NCOMPATIBLE INSECT TECHNIQUE: A NOVEL AN
NVIRONMENTALLY FRIENDLY TECHNOLOGY FO
INSECT PEST POPULATION CONTROL

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Synopsis of the Presentation

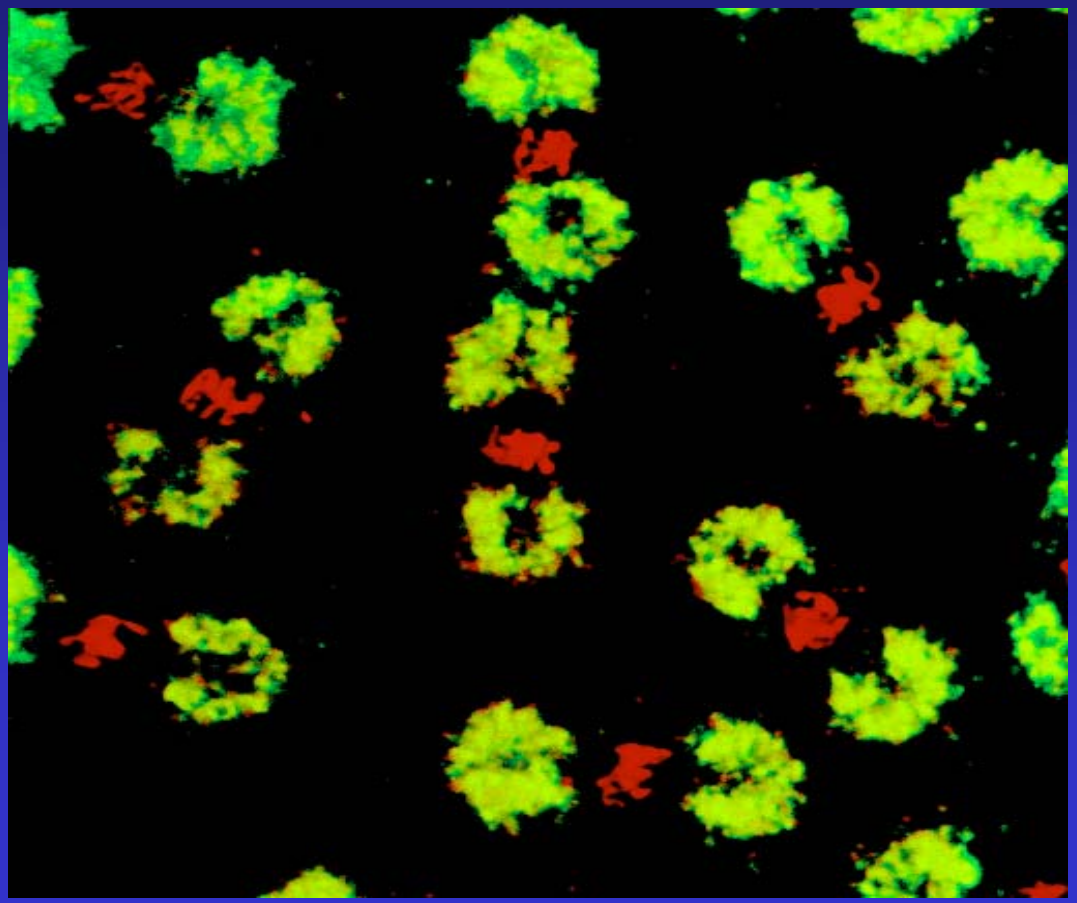
rt A: Introduction to Wolbachia Biology

rt B: Wolbachia Applications

rt C: Conclusions and Future Goals

Part A: Introduction to Wolbachia Biology

olbachia: an obligatory intracellular and maternal ansmitted alpha-proteobacterium that interacts wi host microtubules



zis' Lab]

Host Distribution of *Wolbachia* Strains

Arthropods

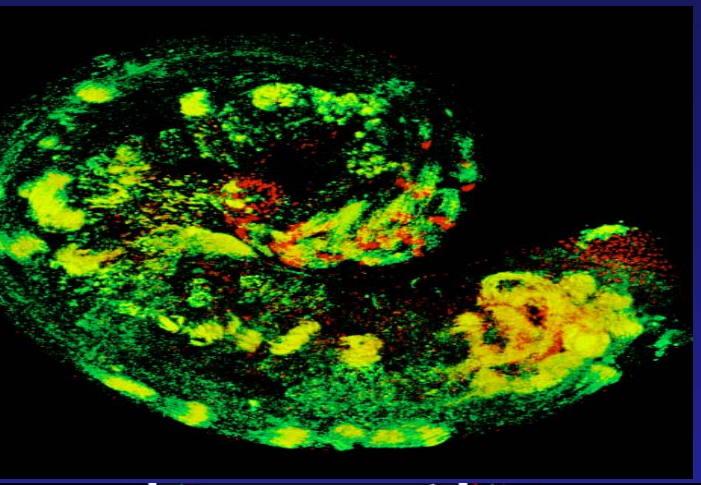
- Insects
- Mites
- Isopods
- Spiders
- Springtails

