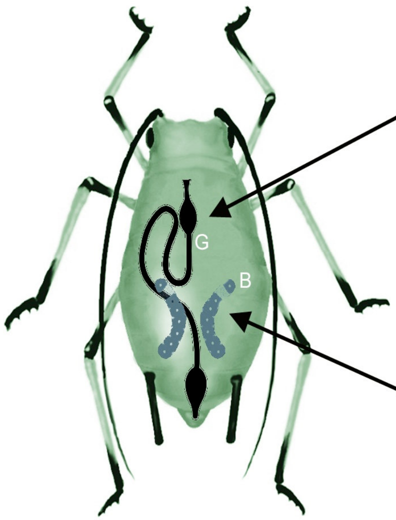
UNIMORE
UNIVERSITÀ DEGLI STUDI DI
MODENA E REGGIO EMILIA

Insetti e batteri: il ruolo della simbiosi nell'evoluzione degli insetti

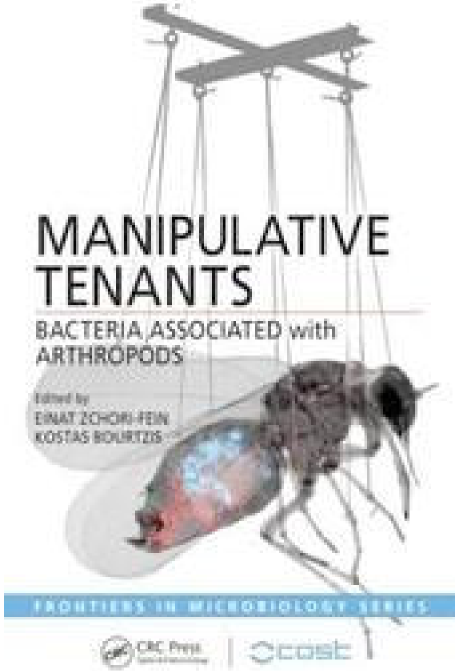
Prof. Mauro Mandrioli
Dipartimento di Scienze della Vita, UNIMORE

Reggio Emilia, 7 maggio 2015 - "Gli Insetti: un mondo di dominatori"



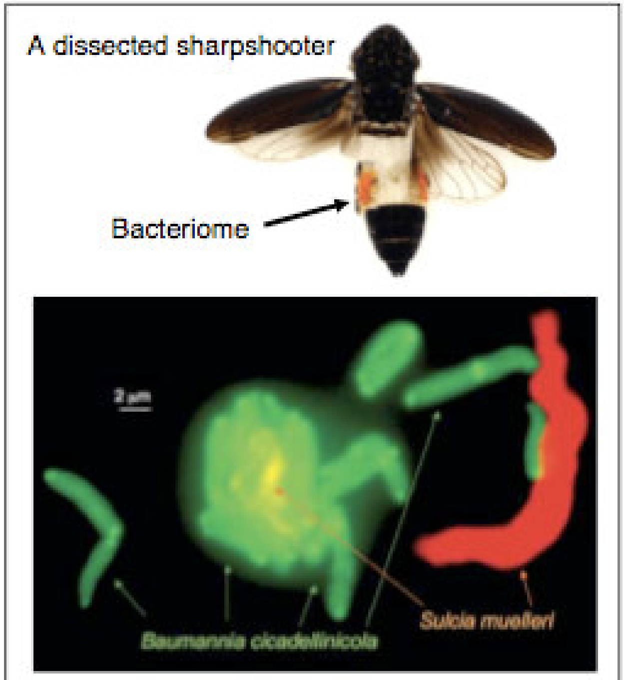
Secondary symbionts:
Hamiltonella defensa, Regiella insecticola, Erwinia aphidicola, Serratia symbiotica, Pseudomonas aeruginosa, Wolbachia pipientism Rickettsiella sp., Rickettsia sp., Spiroplasma sp., Arsenophonus sp., Photorhabdus sp., Xenorhabdus sp.

