Mutualistic parasites

Francisco Dionisio[1,2,3]*, João Alves Gama[1,2], Iolanda Domingues[1,2], Ana Maria Reis[3,4], Helena Mendes-Soares[5], Ana Margarida Matos[4], André Filipe Paulino Carvalho[1,2]

[1]Instituto Gulbenkian de Ciência, Oeiras, Portugal, [2]Centro de Biologia Ambiental, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal, [3]Departamento de Biologia Vegetal, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal, [4]Center for Biodiversity, Functional and Integrative Genomics, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal, [5]Department of Biological Sciences and the Institute for Bioinformatics and Evolutionary Studies, University of Idaho, Moscow, Idaho, United States of America

*E-mail: francisco.dionisio@gmail.com; tel.: +351-966408422

Published work: Gama JA, Reis AM, Domingues I, Mendes-Soares H, Matos AM, et al. (2013) Temperate Bacterial Viruses as Double-Edged Swords in Bacterial Warfare. PLoS ONE 8(3): e59043. doi:10.1371/journal.pone.0059043

Introduction

A recent hypothesis, supported by mathematical models and computational simulations, suggests that a chronically infected host may have advantage in using its own pathogens to harm non-kin hosts[1,2,3].

We aimed to experimentally test this hypothesis. In our experimental system (Figure 1) we consider chronically infected *E. coli* individuals (lysogens) and non-infected individuals. Lysogens have the λ virus stably integrated in their genome and its vertical transmission is ensured. Under certain conditions a few lysogens produce viral progeny and die while releasing it to the environment. The free viruses are then able to invade new hosts. Although new viruses invade lysogens, the integrated virus renders these cells immune to new infections. When infecting susceptible individuals, the virus, either kills the host and releases its progeny, or integrates in their genome originating new lysogens.

We also analyzed the effect of different habitats on this behavior. It is expected that in structured habitats viruses spread in the neighborhood of lysogens and kill near competitors, leading to the accumulation of resources nearby while in unstructured habitats both viruses and resources are randomly distributed (thus benefits instead of directed to lysogens only are shared by the whole population).

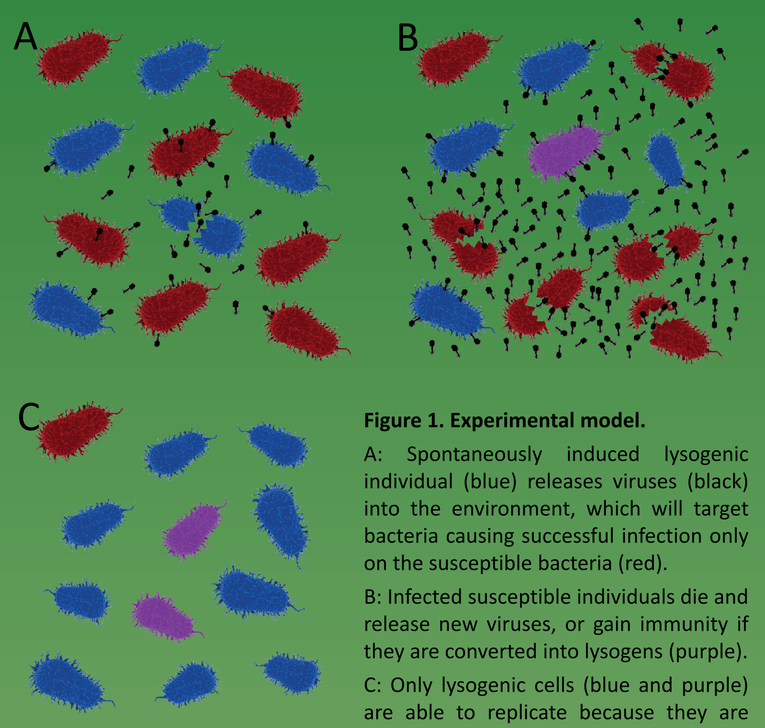

Figure 1. Experimental model.

A: Spontaneously induced lysogenic individual (blue) releases viruses (black) into the environment, which will target bacteria causing successful infection only on the susceptible bacteria (red).

B: Infected susceptible individuals die and release new viruses, or gain immunity if they are converted into lysogens (purple).

C: Only lysogenic cells (blue and purple) are able to replicate because they are immune to the virus.

Conclusions

- **The frequence of lysogens increases relatively to their competitor's frequency showing that the use of viruses as a weapon is of biological importance (Figure 2).**
- **When initially rare, the density of lysogens increases over time while the density of susceptible competitors decreases (Figure 3. A/B). However, when lysogens are initially dominant, their density is stably manintained and the density of the competitors decrease (Figure 3. C/D).**
- **At long term, the effect of the virus is less benefical to lysogens as competitors are gaining immunity, or resistance is selected (Figure 3. A/B/C).**
- **Viral amplification is greater when lysogens are initially rare due to the relative higher density of competitor cells where the virus is able to replicate (Figure 4).**

Infected hosts can use their pathogens to arm non-kin conspecifics and consequently outcompete them. This might explain the high presence of viruses in bacterial genomes[4] and retro-viruses in human genomes[5].