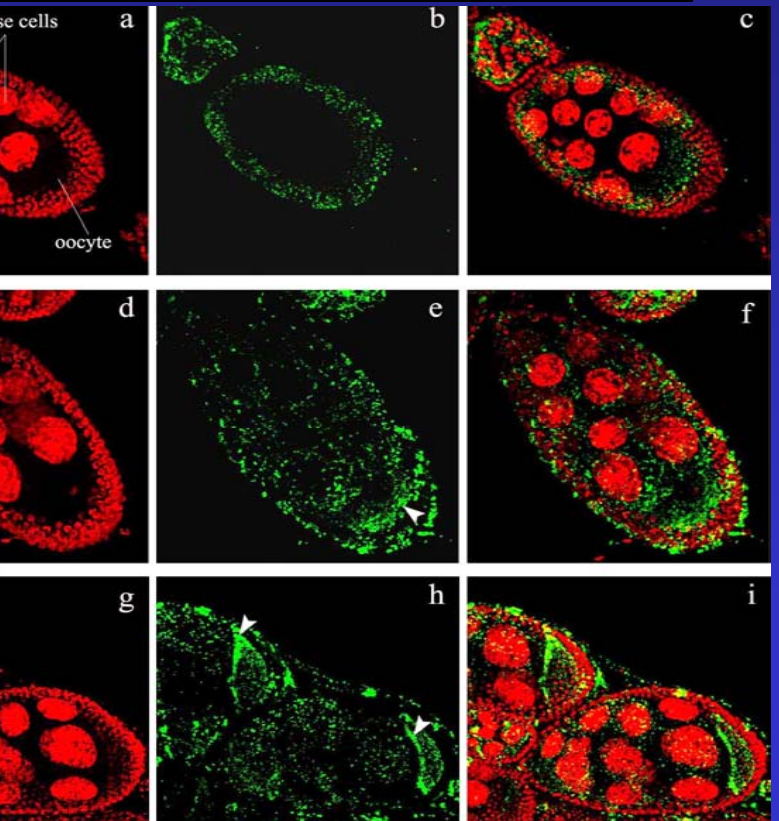
Nematodes

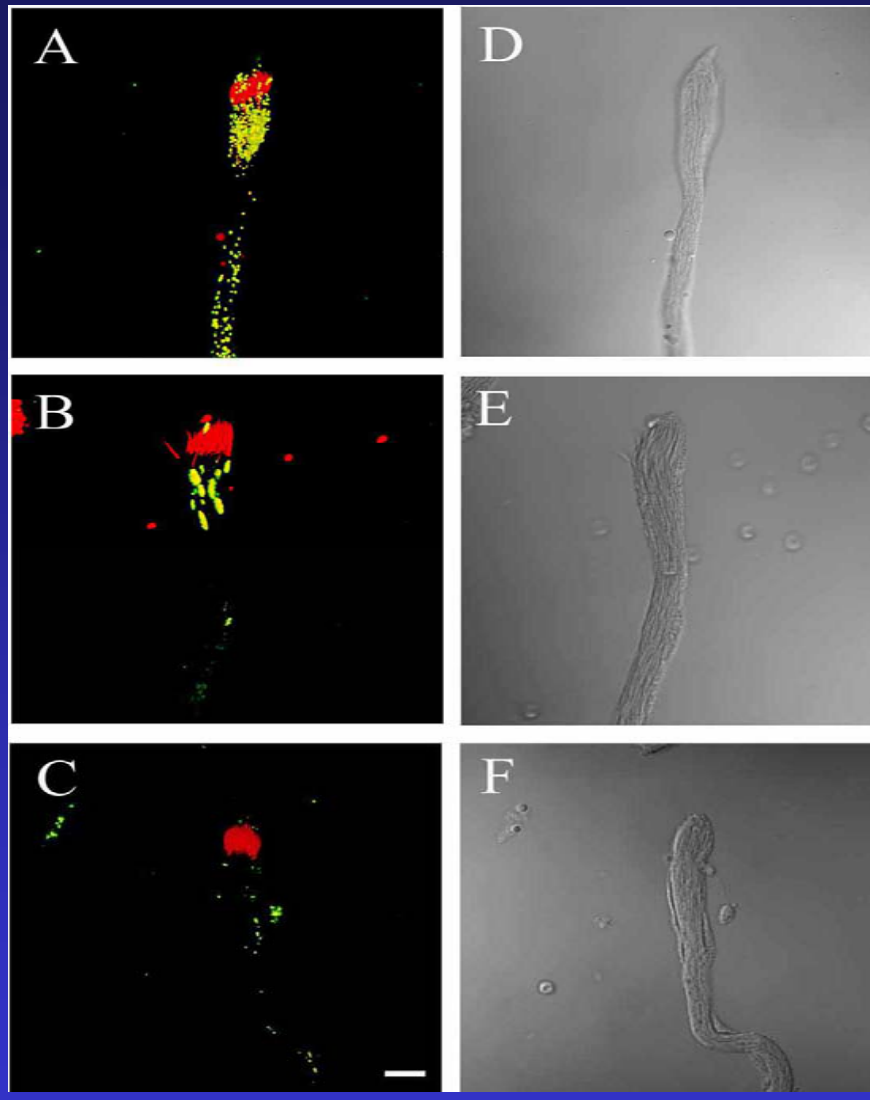
- Filarial worms

vever, many species of agricultural importance (such as the med fly
atitis capitata and the olive fly *Bactrocera oleae*) and species of medica
ortance (such as *Aedes aegypti* and *Anopheles gambiae*) are not infecte
Wolbachia