Primary symbiont:
Buchnera aphidicola

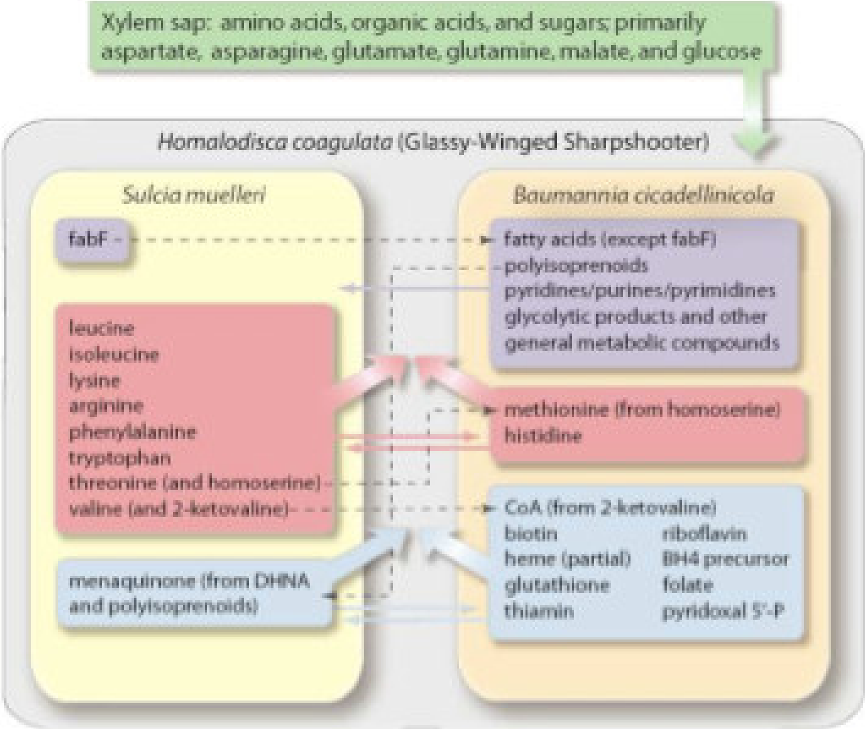

MANIPULATIVE
TENANTS
BACTERIA ASSOCIATED with
ARTHROPODS
Edited by
EINAT ZCHORI-FEIN
KOSTAS BOURTZIS
FRONTIERS IN MICROBIOLOGY SERIES
CRC Press
cost

PRIMARY SYMBIONTS

They provide to the host essential factors that are lacking in the natural diet *(Dale & Moran 2006, Cell 126:453-465)*



(Moran, 2007, PNAS, 104:8627-8633)



Functional Ecology

British Ecological Society

Functional Ecology 2009, 23, 38–47 doi: 10.1111/j.1365-2435.2008.01442.x

NUTRITIONAL ECOLOGY

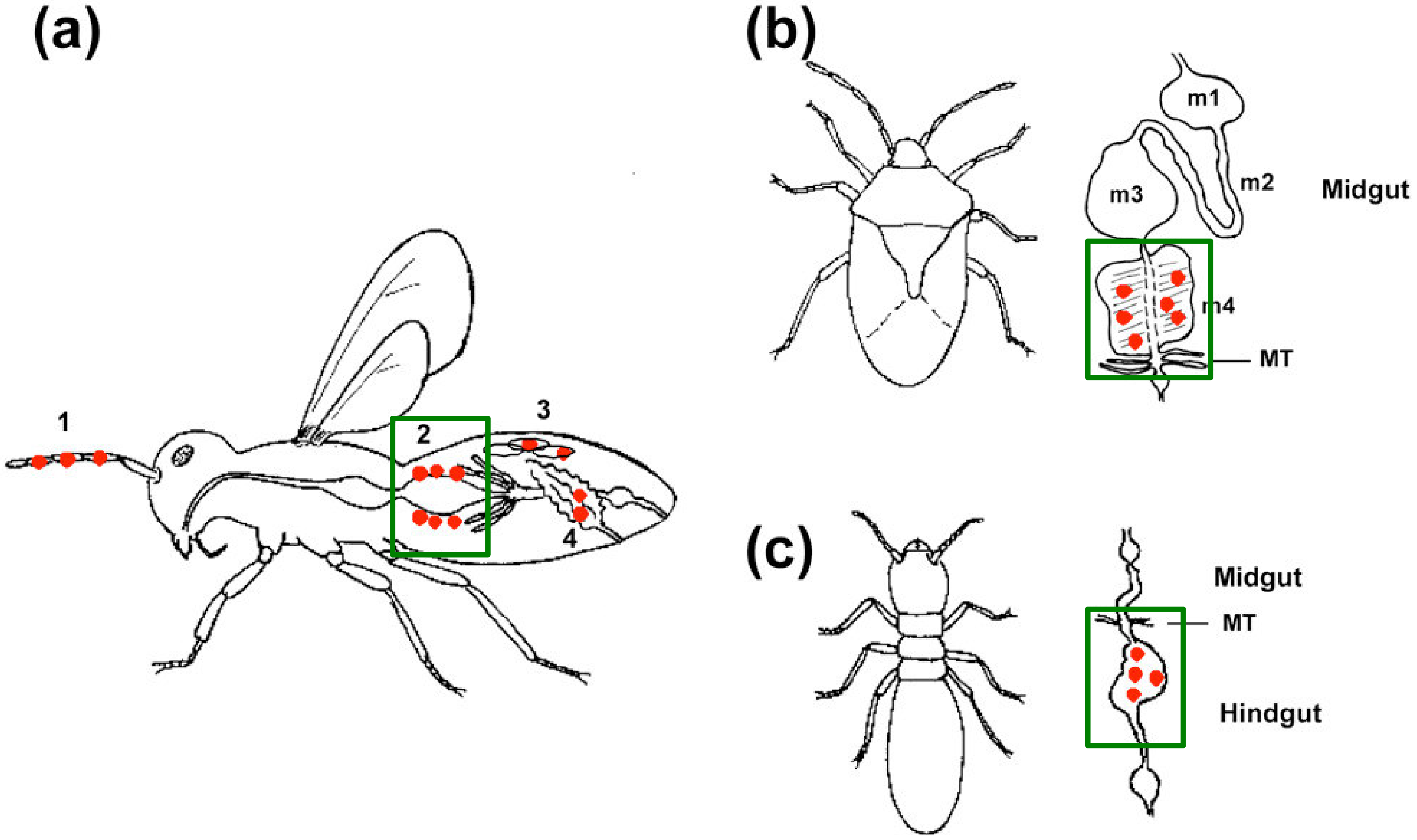
The microbial dimension in insect nutritional ecology

