupt in deadly population explosions in the brain and other tissues of the fruit fly, two scientists now report.

Seymour Benzer and Kyung-Tai Min, geneticists at the California Institute of Technology in Pasadena, came across this unexpected discovery when they noticed that some fruit flies they had bought recently weren't living as long as other members of the species, *Drosophila melanogaster*.

The progeny of the insects also died young, suggesting an inherited reduction in life span. Yet when male short-lived flies bred with normal fruit flies, this premature death sentence did not pass on, indicating that it can be inherited only from female short-lived flies.

The investigators, who were originally looking for genetic mutations that trigger neurodegeneration, used an electron microscope to study the brains of the short-lived flies. They saw strange particles in the cells of the brain, as well as the retina, ovary, and other tissues.

The number of particles increased as the insects grew older. "With advancing age, the cell, including the nucleus, becomes packed with them, akin to a bag filled with popcorn," the two scientists report in the Sept. 30 PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES (PNAS).

Benzer and Min suspected that the particles might be intracellular bacteria, particularly those known as *Wolbachia*, which are inherited from the mother and populate the ovaries.

The researchers confirmed their hunch by isolating *Wolbachia* DNA from the fruit flies and showing that an antibody that binds to the bacterium marks the particles. The investigators observed that treating the flies with an antibiotic gave the insects a normal life span.

"The evidence is pretty convincing that it's *Wolbachia*," says John H. Werren of the University of Rochester (N.Y.), who has written a commentary for the next issue of PNAS.

The new finding startled *Wolbachia* scientists, who had previously regarded the bacterium as a "prudent parasite," says Werren. Since the bacteria live in an insect's eggs, they spread only if the female reproduces. This strategy should drive the bacteria to evolve into forms that do not harm their host, he explains.

Wolbachia has gained fame for the unusual ways it manipulates the reproduction of insects and other arthropods without hurting them (SN: 11/16/96, p. 318). In some insects, for example, infected males can reproduce only with infected females, ensuring the bacterium's spread.

Beyond these reproductive machinations, "no one had found any negative repercussions of significance to having a *Wolbachia* infection. This [finding] suggests there can be some," says Scott L. O'Neill of Yale University Medical School.

The investigators do not yet know whether this lethal strain of *Wolbachia*, dubbed *popcorn* by Benzer and Min, stems from a single genetic alteration in the normal form or represents a variant that has undergone more complex changes to produce a different life cycle. In the latter case, *popcorn* may have evolved the ability to spread directly between fruit flies, rather than depending solely on the flies' reproduction.

"What we want to know is how closely related the *popcorn* strain is to the strain normally found," Werren says.

Benzer and Min plan to study why *popcorn* begins its population explosion only in adult flies—it seems to live peacefully inside earlier stages of the insect life cycle. The researchers suggest that further studies of *Wolbachia*, including the *popcorn* strain, may provide insight into related bacteria that cause such human diseases as typhus.

Finally, Benzer notes that many researchers have trouble keeping their fruit flies alive in the laboratory and that *Wolbachia* may sometimes be responsible for these so-called weak populations. —*J. Travis*

An alphabet for a letter-perfect protein

Just 26 letters, linked together in a myriad of permutations, capture all the richness of the English language. Likewise, all proteins are formed from only 20 amino acids strung into long chains and folded upon themselves into functional, three-dimensional shapes.

Now, researchers at the University of Washington in Seattle have found that a reduced alphabet of just five amino acids is enough to make much of a working protein. They successfully replaced the bulk of a small, biologically important protein with an equivalent structure made only of the amino acids isoleucine, lysine, glutamate, alanine, and glycine. Their findings appear in the October NATURE STRUCTURAL BIOLOGY.

"It's a splendid paper," says William F. DeGrado of the University of Pennsylvania in Philadelphia. "It dovetails nicely with what's known about the rules of protein folding. It shows that it's a simpler process than one would think."

That's good news for scientists interested in designing new proteins. It also supports the argument that life on Earth could have started with simple biochemical processes and gradually built to greater complexity (SN: 7/23/94, p. 58). The new results suggest that early life forms could have created functional proteins with only a few amino acids, says study coauthor David Baker.

Baker and his colleagues focused on a 57-unit chain, containing 18 different amino acids, from a protein subunit called the SH3 domain. This sequence is found in many large proteins that act as chemical messengers within and between cells. The chain folds back and forth on itself to create a structure, called a beta sheet, that resembles accordion pleats. Similar sheets occur in many proteins, helping define their three-dimensional forms.

The researchers created millions of candidate proteins by considering each amino acid position and choosing potential substitutes from a small set. They then screened the different combinations for ones that folded into the proper configuration. Isoleucine repels water, making it a good choice for the amino acids that point inward toward the protein core. Lysine and glutamate both attract water, making them ideal for the amino acids on the protein's outer surface.

Previous work had shown that combinations of just three amino acids are sufficient to form bundles of spiral-shaped structures called alpha helices. The bundles are rather formless, however. To make the more ordered beta sheet, the researchers found they had to use alanine and glycine at certain positions.

The researchers screened the candidate structures for the ability to bind to a peptide that attaches at a specific binding site within the SH3 domain. They did not try to replace the amino acids forming the binding site, Baker says.

"It's wise not to do that on the first pass," says Michael H. Hecht of Princeton University. That way, they could be sure that the new sequences had the same overall shape "because it places the binding site in the same place," he explains.

The reconstructed protein also folded at the same rate as the natural protein, Baker says, suggesting that today's protein sequences didn't evolve through a race to bend more efficiently.

The work is an "eye-opener," says Hecht. He compares the natural and simplified protein sequences to two very different languages. "If you only look at China, you may think it takes hundreds of characters to write a language. But then if you go to England, you see that you can write a perfectly good, functional language with only 26 characters."

Although five amino acids seem to be enough to form beta sheets, "proteins have to do more than fold," says Baker. More amino acids "allow enzymes to carry out more specialized tasks." As part of a biochemical language, proteins must benefit from being able to choose from a complete alphabet. —*C. Wu*