Results

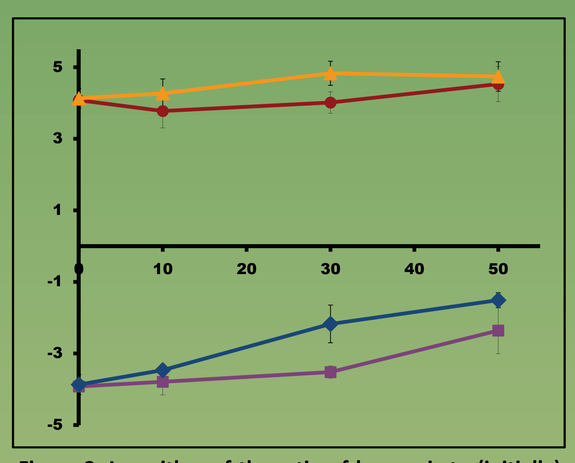

Figure 2. Logarithm of the ratio of lysogenic to (initially) susceptible strains during competitions.

Vertical axes represent the log10 of ratio of lysogenic to (initially) susceptible cells at 0, 10, 30 and 50 generations of competition. Each data set represents the variation of ratio of the strains under the different experimental conditions: ratio = 10^4:1, structured habitat (yellow triangles); ratio = 10^4:1, unstructured habitat (red circles); ratio = 1:10^4, structured habitat (blue diamonds); ratio = 1:10^4, unstructured habitat (purple squares). Error bars represent twice the standard error of the mean of three replicates. Linear regressions reveal that all lines have negative slopes (ANOVA, d.f. = (1,10), p<0,05).

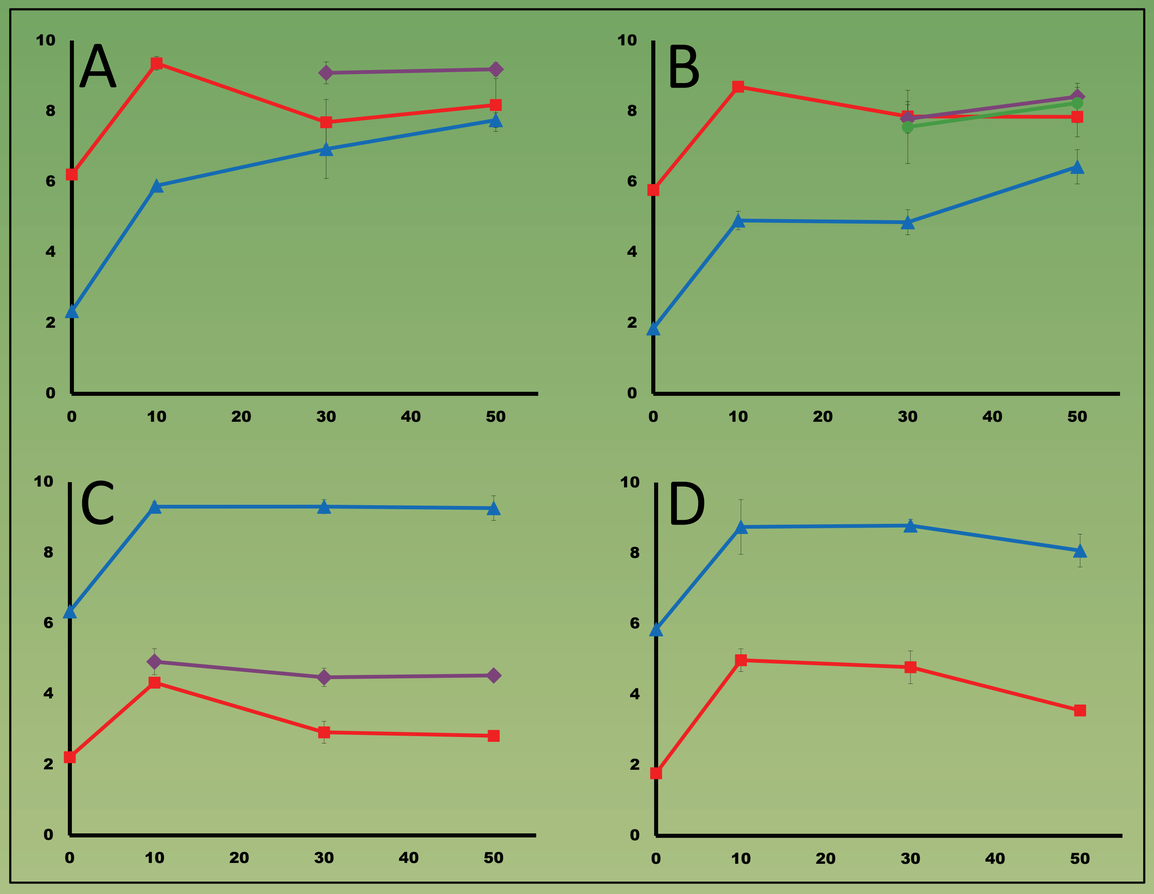

Figure 3. Logarithm of the densities of bacterial strains and virus during competition.