Wolbachia during *Drosophila* Spermatogenesis & Ooge



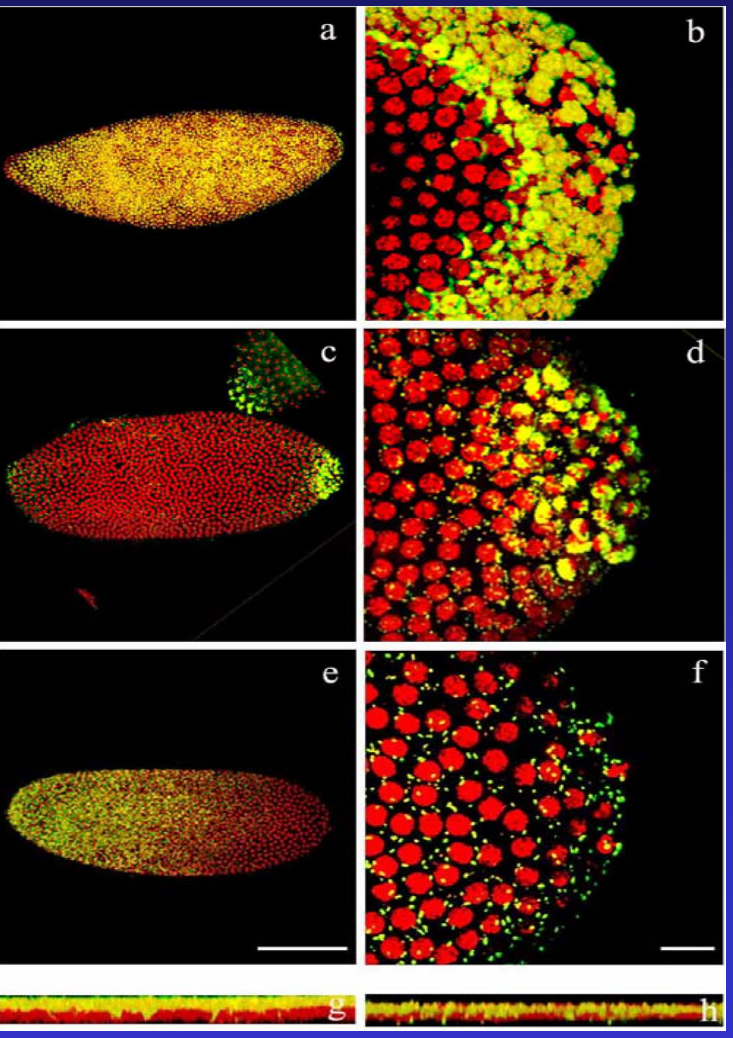


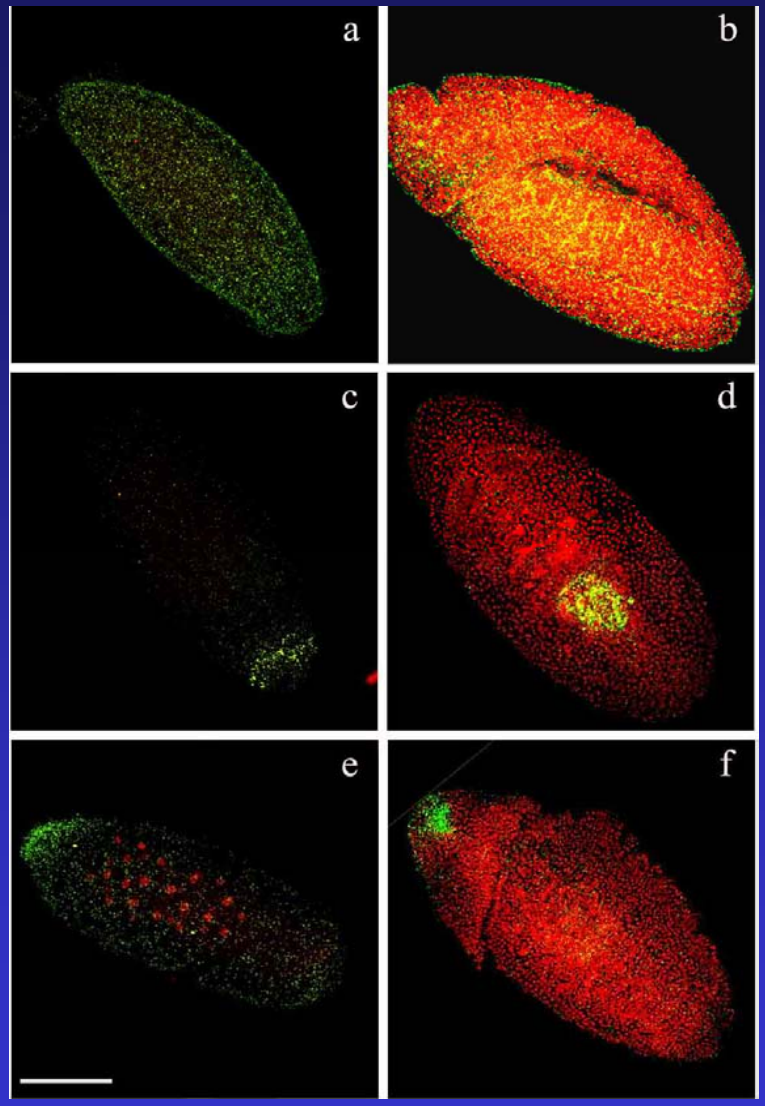


[Veneti et al., Genetics, 2003]

[Veneti et al., Appl Env Microbiol, 2

Wolbachia during *Drosophila* Embryogenesis





ti et al., Appl Env Microbiol, 2004]

Wolbachia-Induced Reproductive Abnormalities

lbachia induce a number of reproductive alterations, such as:

