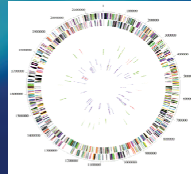


Basic and Applied Aspects of Insect Symbiosis



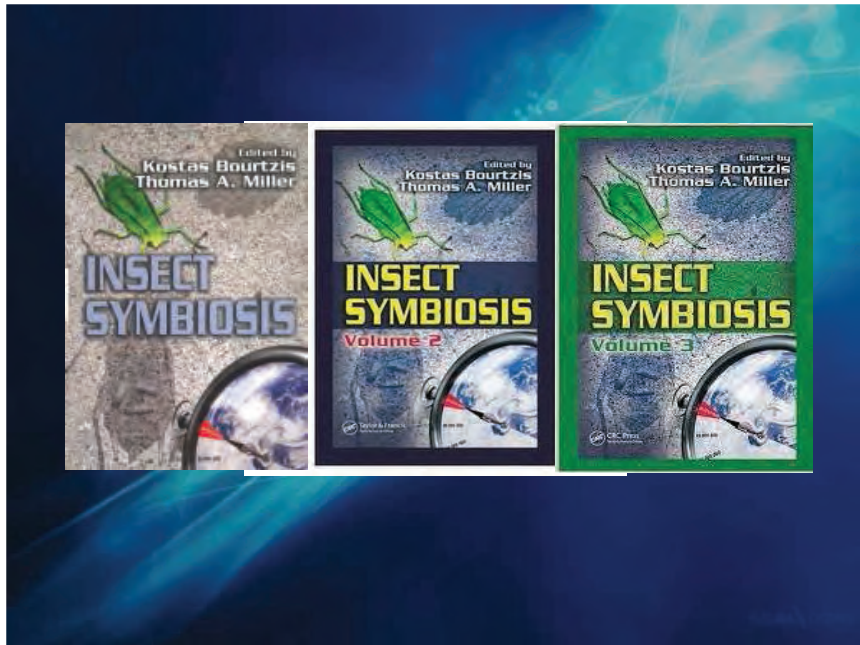
Kostas Bourtzis

Department of Environmental and Natural Resources Management
University of Ioannina, Greece

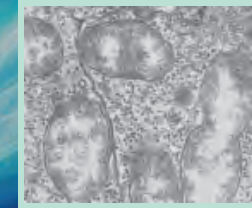
Outline of the Presentation

- Introduction to MicroBioKosmos and SymbioKosmos
- Insect Symbionts
 - different classes
 - biological role
 - applications
 - future of Insect Symbiosis in Europe?

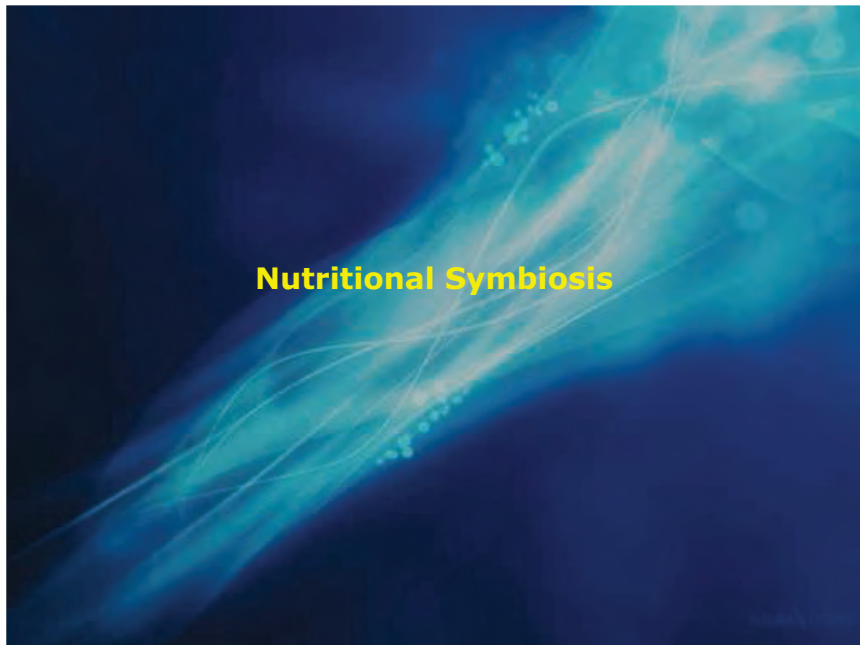




What do symbiotic bacteria do in arthropods?



- Supplement nutrition deficiencies
- Help cope with environmental factors (biotic & abiotic)
- Reproductive manipulations



Nutritional Symbiosis I

Buchnera: nutritional symbiont (or organelle) in aphids

Genome sequence of the endocellular bacterial symbiont of aphids *Buchnera* sp. APS

Genome sequence of the endocellular bacterial symbiont of aphids, *Buchnera* sp. APS
 M. Saito et al. *Genome Research* 14:1111-1122 (2004)
 Copyright © 2004 Cold Spring Harbor Laboratory Press
 10.1101/241502

Letters to nature

Direct, host, and symbiont DNA in the CG of these aphids are associated with the host's symbiont population and to the host's feeding behavior (the host's diet). The data also show an association between the host's feeding behavior and the host's population dynamics. We also found a strong association between the host's feeding behavior and the host's population dynamics. We also found a strong association between the host's feeding behavior and the host's population dynamics.

We identified 101 genes in the genome with an average size of 366 bp, covering 80% of the total genome (Fig. 1). The majority of the genes had bacterial origins (Fig. 2). The majority of the genes were found in the genome of the host's population dynamics.

Jan O. Andersson

Evolutionary genomics: Is *Buchnera* a bacterium or an organelle?

Jan O. Andersson

Portiera: in whitefly

The first genome sequence of an intracellular bacterial symbiont of a eukaryotic cell has been determined. The *Buchnera* genome shares features with the genomes of both intracellular pathogenic bacteria and eukaryotic organelles, and it may represent an intermediate between the two.

Address: Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4R2
 Email: janders@dal.ca

Genome Biology 2003, 4(12):R48

DOI: 10.1186/gb-2003-4-12-r48
 © 2003 Andersson et al; licensee BioMed Central Ltd. All rights reserved.

the whitefly genome [1]. Indeed, the presence of prokaryotic-like features, such as *Mycobacterium leprae* and *Rickettsia*, indicates that they have very dynamic genomes with frequent gene loss. In contrast, the genome size ratio within *Buchnera* strains is low like the ones from the various divergent eukaryotes that the pathogenic bacteria. This suggests that the pathogenic bacteria have a limited amount of genes and that the genome is not in the *Buchnera* genome [5]. For this reason, the eight chromosomes of *Buchnera* are not considered as such in the *Buchnera* genome map presented [1].