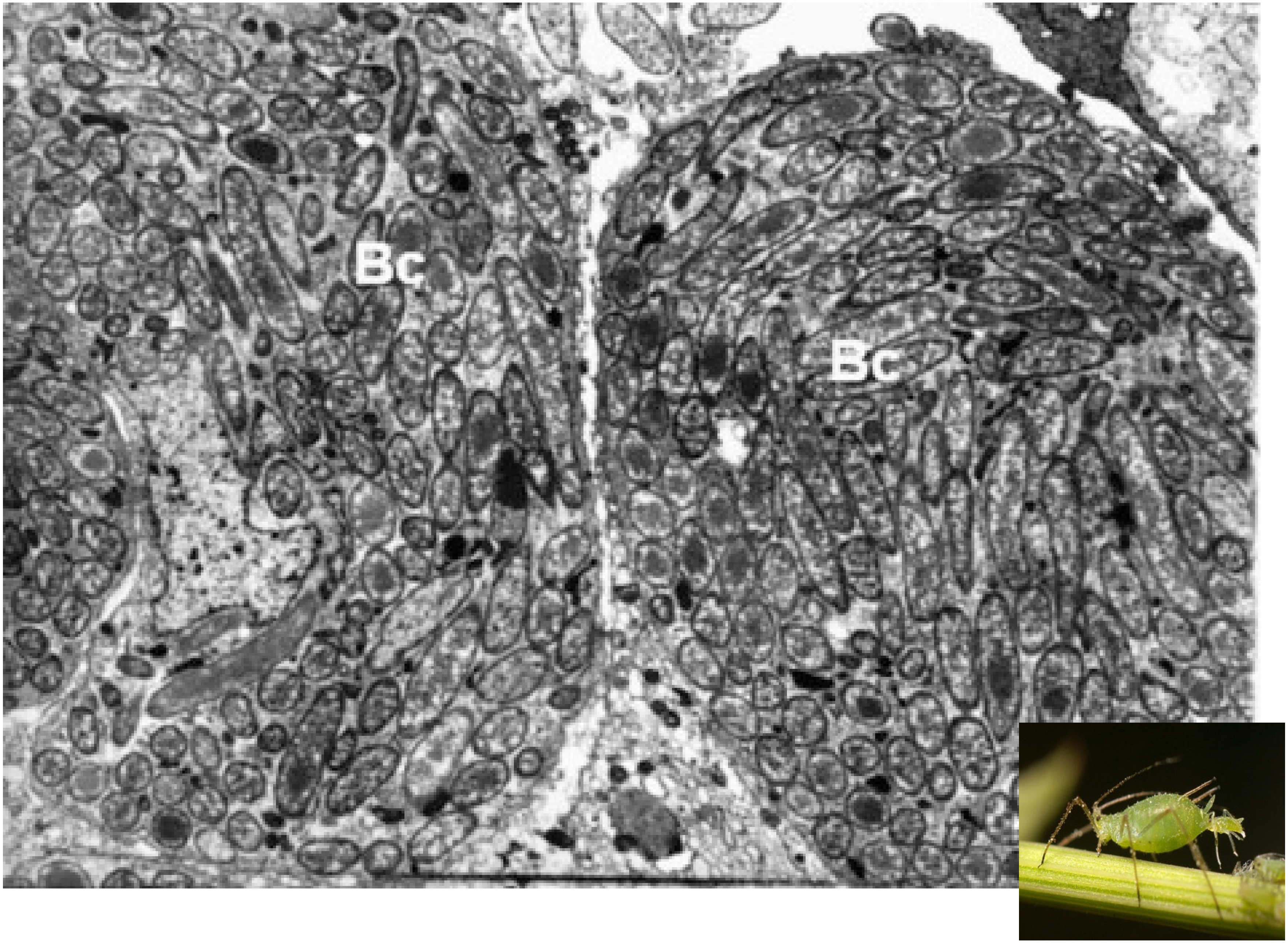
A. E. Douglas*

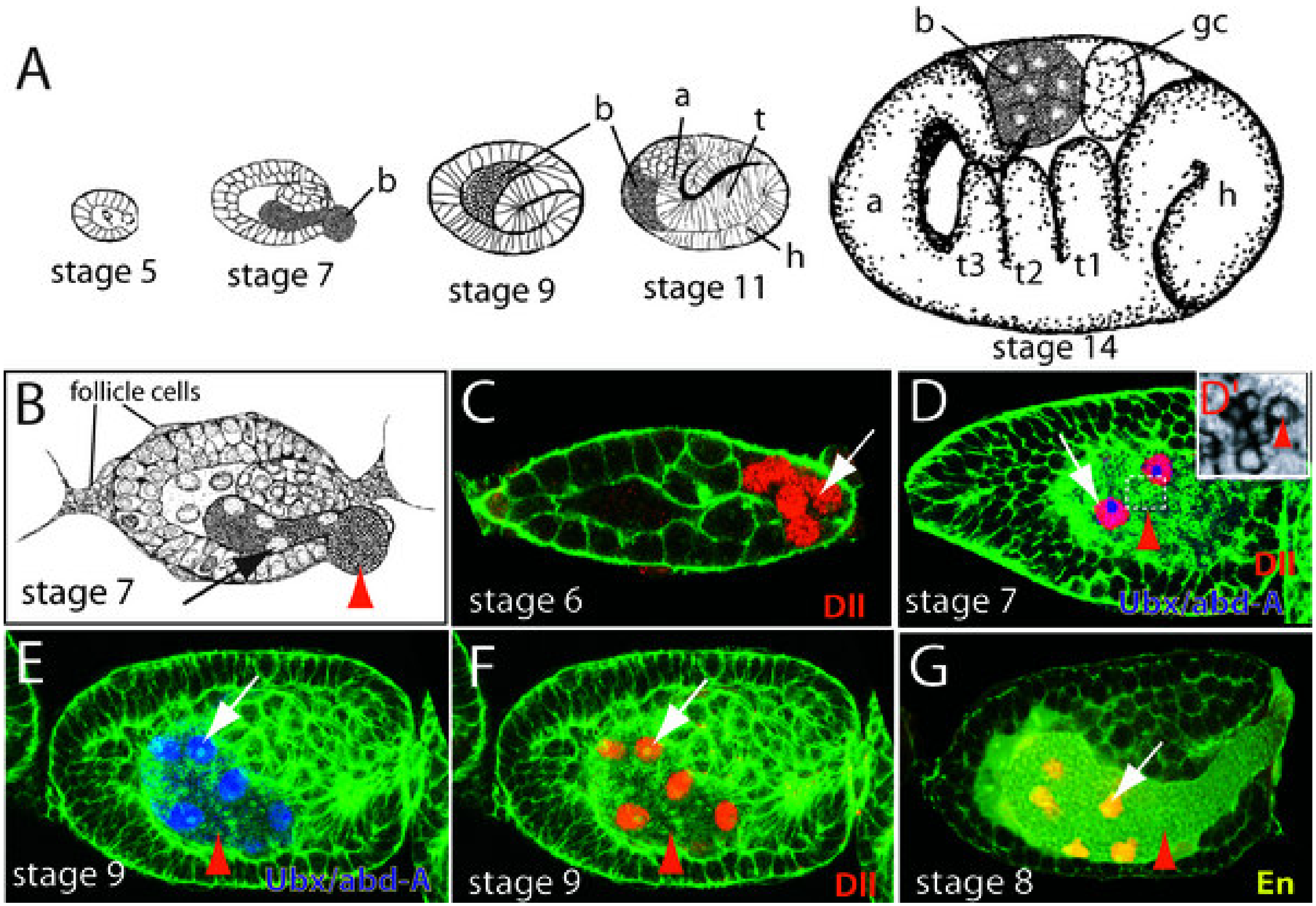
Department of Biology, University of York, York YO10 5YW, UK