eminization

arthenogenesis

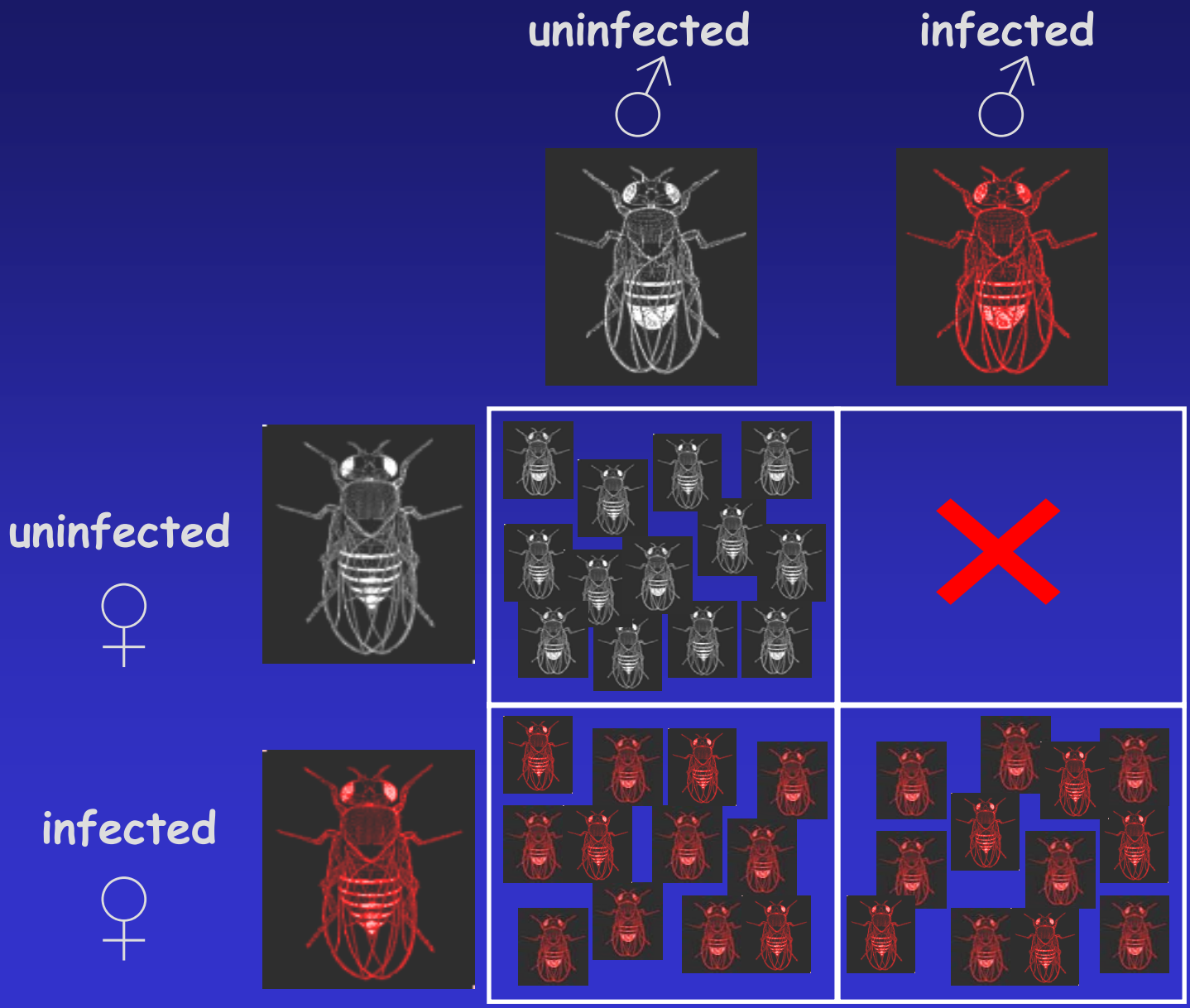
Iale-killing

ytoplasmic Incompatibility

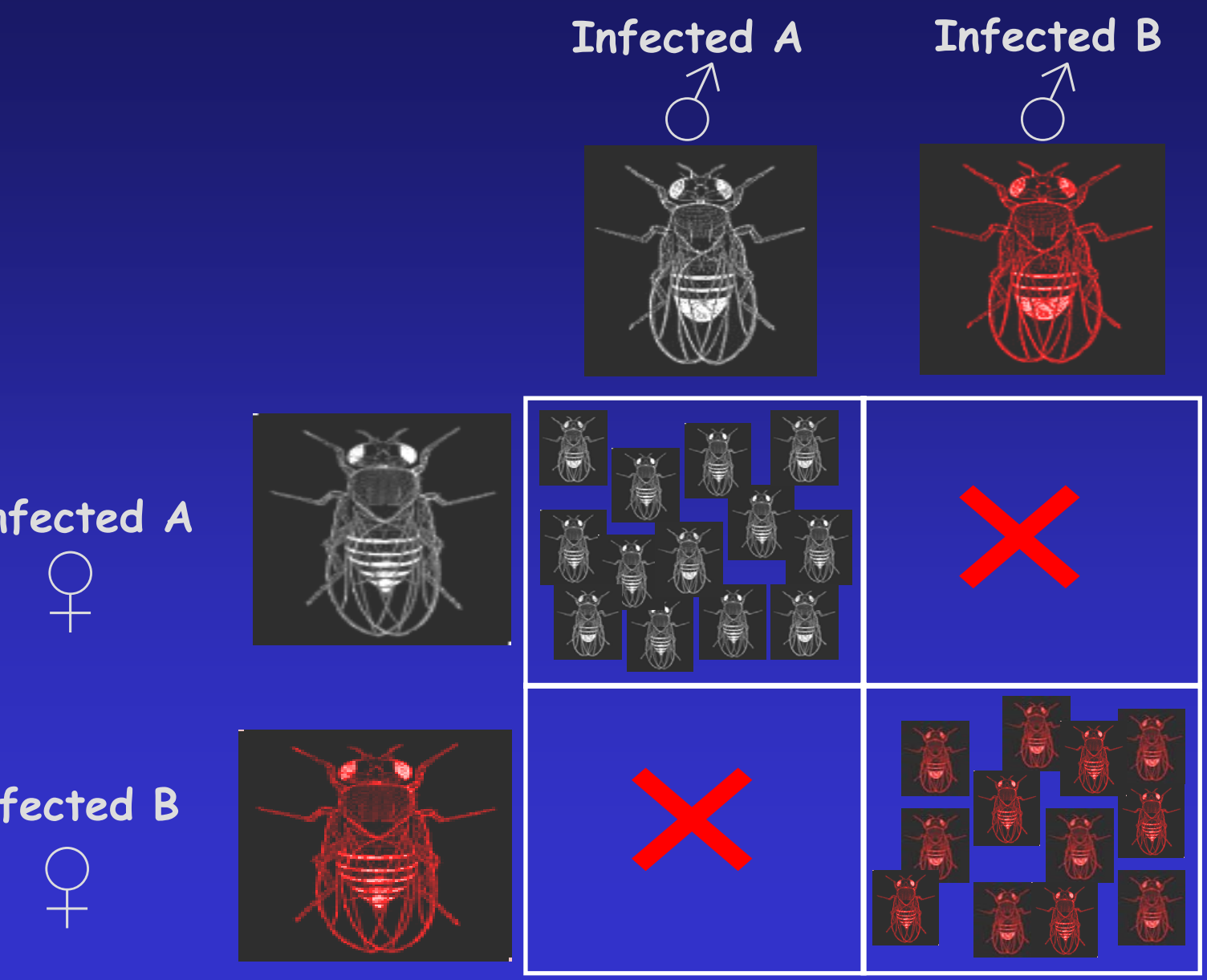
of the above phenotypes "help" *Wolbachia* spreading since they favor t
uction of infected females.

l cases, normal reproduction can be restored by curing the host with
biotics, thus confirming the connection between *Wolbachia* and these
notypes.