Help cope with environmental factors I

OPEN ACCESS Freely available online

PLoS BIOLOGY

Aphid Thermal Tolerance Is Governed by a Point Mutation in Bacterial Symbionts

Helen E. Dunbar, Alex C. C. Wilson¹, Nicole R. Ferguson, Nancy A. Moran¹

Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona, United States of America

Symbiosis is a ubiquitous phenomenon generating biological complexity, affecting adaptation, and expanding ecological capabilities. However, symbionts, which can be subject to genetic limitations such as clonality and genomic degradation, also impose constraints on hosts. A model of obligate symbiosis is that between aphids and the bacterium *Buchnera aphidicola*, which supplies essential nutrients. We report a mutation in *Buchnera* of the aphid *Acyrtosiphon pisum* that recurs in laboratory lines and occurs in field populations. This single nucleotide deletion affects a homopolymeric run within the heat-shock transcriptional promoter for *hspA*, encoding a small heat-shock protein. This *Buchnera* mutation virtually eliminates the transcriptional response of *hspA* to heat stress and lowers its

Help cope with environmental factors II

Facultative bacterial symbionts in aphids confer resistance to parasitic wasps

Kerry M. Oliver¹, Jacob A. Russell¹, Nancy A. Moran¹, and Martha S. Hunter^{1*}

Departments of ¹Entomology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721

Edited by Lynn Margulis, University of Massachusetts, Amherst, MA, and approved December 16, 2002 (received for review August 28, 2002)

Symbiotic relationships between animals and microorganisms are common in nature, yet the factors controlling the abundance and distributions of symbionts are mostly unknown. Aphids have an obligate association with the bacterium *Buchnera aphidicola* (the primary symbiont) that has been shown to contribute directly to aphid fitness. In addition, aphids sometimes harbor other vertically transmitted bacteria (secondary symbionts), for which few benefits of infection have been previously documented. We carried out experiments to determine the consequences of these facultative symbioses in *Acyrtosiphon pisum* (the pea aphid) for vulnerability of the aphid host to a hymenopterous parasitoid, *Aphidius ervi*, a major natural enemy in field populations. Our results show that, in a controlled genetic background, infection confers resistance to parasitoid attack by causing high mortality of developing parasitoid larvae. Compared with uninfected controls, experimentally

A. pisum clones vary greatly in their resistance to *A. ervi* development following oviposition. To determine the possible contribution of SS to this variation, we established genetically uniform aphid lineages that differed only in their SS infection status. To eliminate any effects of genetic variation of the aphids in resistance to parasitism, we introduced an uninfected *A. pisum* clone with SS from body fluids of clones harboring each of the 7-3 proteobacteria SS types, thus creating three genetically uniform lineages of aphids that differed from the original only by the presence of a particular SS. These lineages were used in experiments to determine the consequences of these facultative symbioses for aphid vulnerability to an important hymenopterous parasitoid. The major protective effects that we documented may contribute to the maintenance and spread of facultative symbionts in host populations.

Hamiltonella defensa, genome evolution of protective bacterial endosymbiont from pathogenic ancestors

Patrick H. Degnan¹, Yeisoo Yu², Nicholas Siseros¹, Rod A. Wing¹, and Nancy A. Moran¹

¹Department of Ecology and Evolutionary Biology, ²Arizona Genomics Institute, University of Arizona, Tucson, AZ 85721

Edited by Edward F. DeLong, Massachusetts Institute of Technology, Cambridge, MA, and approved April 14, 2009 (received for review January 7, 2009)

Eukaryotes engage in a multitude of beneficial and deleterious interactions with bacteria. *Hamiltonella defensa*, an endosymbiont of aphids and other sap-feeding insects, protects its aphid host from attack by parasitoid wasps. Thus *H. defensa* is only condi-

tionally transmitted either intraspecifically [e.g., sexually (22)] or interspecifically (12, 17). Moreover, protection by *H. defensa* has been shown to be transferable between distantly related aphid species (19).

Help cope with environmental factors III

Variation in resistance to parasitism in aphids is due to symbionts not host genotype

Kerry M. Oliver¹, Nancy A. Moran¹, and Martha S. Hunter^{1*}

Departments of ¹Entomology and ²Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721

Contributed by Nancy A. Moran, July 20, 2005

Natural enemies are important ecological and evolutionary forces, and heritable variation in resistance to enemies is a prerequisite for adaptive responses of populations. Such variation in resistance has been previously documented for pea aphids (*Acyrtosiphon pisum*) attacked by the parasitoid wasp *Aphidius ervi*. Although the variation was presumed to reflect genotypic differences among the aphids, another potential source of resistance to *A. ervi* is infection

transmitted (mother to offspring) facultative ("secondary") symbionts (SS) in addition to the obligate primary symbiont *Buchnera aphidicola*. Although the nutritional function of *Buchnera* is relatively well understood (17, 18), the roles of these SS in *A. pisum* are only now coming to light. *Regiella insecticola* (formerly the U-type or PAUS) has been implicated in host-plant specialization in Japanese *A. pisum* (ref. 16; but see ref. 19).

Symbionts and Host Selection Behavior

A newly discovered bacterium associated with parthenogenesis and a change in host selection behavior in parasitoid wasps

E. Zchori-Fein¹, Y. Gottlieb², S. E. Kelly³, J. K. Brown¹, J. M. Wilson¹, T. L. Karr⁴, and M. S. Hunter^{1*}

¹Department of Plant Sciences, 303 Forbes Building, University of Arizona, Tucson, AZ 85721; ²Department of Organismal Biology and Anatomy, 1027 East 57th Street, University of Chicago, Chicago, IL 60637; ³Department of Entomology, 410 Forbes Building, University of Arizona, Tucson, AZ 85721; and ⁴Department of Cell Biology and Anatomy, P.O. Box 245044, University of Arizona, Tucson, AZ 85721

Communicated by Margaret G. Kibvel, University of Arizona, Tucson, AZ, September 4, 2001 (received for review February 6, 2001)