Vertical axes represent the log10 of the density of lysogenic cells (blue triangles), of susceptible cells (red squares), of lysogenic (initially susceptible) cells (purple diamonds) and of resistant (initially susceptible) cells susceptible (green circles) in the beginning of competitions and after 10, 30 and 50 generations. Error bars represent twice the standard error of the mean of three replicates. In all competitions, the initial total bacterial density was around 10^6 cells/ml. We varied the initial ratio of lysogenic to susceptible cells and the structure of the habitat: A: ratio = 1:10^4, structured habitat; B: ratio = 1:10^4, unstructured habitat; C: ratio = 10^4:1 structured habitat; D: ratio = 10^4:1, unstructured habitat.

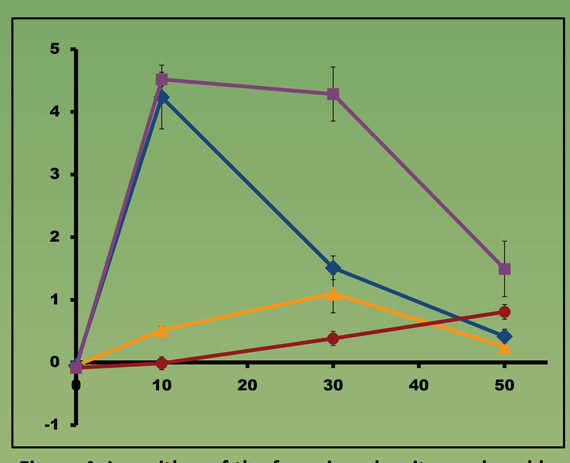

Figure 4. Logarithm of the free virus density produced by susceptible cells during competitions.

Vertical axes represent the log10 of free virus density produced by susceptible cells at 0, 10, 30 and 50 generations. These values were calculated by discounting the expected amount of virus produced by spontaneous induction of lysogens (assuming that there a virus per 3.13x10^4 lysogens cells in pure) to the total values of free virus measure at each point. Each data set represents the viral density under the different experimental conditions: ratio = 10^4:1, structured habitat (yellow triangles); ratio = 10^4:1, unstructured habitat (red circles); ratio = 1:10^4, structured habitat (blue diamonds); ratio = 1:10^4, unstructured habitat (purple squares). Error bars represent twice the standard error of the mean of three independent replicates.

Methods

We competed two *E. coli* strains with identical growth rates. They were isogenic except for selective markers and for the presence of the integrated virus. Competitions varied in the initial frequency of lysogens and habitat structure. Competitions in unstructured habitats were performed in Luria Broth (LB) at 37ºC with agitation (170 rpm) during 50 generations, with serial transfer occurring every 10 generations. Competitions in structured habitats were identical; however bacteria were embedded in a static soft-agar matrix instead of liquid LB.

We measured the titer of each strain (by plating in selective media) and free virus (spot test in lawns of the susceptible strain) and the virus-sensitivity of the susceptible strain (cross-streak immunity test) after 10, 30 and 50 generations.

References

[1] Rozsa, L. (1999) Influencing random transmission is a neutral character in hosts, *Journal of Parasitology*, 85, 1032-1035.

[2] Rozsa, L. (2000) Spite, xenophobia, and collaboration between hosts and parasites, *Oikos*, 91, 396-400.

[3] Dionisio, F. (2007) Selfish and spiteful behaviour through parasites and pathogens, *Evolutionary Ecology Research*, 9, 1199-1210.

[4] Ohnishi, M., Kurokawa, K. & Hayashi, T. (2001) Diversification of Escherichia coli genomes: are bacteriophages the major contributors?, *Trends in Microbiology*, 9, 481-485.

[5] Collins, F. S., Lander, E. S., Rogers, J., Waterston, R. H. & Conso, I. H. G. S. (2004) Finishing the euchromatic sequence of the human genome, *Nature*, 431, 931-945.

Acknowledgments

This work was supported by Fundação para a Ciência e Tecnologia: a Ph.D. fellowship SFRH/BD/82375/2011 and projects EXPL/BIA-EVF/1281/2012, PTDC/BIA-BDE/66180/2006 and TECH/2007/0001, as well to the TECT-European Science Foundation program.