Uni-Directional CI



Bi-Directional CI



Major Goals of Bourtzis' Lab

- To dissect host-*Wolbachia* symbiosis towards the elucidation of the mechanism of Cytoplasmic Incompatibility (CI)

- To use the mechanism of CI for applied purposes

The EUWOL Consortium

THE EUROPEAN *WOLBACHIA* PROJECT:
ARDS NOVEL BIOTECHNOLOGICAL APPROACHES FOR CONT
OF ARTHROPOD PESTS AND MODIFICATION OF BENEFICIAL
ARTHROPOD SPECIES BY ENDOSYMBIOTIC BACTERIA

1. Bourtzis lab / Savakis lab, Greece
2. Andersson lab, Sweden
3. Garrett lab, Denmark
4. Braig lab, United Kingdom
5. Ashburner lab, United Kingdom
6. Stouthamer lab, The Netherlands [Fabrice Vavre]
7. Martin lab, France
8. Synaptic Ltd, Greece

The EUWOL Project

enome Sequencing of:

- *w*Ri, cytoplasmic incompatibility, *Drosophila simulans*
- *w*Uni, parthenogenesis, *Muscidifurax uniraptor*
- *w*Vul, feminization, *Armadillidium vulgare*

Wolbachia Comparative Genomics

pleted Genomes: *w*Mel and *w*Bm
anced Stage: *w*Ri, *w*Uni, *w*Vul, *w*Pip, *w*Ovo, *w*Dim
al Genomes: *w*Sim, *w*Ana, *w*Wil

SUGGESTS THAT

"core" *Wolbachia* genome is very similar between different strains (single rDN
, limited amino acid and nucleotide synthesis genes, limited number of regula

difference is mainly due to the different number of mobile elements, repeats a
n-repeat genes

ge elements & ankyrin-repeat genes include the most divergent genes

ge genes and/or ankyrin-repeat genes may be implicated in the induction of th
uctive abnormalities; this conclusion is also supported by the fact that the *w*Br
e contains no prophage and has a reduced number of ankyrin genes

group is currently studying phage and ankyrin genes in a number of Drosophi
chia associations exhibiting different CI phenotypes. Hopefully, we may know
er these genes are involved in the induction of CI and/or other phenotypes.

Part B: Wolbachia Applications

Wolbachia and Applied Biology

For example:

1. Asexuality

2. As an expression vector

3. As a spreading mechanism

4. As a population suppression mechanism

Wolbachia-induced Cytoplasmic Incompatibility as a tool to suppress med fly populations

Wolbachia transfer to the med fly *Ceratitis capitata*

he entire work was done at the IMBB, Crete.

ecipient: Benakeion strain

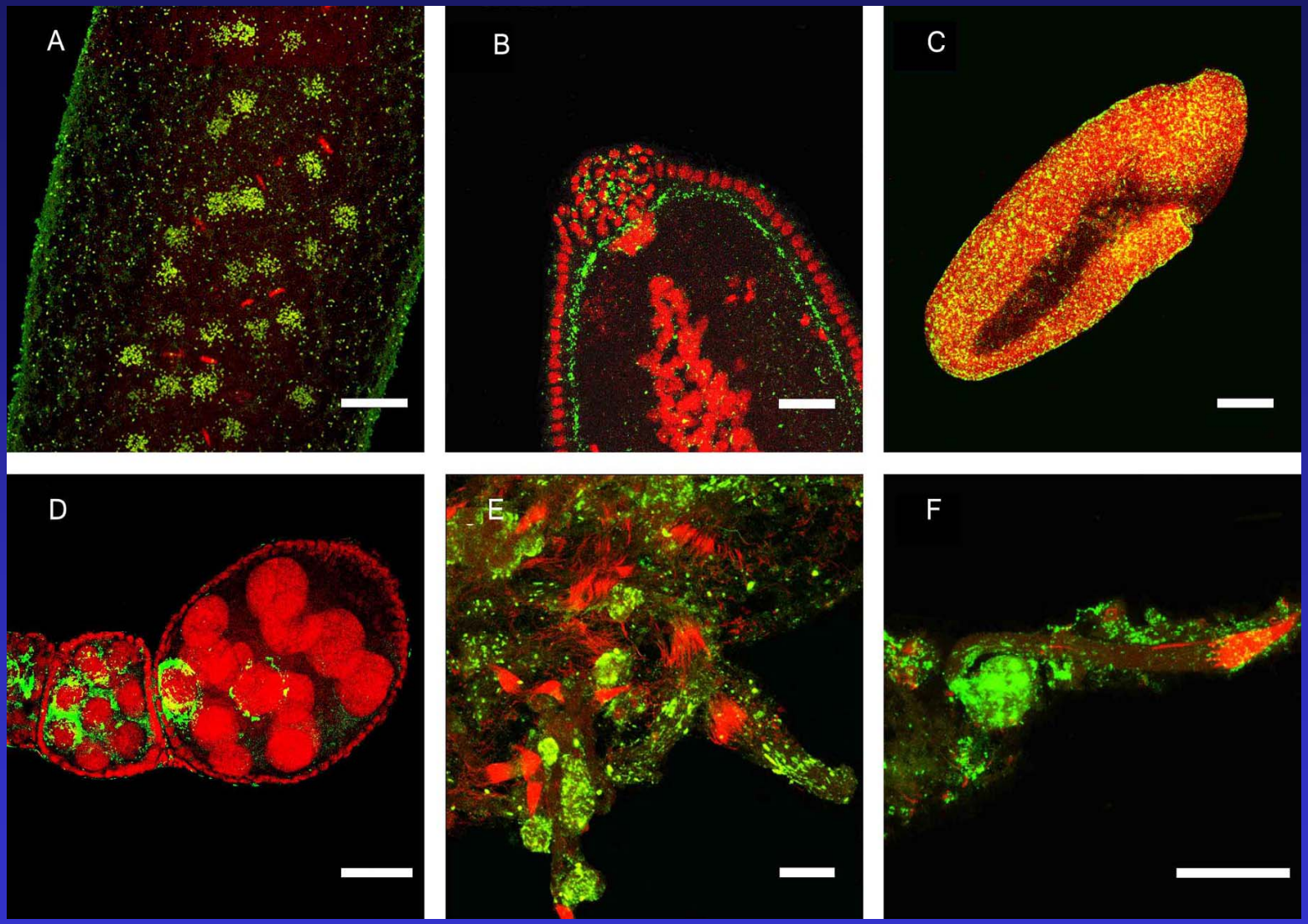
onor species was two populations of *Rhagoletis cerasi:*
from Austria (*w*Cer1 + *w*Cer2) and one from Sicily (*w*Cer1 + *w*Ce
r4) [supplied by M. Riegler and C. Stauffer]