The symbiotic bacterium *Wolbachia pipiensis* has been considered unique in its ability to cause multiple reproductive anomalies in its arthropod hosts. Here we report that an undescribed bacterium is vertically transmitted and associated with thelytokous parthenogenetic reproduction in *Encarsia*, a genus of parasitoid wasps. Although *Wolbachia* was found in only one of seven parthenoge-

might expect selection on both bacterial and wasp genomes to act to prevent infected females from accepting hosts that may be suitable for male but not female development. In most cases, these behavioral refinements may be too subtle to measure, but they are likely to be very important in those parasitoids in which males and females generally develop in different host environments (14).

doi:10.1111/j.1469-7520.2009.02024.x

Manipulation of oviposition choice of the parasitoid wasp, *Encarsia pergandeia*, by the endosymbiotic bacterium *Candidium*

S. G. KENYON¹ & M. S. HUNTER¹

¹Department of Entomology, University of Arizona, Tucson, Arizona, United States of America; ²Department of Entomology, University of Arizona, Tucson, AZ, USA

J. Evol. Biol.

PNAS

PNAS

PNAS

PNAS

Symbionts and Fitness Effects

Hereditas, (7 July 2010) | doi:10.1038/hdy.2010.89

Endosymbiont costs and benefits in a parasitoid infected with both *Wolbachia* and *Cardinium*

J A White, S E Kelly, S N Cockburn, S J Periman and M S Hunter

Theory suggests that maternally inherited endosymbionts can promote their spread and persistence in host populations by enhancing the production of daughters by infected hosts, either by improving overall host fitness, or through reproductive manipulation. In the doubly infected parasitoid wasp *Encarsia inaron*, *Wolbachia* manipulates host reproduction through cytoplasmic incompatibility (CI), but *Cardinium* does not. We investigated the fitness costs and/or benefits of infection by each bacterium in differentially cured *E. inaron* as a potential explanation for persistence of *Cardinium* in this population. We introgressed lines infected with *Wolbachia*, *Cardinium* or both with the cured line to create a similar genetic background, and evaluated several parasitoid fitness parameters. We found that symbiont infection resulted in both fitness costs and benefits for *E. inaron*. The cost was lower initial egg load for all infected wasps. The benefit was increased survivorship, which in turn increased male production for wasps infected with only *Cardinium*. Female production was unaffected by symbiont infection; we therefore have not yet identified a causal fitness effect that can explain the persistence of *Cardinium* in the population. Interestingly, the *Cardinium* survivorship benefit was not evident when *Wolbachia* was also present in the host, and the reproduction of doubly infected individuals did not differ significantly from uninfected wasps. Therefore, the results of our study show that even when multiple infections seem to have no effect on a host, there may be a complex interaction of costs and benefits among symbionts.

Fecundity
Fertility
Longevity
Mating behavior
etc

Symbionts and Insecticide Resistance

Rapid Report

The presence of *Rickettsia* is associated with increased susceptibility of *Bemisia tabaci* (Homoptera: Aleyrodidae) to insecticides

Svetlana Kotsedalov¹, Einat Zchori-Fein²,
Elad Chiel^{2,3}, Yuval Gottlieb¹, Moshe
Inbar³, Murad Ghanim^{1,*}

Issue

Article first published online: 23 APR 2008

DOI: 10.1002/ps.1595

Copyright © 2008 Society of Chemical Industry



Pest Management Science
Volume 64, Issue 8, pages
789–792, August 2008

Help cope with environmental factors VII

Mainly vertically (maternal transmission) but also horizontally!

Almost There: Transmission Routes of Bacterial Symbionts between Trophic Levels

Elad Chiel^{1,2,3,*}, Einat Zchori-Fein², Moshe Inbar¹, Yuval Gottlieb⁴, Tetsuya Adachi-Hagimori^{3,5}, Suzanne E. Kelly³, Mark K. Asplen⁶, Martha S. Hunter³

¹ Department of Evolutionary and Environmental Biology, University of Haifa, Haifa, Israel, ² Department of Entomology, Neve-Ya'ar Research Center, ARO, Ramat-Yishai, Israel, ³ Department of Entomology, University of Arizona, Tucson, Arizona, United States of America, ⁴ Department of Entomology, the Volcani Center, ARO, Beit-Dagan, Israel, ⁵ Graduate School of Biosphere Sciences, Hiroshima University, Higashi-Hiroshima, Hiroshima, Japan, ⁶ Department of Entomology, University of Minnesota, St. Paul, Minnesota, United States of America

PLoS One

Symbiont-induced Reproductive Manipulations

Reproductive manipulations

Wolbachia
Cardinium
Arsenophonus
Rickettsia
Spiroplasma

Feminization
Parthenogenesis
Male killing
Cytoplasmic Incompatibility

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Microbiology

Wolbachia: more than just a bug in insects genitals
Aggeliki Saridaki and Kostas Bourtzis

Research on the intracellular bacterial symbiont *Wolbachia* has grown on many levels, providing interesting insights on various aspects of the mosquito's biology. Although data from fully sequenced genomes of different *Wolbachia* strains and from experimental studies of host-microbe interactions continue to arise, most of the molecular mechanisms employed by *Wolbachia* to manipulate the host cytoplasmic machinery and to ensure vertical transmission are yet to be discovered. Apart from the well-established role of *Wolbachia* in triggering reproductive alterations, a new fascinating aspect is emerging, related to the ecological benefits that the symbiont provides to

been detected in many host tissues (Figure 1). *Wolbachia* has evolved several strategies to ensure vertical transmission through the manipulation of host reproductive system. These strategies include feminization, parthenogenesis, male killing and cytoplasmic incompatibility. All the above phenotypes, commonly referred to as 'reproductive parasitism', increase the frequency of infected females in the host population.

