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HAVING ENEMIES MAKES YOU STRONGER, FINDS A STUDY OF FLIES CO-EVOLVED WITH BACTERIAL PATHOGEN

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Lab practice: We manually infect each individual fly using a sterile needle dipped in bacterial solution every single time, says Neetika Ahlawat | Photo Credit: Vanika Gupta, Vinesh Shenoi

The natural world is rife with pairs of antagonists. Plants and viruses, insects and pathogens, bacteria and their phages, and so on. In these systems, it is an interesting question to study how the resistance to a pathogen, in the case of the host, and virulence towards the host, in the case of a pathogen, evolve. Towards understanding this better, Indian Institute of Science Education and Research Mohali researchers have taken up the system of the fruit fly *(Drosophila melanogaster)* and a bacterial pathogen that affects the fruit fly, sometimes even causing death – *Pseudomonas entomophilia* – have been co-evolved to study the pathway of evolution taken by the system of antagonists. In this case, they find that being surrounded by enemies actually makes the organism stronger, or fitter, to combat the enemy.

How does one set up a co-evolving system experimentally? A population of flies are infected by the pathogen and the infection is allowed to take its course. Among the infected flies, only those that survive the infection, namely the ones that have the best immune systems to combat the pathogen, are taken to breed the next generation. Similarly, bacteria are collected from the flies that die due to the infection. These are the bacteria that have the virulence sufficiently strong to cause death in the present population. These bacteria are taken to breed and also infect the flies in the next generation. Thus, both the host (fruit fly) and the pathogen (bacteria) are 'selected' for having the maximum fitness.

The methodology of the experiment is like this: Four types of populations were bred in the lab. One in which, as described above, the host and pathogen both co-evolved. The second was a population in which only the host was selected from the flies that did not die due to the infection. Every generation, infection was done from a stock of ancestral bacteria which was not evolving. The third and fourth were two types of control populations.

This methodology allowed the researchers to compare the evolution process in hosts that were co-evolved against their pathogen and the hosts that were adapted against a static, non-evolving pathogen. They found that the former category evolved higher survivorship against the co-evolved pathogen than the hosts that was adapted against a non-evolving, static pathogen. Further they also found that the co-evolved hosts showed higher survivorship with respect to ancestral, unevolved pathogens than their counterparts who have been pitted against static pathogens.

From the point of view of the pathogens also a similar improvement in fitness was seen – the co-evolved pathogens develop greater virulence and capacity to cause death than the ancestral, unevolved pathogens. "This evolved virulence of co-evolving pathogens was higher towards both their local hosts (against which they coevolved each generation) and nonlocal hosts," says Dr. Neetika Ahlawat, from the Department of Biological Sciences at IISER Mohali, who led the research. The work has been published in the journal E *cology and Evolution.*

The evolution pathways described above then lead to the conclusion that having enemies makes the organism stronger. Just as the host evolves a stronger, more resistant immune system when pitted against a co-evolving pathogen, the pathogen also becomes more virulent when allowed to evolve against a co-evolving host.

The experiment is the first to study co-evolving insect–pathogen pair, that is, *Drosophila melanogaster* and *Pseudomonas entomophilia.* Earlier studies have either studied insects evolving against static pathogens or have studied bacteria and virus pairs that are co-evolving. “This is also the first study to compare insect–host evolution to a co-evolving pathogen versus host evolution towards a non-evolving or static pathogen,” says Prof. N.G. Prasad, senior author in the study.

The interactions being very complex, it was hard to predict the outcomes, and there were surprises: The researchers expected the co-evolving pathogens to show specificity towards their local co-evolving hosts as these two antagonists were allowed to co-evolve for several generations in closed association. “However, contrary to our expectations, we observed a rather more general effect of evolved virulence of the co-evolving pathogens,” he clarifies.

When the researchers started, they anticipated major challenges in conducting the study. There were four selection regimes (host–pathogen co-evolution, one-sided host evolution to a static pathogen and two controls) each with four population sets to evolve. “In view of the fly mortality caused due to pathogenic infection, we had to pick a relatively larger fly population size in both the selected populations,” says Dr. Ahlawat.

“In our lab, we manually infect each individual fly using a sterile needle dipped in bacterial solution every single time,” she says. Infecting about 1,000 flies individually for each group in one single day was a major challenge.

Each replicate (group) containing four populations (two selected and two control) were handled on different days. “This means 1,000 fly infections daily for four days. Therefore, we had to infect 4,000 flies each generation, over 20 generations,” says Dr. Ahlawat.

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