vo stably transinfected lines were produced: 88.6 (*w*Cer2) and S
er4).

0 % infection rates (for over 3 years now, 40 generations).

lou et al., PNAS, 2004]

Wolbachia Distribution in Transinfected Medfly



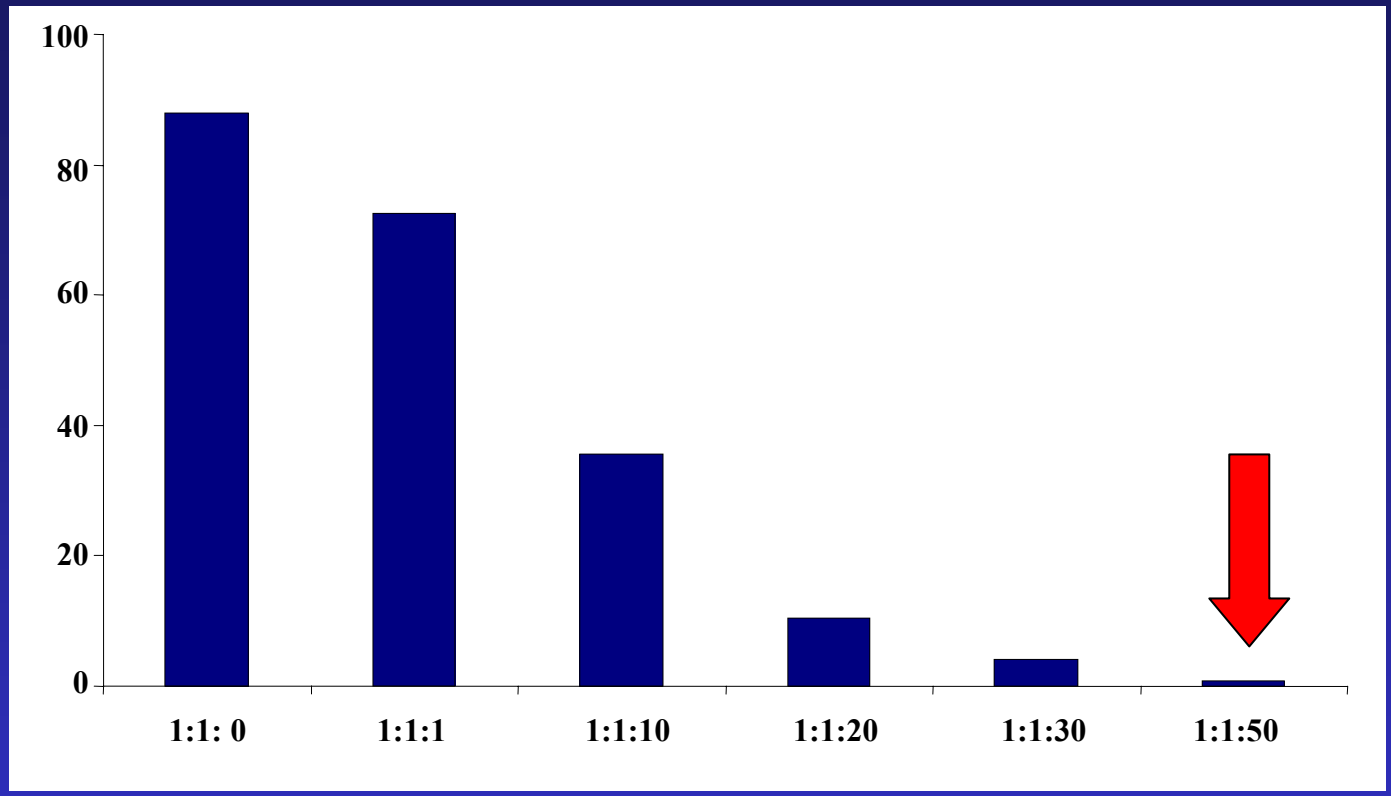
ou et al., PNAS, 2004]