PCR-based screening methods revealed the widespread distribution of the symbiont in diverse hosts, covering

Introduction to Wolbachia biology

Wolbachia manipulates oogenesis thru apoptosis

Removing symbiotic *Wolbachia* bacteria specifically inhibits oogenesis in a parasitic wasp

Franck Dedeine^{1*}, Fabrice Vavre², Frédéric Fleury³, Benjamin Loppin⁴, Michael E. Hochberg⁵, and Michel Boulétreau⁴

¹Biometrie et Biologie Evolutive, Unité Mixte de Recherche-Centre National de la Recherche Scientifique, 5558 Université Lyon 1, 43, Boulevard du 11 Novembre 1918, 69622 Villeurbanne Cedex, France; ²Centre de Génétique Moléculaire et Cellulaire, Unité Mixte de Recherche-Centre National de la Recherche Scientifique, 5534 Université Lyon 1, 43, Boulevard du 11 Novembre 1918, 69622 Villeurbanne Cedex, France; and ³Institut des Sciences de l'Evolution, Unité Mixte de Recherche-Centre National de la Recherche Scientifique, 5554 Université Montpellier II, Place Eugène Bataillon, 34095 Montpellier Cedex 5, France

Edited by Lynn Margulis, University of Massachusetts, Amherst, MA, and approved March 16, 2001 (received for review June 30, 2000)

Parasitic inhibition of cell death facilitates symbiosis

Bart A. Pannebakker^{1,2*}, Benjamin Loppin³, Coen P. H. Elemans⁴, Lionel Humbiot⁵, and Fabrice Vavre⁶

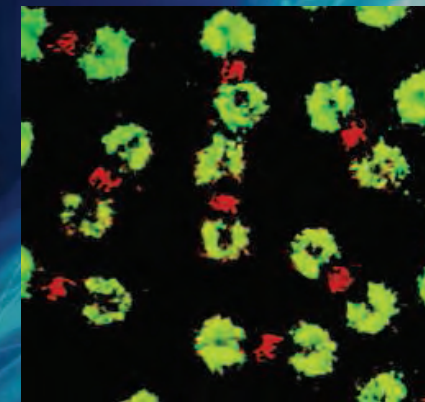
¹Laboratoire de Biométrie et Biologie Evolutive, Unité Mixte de Recherche 5558, and ²Centre de Génétique Moléculaire et Cellulaire, Unité Mixte de Recherche 5534, Centre National de la Recherche Scientifique, Université Claude Bernard Lyon 1, 43, Boulevard du 11 Novembre 1918, 69622 Villeurbanne Cedex, France; ³Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Arthur Keith Laboratory, King's Buildings, West Main Road, Edinburgh EH9 3JF, Scotland, United Kingdom, and ⁴Department of Biology, University of Utah, Salt Lake City, UT 84112

Edited by Nancy A. Moran, University of Arizona, Tucson, AZ, and approved November 9, 2006 (received for review September 7, 2006)

Symbiotic microorganisms have had a large impact on eukaryotic evolution, with effects ranging from parasitic to mutualistic. Mitochondria and chloroplasts are prime examples of symbiotic microorganisms that have become obligate for their hosts, allowing them to colonize a wide range of suitable habitats. Out of the

chambers, while at this stage apoptosis does not occur in the ovarioles of symbiotic control females (Fig. 2) (Mann-Whitney *U* test: $W = 464$; $n = 45$; $P < 0.001$). Apoptosis is an essential component of insect oogenesis and occurs at several stages

Wolbachia interacts with host microtubules



[Bourtzis' Lab]

Host Distribution of Wolbachia Strains

Arthropods

- Insects
- Mites
- Isopods
- Scorpions
- Spiders
- Springtails

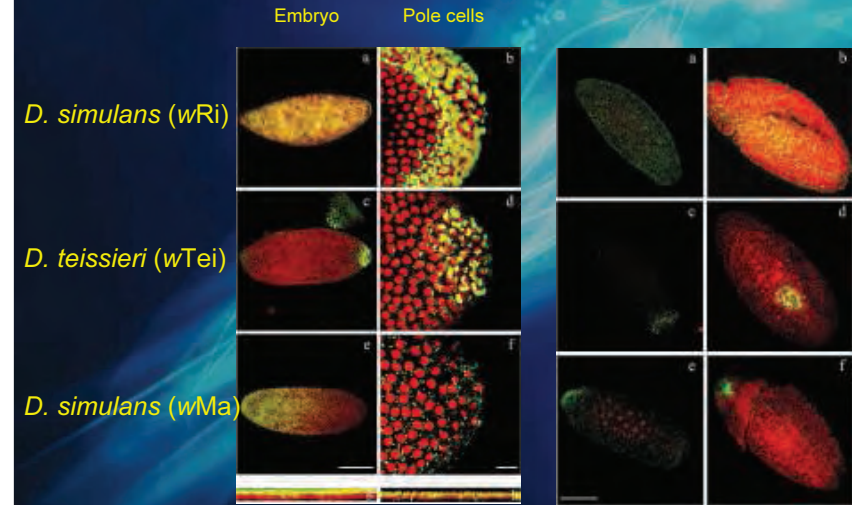
Nematodes

- Filarial worms (mainly)

Not infected:

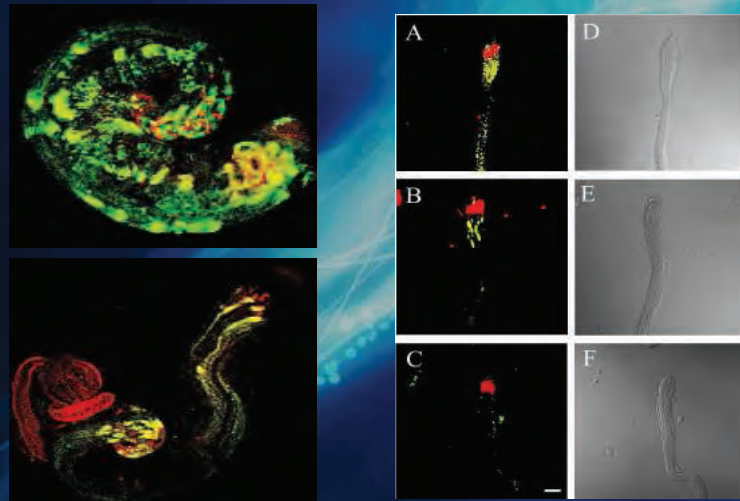
- many species of agricultural importance (e.g. *Bactrocera oleae*)
- many species of medical importance (e.g. *Anopheles gambiae*)
- many species of environmental importance (e.g. *Dendroctonus* spp.)

Wolbachia during *Drosophila* embryogenesis



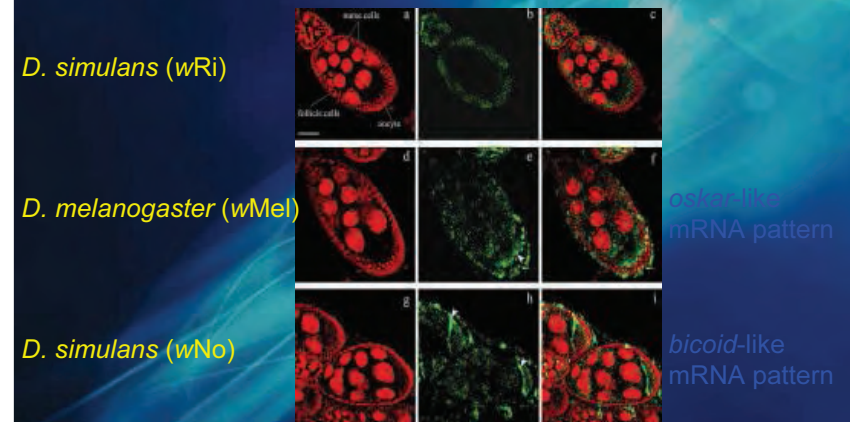
[Veneti et al. (2004) Appl. Env. Microbiol. 70: 5366-5372]

Wolbachia during *Drosophila* spermatogenesis



[Veneti et al. (2003), Genetics 164: 545-552; Clark et al. (2002), Mech. Devel. 111: 3-15; Clark et al. (2003), Mech. Devel. 120: 85-98]

Wolbachia during *Drosophila* oogenesis



oskar-like mRNA pattern

bicoid-like mRNA pattern

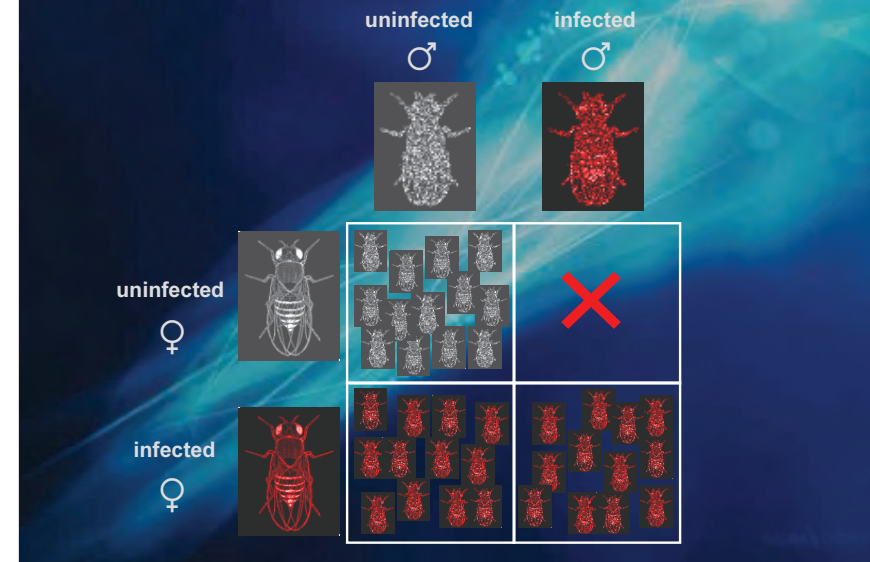
[Veneti et al. (2004) Appl. Env. Microbiol. 70: 5366-5372]

Wolbachia-Induced Reproductive Abnormalities

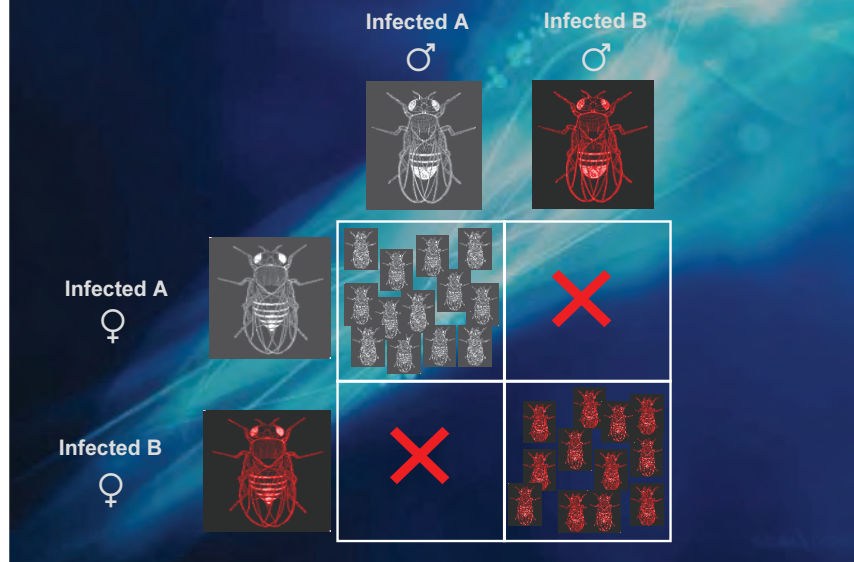
Wolbachia induce a number of reproductive alterations, such as:

- Feminization
 - Parthenogenesis
 - Male-killing
 - Cytoplasmic Incompatibility
- Spreading
 - Curing - Antibiotics

Uni-Directional CI



Bi-Directional CI



Wolbachia Genomics

The mosaic genome structure of the *Wolbachia* wRi strain infecting *Drosophila simulans*

Lisa Klasson¹, Joakim Westberg¹, Panagiotis Sapountzis², Kristina Näslund³, Ylva Lutnaes⁴, Alistair C. Darby^{4,2}, Zoe Veneti⁵, Lanming Chen^{6,1}, Henk R. Braig⁷, Roger Garrett⁸, Kostas Bourtzis^{9,1}, and Siv G. E. Andersson^{4,1}

¹Department of Molecular Evolution, Evolutionary Biology Centre, Uppsala University, SE-752 38 Uppsala, Sweden; ²Department of Environmental and Natural Resources Management, University of Gothenburg, SE-400 29 Gothenburg, Sweden; ³Institute of Molecular Biology and Biotechnology, FORTH, 71110 Heraklion, Crete, Greece; ⁴Centre for Comparative Genomics, Department of Biology, University of Copenhagen, DK-2200 Copenhagen R, Denmark; and ⁵School of Biological Sciences, Bangor University, Wales, LL57 2UW, United Kingdom

Edited by Nancy A. Moran, University of Arizona, Tucson, AZ, and approved February 20, 2009 (received for review October 24, 2008)

PNAS

Wolbachia-based applications

Wolbachia and Applied Biology

For example:

1. Asexuality
 2. As an expression vector
 3. As a tool for the modification of population age structure
 4. As a spreading mechanism
 5. As a tool for population suppression of insect pests
- and
6. Wolbachia and Immunity