CI assays with transinfected *Ceratitis capitata*

(females x males)	Embryos scored	Embryonic mortal (%)
ected Benakeion x WolMed 88.6 (*w*Cer2)	3000	100 ± 0
ected Benakeion x WolMed S10.3 (*w*Cer4)	3000	100 ± 0
ed 88.6 (*w*Cer2) x Uninfected Benakeion	3000	16.73 ± 0.68
ed S10.3 (*w*Cer4) x Uninfected Benakeion	3000	32.03 ± 0.85
ed S10.3 (*w*Cer4) x WolMed 88.6 (*w*Cer2)	3000	100 ± 0
ed 88.6 (*w*Cer2) x WolMed S10.3 (*w*Cer4)	3000	100 ± 0
ed 88.6 (*w*Cer2) x WolMed 88.6 (*w*Cer2)	3000	64.77 ± 0.87
ed S10.3 (*w*Cer4) x WolMed S10.3 (*w*Cer4)	3000	67.25 ± 0.87
ected Benakeion x Uninfected Benakeion	3000	12.17 ± 0.60
olMed S10.3 tet x WolMed S10.3 tet	1890	23.44 ± 0.97
olMed S10.3 tet x WolMed S10.3 tet	3000	11.80 ± 0.59
olMed 88.6 tet x WolMed 88.6 tet	2283	25.10 ± 0.91

ou et al., PNAS, 2004]

ppression of medfly populations using *Wolbachia*-induced



Number of adults	300	300	300	306	290	520
Number of eggs scored	3000	3000	2097	1688	858	700

Part C: Conclusions and Future Goals

Incompatible Insect Technique (I.I.T.): chnology analogous to Sterile Insect Technique (S.

.T. is based on the mechanism of Wolbachia-induced CI

.T. is a method of genetic pest control by means analogous to S.I.T.

n effective sexing system is necessary, as is the case for S.I.T.

.T. is an environmentally friendly technology

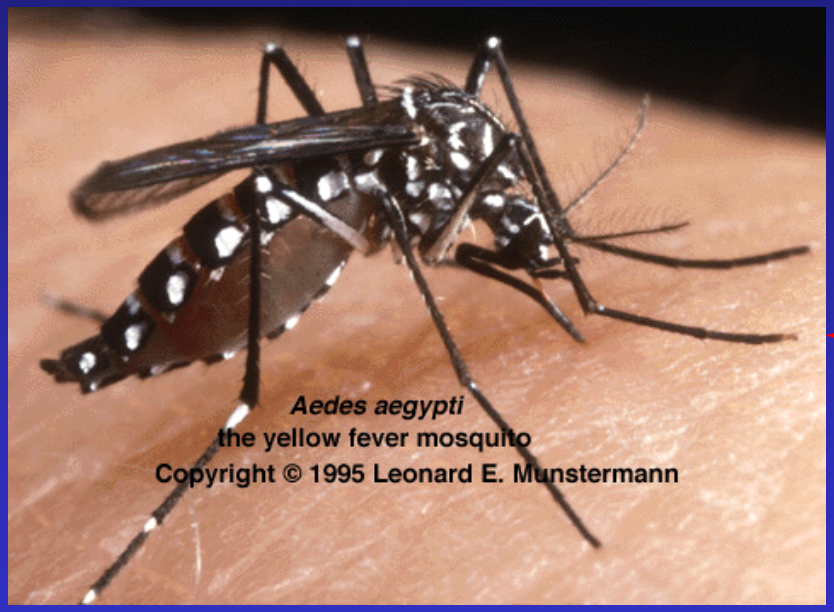
ow technological imput is sufficient; Low cost technology

gher competitiveness of released males compared to other sterility syst

has been used successfully in the past in field trials (for example, for th
cation of *Cx pipiens* in India, a project funded by WHO). At that time,
it was not known that CI is induced by Wolbachia

Next target species for I.I.T. applications are:

e olive fly, *Bactrocera oleae*



- the mosquito, *Aedes aeg*
  (then *Anopheles* sp.)

We welcome suggestions for collaborations
apply the I.I.T. to your favourite insect pest specie

The mechanism of CI?

Our current data,
ed on comparative genomics and proteomics approach
suggest that phage genes and/or ankyrin-repeat genes
may be implicated in the CI mechanism

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