Wolbachia and Applied Biology

For example:

1. Asexuality
 2. As an expression vector
 3. As a tool for the modification of population age structure
 4. As a spreading mechanism
 5. As a tool for population suppression of insect pests
- and
6. Wolbachia and Immunity

Wolbachia-induced cytoplasmic incompatibility as a means for insect pest population control

Sofia Zabalou^{1*}, Markus Riegler^{2,3}, Marianna Theodorakopoulou¹, Christian Stauffer⁴, Charalambos Savakis⁵, and Kostas Bourtzis^{6,4,1†}

¹Department of Medical Sciences, Medical School, and ²Department of Biology, University of Crete, Heraklion 711 10, Crete, Greece; ³Technological Educational Institute of Crete, Heraklion 711 10, Crete, Greece; ⁴Institute of Forest Entomology, Forest Pathology and Forest Protection, Department of Forest and Soil Sciences, BOKU, University of Natural Resources and Applied Life Sciences, 1180 Vienna, Austria; ⁵Department of Zoology and Entomology, University of Queensland, St. Lucia QLD 4072, Australia; ⁶Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology, Hellas, Vassilika Vouton, P.O. Box 1527, Heraklion 711 10, Crete, Greece; and ^{**}Department of Environmental and Natural Resources Management, University of Ioannina, 2 Salfeti Street, Agrinio 26100, Greece

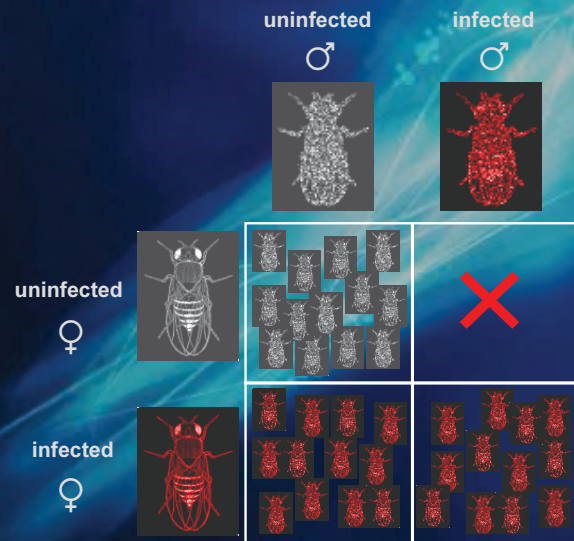
Edited by John H. Law, University of Georgia, Athens, GA, and approved September 9, 2004 (received for review May 31, 2004)

Biological control is the purposeful introduction of parasites, predators, and pathogens to reduce or suppress pest populations. *Wolbachia* are inherited bacteria of arthropods that have recently attracted attention for their potential as new biocontrol agents. *Wolbachia* manipulate host reproduction by using several strategies, one of which is cytoplasmic incompatibility (CI) [Stouthamer, R., Breeuwer, J. A. J. & Hurst, G. D. D. (1999) *Annu. Rev. Microbiol.* 53, 71–102]. We established *Wolbachia*-infected lines of the medfly *Ceratitis capitata* using the infected cherry fruit fly *Rhagoletis cerasi* as donor. *Wolbachia* induced complete CI in the novel host. Laboratory cage populations were completely suppressed by single releases of infected males, suggesting that *Wolbachia*-induced CI could be used as a novel environmentally friendly tool for the control of medfly populations. The results also encourage the introduction of *Wolbachia* into pest and vector species of economic and hygienic relevance to suppress or modify natural populations.

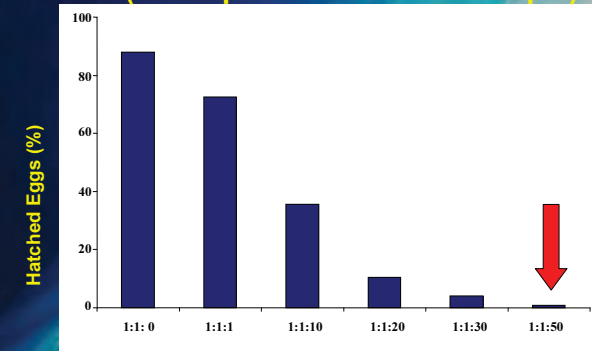
transfer of natural bacterial symbionts from a related species, *R. cerasi* (Diptera, Tephritidae) (18), to an uninfected laboratory strain of medfly *C. capitata* (Diptera, Tephritidae), the Benackon strain.

Previous studies have demonstrated high levels of incompatibility between natural populations of *R. cerasi* (10, 19), the basis of which was recently shown to be *Wolbachia* (18). Populations of *R. cerasi* are either infected by a single *Wolbachia* variant, wCer1, or coinfecting by two variants, wCer1 and wCer2. Incompatibility occurs between males from doubly infected populations and females from singly infected populations, suggesting the wCer2 infection as the cause of CI (18). Additionally, transfer of wCer2 in *Drosophila simulans* also resulted to the induction of CI (17). An additional, yet uncharacterized, *Wolbachia* strain (wCer3) has been recently found in Sicilian populations of *R. cerasi* (M.R. and C. Stauffer, unpublished results).

Uni-Directional CI



Suppression of medfly populations using *Wolbachia*-induced CI (Incompatible Insect Technique)



	1:1:0	1:1:1	1:1:10	1:1:20	1:1:30	1:1:50
Number of adults	300	300	300	306	290	520
Number of eggs scored	3000	3000	2097	1688	858	700

[Zabalou et al. (2004), PNAS, 101: 15042-15045]

IIT like SIT

100% CI (100% embryonic mortality)

Incompatible insect technique: incompatible males from a *Ceratitis capitata* genetic sexing strain

S. Zabalou^{1,2}, A. Apostolaki¹, I. Livadaras¹, G. Franz³, A. S. Robinson³, C. Savakis^{1,4} & K. Bourtzis^{5*}

¹Institute of Molecular Biology and Biotechnology, FORTH, Yerosolima Vasilia, PO Box 1327, Heraklion 71110, Crete, Greece;

²Technological Educational Institute of Crete, Heraklion 71110, Crete, Greece; ³Entomology Unit, FAO/ILRI Agriculture and Biotechnology Laboratory A-2444 Siberaof, Austria; ⁴Medical School, University of Crete, Heraklion 71110, Crete, Greece; and ⁵Department of Environmental and Natural Resources Management, University of Ioannina, 2 Sefeei Street, Agrinio 26070, Greece

Accepted: 25 May 2009

Key words: *Wolbachia*, cytoplasmic incompatibility, SIT, IIT, medfly, pest control, Diptera, Tephritidae

Entomol. Exp. Appl.

Incompatible Insect Technique (I.I.T.)

- Based on the mechanism of *Wolbachia*-induced CI
- Analogous to S.I.T.
- Effective sexing system is necessary
- Environmentally friendly technology
- Low technological input
- Low cost technology
- Higher competitiveness of released males
- Successful applications (Medfly, Olive fly, *C. pipiens*, *Cadra cautella*)
- We welcome suggestions for collaborations to apply the I.I.T. to your favourite insect pest species!

Wolbachia and Applied Biology

For example:

- 1. Asexuality
- 2. As an expression vector
- 3. As a tool for the modification of population age structure
- 4. As a spreading mechanism
- 5. As a tool for population suppression of insect pests and
- 6. Wolbachia and Immunity

Wolbachia and Virus Protection in Insects

Lucy M. Hejblum, Jeremy C. Strickland, Sarah J. O'Neill, Kaye N. Johnson

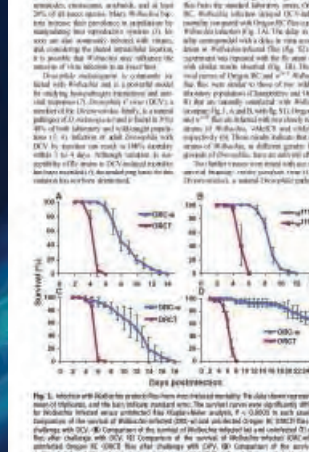


Fig. 1. Mortality of Wolbachia-infected mosquitoes. Survival curves were generated for mosquitoes that were challenged with a lethal dose of virus. The survival curves were generated for mosquitoes that were challenged with a lethal dose of virus.

Wolbachia, a maternally inherited, Gram-negative bacterium, is found in a wide range of arthropods. In insects, it is often associated with asexuality, reproductive manipulation, and host-plant specialization. Wolbachia is also known to interact with several human pathogens, including dengue and Zika viruses. In this study, we investigated the protective effects of various Wolbachia strains against a lethal challenge in the mosquito *Aedes aegypti*.

We compared the survival of six Wolbachia-infected mosquito lines (wMel, wMelPop, wMel102, wMel104, wMel106, wMel109) against a control group (wO). The survival curves were generated for mosquitoes that were challenged with a lethal dose of virus. The survival curves were generated for mosquitoes that were challenged with a lethal dose of virus.

Immune Activation by Life-Shortening Wolbachia and Reduced Filarial Competence in Mosquitoes

Zakeria Karimiri, Peter E. Cook, Hoang N. Phuc, Steven P. Stöckli

Wolbachia strain wMelPop reduces the longevity of its *Anopheles stephensi* host and, when introduced into the mosquito *Aedes aegypti*, halves its life span. We show that wMelPop induces up-regulation of the mosquito's innate immune system and that its presence inhibits the development of filarial nematodes in the mosquito. These data suggest that wMelPop could be used in the global effort to eliminate lymphatic filariasis and possibly for the control of other mosquito-borne parasites where immune preactivation inhibits their development. The cost of constitutive immune up-regulation may contribute to the life-shortening phenotype.

Wolbachia pipiensis is a maternally inherited intracellular bacterium of arthropods, capable of spreading itself through populations by reproductive manipulation such as cytoplasmic incompatibility (CI). The strain wMelPop or "popcorn" usually reduces the longevity of its *Anopheles stephensi* host (1) and also has been shown to have lifespan when the mosquito *Aedes aegypti* was stably trans-

ferred (2). The wMelPop life-shortening phenotype often the prospect of a disease control system by potentially skewing the population structure toward younger individuals. Vectorial capacity is particularly sensitive to mosquito age because mosquito-borne pathogens require an extrinsic incubation period between ingestion and transmission that is long relative to mean life span in the field, such that only older mosquitoes within

a population are potentially infective. wMelPop was also found to be inherited at high rates and to induce strong CI in *Ae. aegypti*, which provides a reproductive advantage to infected females. The wMelPop strain should be capable of spreading through populations despite the reduction in mean life span, because reproduction by older individuals makes a relatively small contribution to the next generation (2-6).

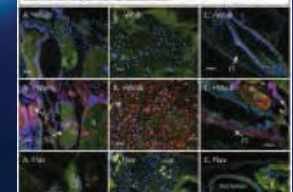
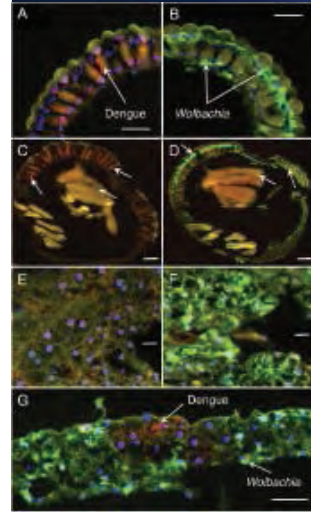
We compared host gene expression using whole-genome microarrays in potentially identical *Ae. aegypti* lines infected and uninfected with wMelPop (7) to examine the mechanism underlying the life-shortening phenotype. Of 199 genes downregulated by more than a twofold threshold, 7% had positive immune-related functions (Fig. 1 and table S2). These included genes that encode T7 (CLIP) domain, serine proteases, rice

Cell

A Wolbachia Symbiont in *Aedes aegypti* Limits Infection with Dengue, Chikungunya, and Plasmodium

Lorenza A. Moreira^{1,2*}, Inaki Barber-Orosco¹, Jason A. Jeffery³, Quang-Hung Luu³, Alexis T. Pyle⁴, Lauren M. Hejblum⁵, Bruno C. Rocha⁶, Sarah J. O'Neill⁷, Andrew Davidson⁸, Markus Wegmann⁹, Leah E. Hege⁷, Kaye N. Johnson¹⁰, Grant H. Riegler¹¹, Elizabeth A. McGraw¹², Andrew F. van der Horst¹³, Dexter A. Ryan¹³, and Scott L. O'Neill^{1*}

¹Centro de Estudos Científicos Avançados, Universidade de Lisboa, Portugal; ²Departamento de Genética, Universidade de Coimbra, Coimbra, Portugal; ³Department of Microbiology and Immunology, The University of Queensland, St. Lucia, Queensland, Australia; ⁴Department of Microbiology and Immunology, The University of Queensland, St. Lucia, Queensland, Australia; ⁵Department of Microbiology and Immunology, The University of Queensland, St. Lucia, Queensland, Australia; ⁶Department of Microbiology and Immunology, The University of Queensland, St. Lucia, Queensland, Australia; ⁷Department of Microbiology and Immunology, The University of Queensland, St. Lucia, Queensland, Australia; ⁸Department of Microbiology and Immunology, The University of Queensland, St. Lucia, Queensland, Australia; ⁹Department of Microbiology and Immunology, The University of Queensland, St. Lucia, Queensland, Australia; ¹⁰Department of Microbiology and Immunology, The University of Queensland, St. Lucia, Queensland, Australia; ¹¹Department of Microbiology and Immunology, The University of Queensland, St. Lucia, Queensland, Australia; ¹²Department of Microbiology and Immunology, The University of Queensland, St. Lucia, Queensland, Australia; ¹³Department of Microbiology and Immunology, The University of Queensland, St. Lucia, Queensland, Australia.



Horizontal Gene(Genome) Transfer(s)

Wolbachia (and other symbionts?) Jumps!

Widespread Lateral Gene Transfer from Intracellular Bacteria to Multicellular Eukaryotes

Julie C. Dunning Hotopp,^{1*}†‡ Michael E. Clark,^{2*} Deodoro C. S. G. Oliveira,² Jeremy M. Foster,³
Peter Fischer,⁴ Mónica C. Muñoz Torres,⁵ Jonathan D. Giebel,² Nikhil Kumar,¹†‡
Nadeeza Ishmael,¹†‡ Shiliang Wang,¹ Jessica Ingram,³ Rahul V. Nene,¹§ Jessica Shepard,¹||
Jeffrey Tomkins,⁵ Stephen Richards,⁶ David J. Spiro,¹ Elodie Ghedin,^{1,7} Barton E. Slatko,³
Hervé Tettelin,¹†|| John H. Werren,²¶

Science 2007

Conclusions

A Single Conclusion

- Do not ignore Symbionts! since they control Insects?....
 - Biology
 - Ecology
 - Evolution

Symbiosis Work in Bourtzis' Lab

MicrobeGR

In the frame of:

- EU FP7 MicrobeGR project (<http://microbegr.env.uoi.gr>)
- EU COST FA0701 “Arthropod Symbiosis: from fundamental studies to pest and disease management” (<http://www.cost-fa0701.com/> & http://w3.cost.esf.org/index.php?id=181&action_number=FA0701)

EU COST ACTION FA0701

“Arthropod Symbiosis: from fundamental studies to pest and disease management”

Prof. Kostas Bourtzis, Chair (Greece)
Dr. Einat Zchori-Fein, Vice Chair (Israel)

Participants

Austria	Latvia
Croatia	Norway
Czech Republic	Portugal
Denmark	Poland
France	Serbia
Germany	Slovenia
Greece	Spain
Hungary	Sweden
Ireland	Switzerland
Italy	The Netherlands
Israel	United Kingdom

Australia
Korea
Russia
Tunisia
United States

111 experts
4 SMEs
20+2 COST countries
5 Non COST countries

Acknowledgements

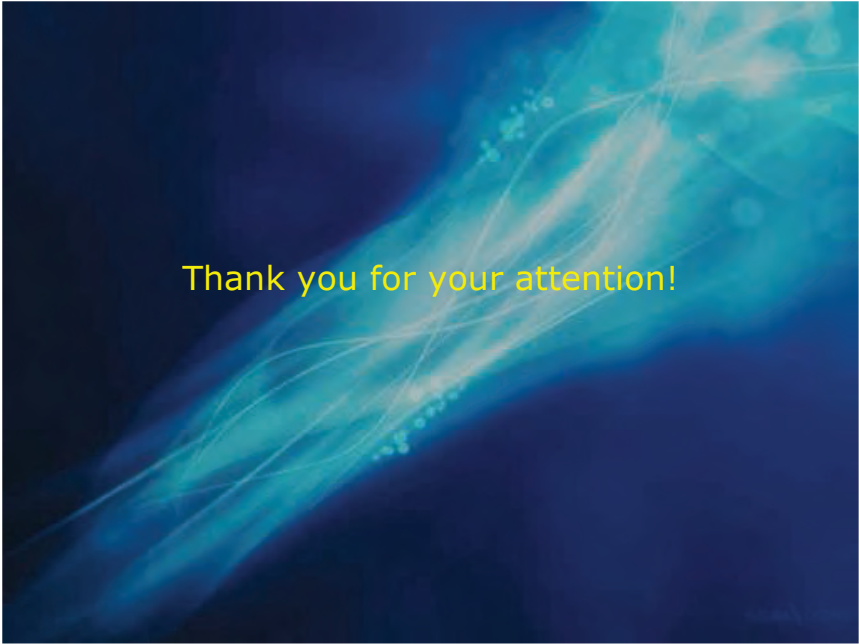
- > **Bourtzis' lab**
- > **IMBB / Fleming**
 - Babis Savakis
- > **FAO/IAEA**
 - Adly Abd-Alla and his colleagues
 - All CRP members provided samples
- > **Yale University**
 - Serap Aksoy
 - Uzma Alam
 - Corey Brelsfoard
- > **University of Pavia**
 - Anna Malacrida and her colleagues
- > **Funding**
 - European Union
 - EU COST Action FA0701
 - FAO / IAEA
 - Greek Secretariat for Research and Technology
 - Greek Ministry of Education
 - Empirikion Foundation

Current Members

- George Tsiamis
- Eva Dionyssopoulou
- Aggeliki Saridaki
- Antonios Avgoustinos
- Athina Hamalaki
- Evangelos Doudoumis
- Evangelos Diakoumis

Past Members

- Sofia Zabalou
- Zoe Veneti
- Stefan Oehler
- Aggeliki Apostolaki
- George Papafotiu
- Ioannis Livadaras

An abstract digital background featuring a gradient of dark blue to teal. Overlaid on this are several thin, glowing white and light blue lines that appear to be part of a network or data flow. There are also small, bright blue and white particles or nodes scattered throughout, particularly in the upper right and lower right areas. The overall effect is that of a futuristic or scientific visualization.

Thank you for your attention!