Summary

1. Many insects derive nutritional advantage from persistent associations with microorganisms that variously synthesize essential nutrients or digest and detoxify ingested food. These persistent relationships are symbioses.
2. There is strong experimental evidence that symbiotic microorganisms provide plant sap-feeding insects with essential amino acids and contribute to the digestion of cellulose in some wood-feeding insects, including lower termites. Basic nutritional information is, however, lacking for many associations, including the relative roles of microbial and intrinsic sources of cellulose degradation in many insects and B-vitamin provisioning by microorganisms in blood-feeding insects.
3. Some nutritional interactions between insects and their symbiotic microorganisms vary among conspecifics and closely related species. This variation can, in principle, contribute to nutritional explanations for variation in the abundance and distribution of insects. For example, the plant utilization traits of phloem-feeding aphids and stinkbugs have been demonstrated to depend on the identity of microbial partners. Evidence that associations can evolve rapidly comes from the demonstration that the impact of the bacterium *Wolbachia* on natural populations of its insect host can change from deleterious to beneficial within two decades.


(a)
1
2
3
4
(b)
m1
m2
m3
m4
Midgut
MT
(c)
Midgut
MT
Hindgut

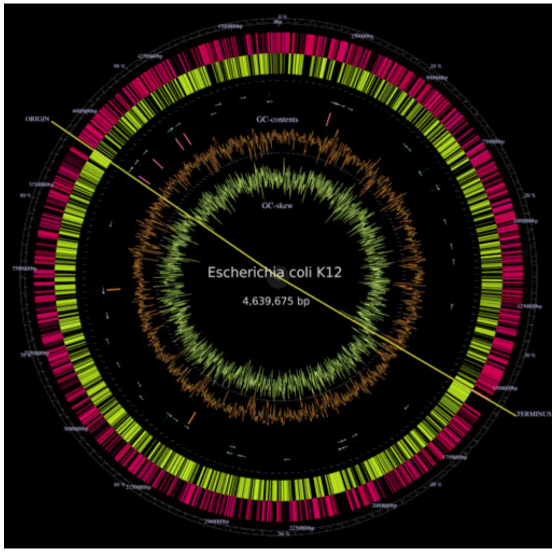

Bc
Bc



Braendle C, Miura T, Bickel R, Shingleton AW, et al. (2003) Developmental Origin and Evolution of Bacteriocytes in the Aphid–Buchnera Symbiosis. PLoS Biol 1(1): e21.

Table 1. Genomic features of primary bacteriocyte endosymbionts of insects

	Blochmannia floridanus	**Blochmannia pennsylvanicus**	***Wigglesworthia glossinidius***	**Buchnera aphidicola Aps**	***Buchnera aphidicola* BCc**	***Buchnera aphidicola* Bp**	***Buchnera aphidicola* Sg**
Phylum	*γ-Proteobacteria*	*γ-Proteobacteria*	*γ-Proteobacteria*	*γ-Proteobacteria*	*γ-Proteobacteria*	*γ-Proteobacteria*	*γ-Proteobacteria*
Host	*Camponotus floridanus*	*Camponotus pennsylvanicus*	*Glossina morsitans*	*Acyrthosiphon pisum*	*Cinara cedri*	*Baizongia pistacea*	*Schizaphis graminum*
Genome size (bp)	705,557	791,654	697,724	640,681	422,434	615,980	641,454
GC content (%)	27.4	29.6	22.5	26.2	20.1	25.3	25.3
Plasmids (total size in bp)	0	0	1 (5280)	2 (11,434)	1 (6054)	1 (2399)	2 (11,547)
Predicted protein coding sequences (number on plasmids)	583	610	617 (6)	562 (9)	357 (5)	504 (3)	550 (9)
rRNAs	3	3	6	3	3	3	3
tRNAs	37	39	34	32	31	32	32
Small RNA genes	2	2	2	2	2	2	2
Pseudogenes	4	4	14	13	3	9	33
ORF average length (bp)	1006	995	990	990	994	990	983
Host nutrition	Omnivorous	Omnivorous	Blood	Phloem	Phloem	Phloem	Phloem
Reference	Gil et al. (2003)	Degnan et al. (2005)	Akman et al. (2002)	Shigenobu et al. (2000)	Perez-Brocal et al. (2006)	van Ham et al. (2003)	Tamas et al. (2002)



Horizontal transfer of genes in bacteria

Paul H. Roy

Symbiont Genes in Host Genomes: Fragments with a Future?

Mark Blaxter[1,*]
[1]Institute of Evolutionary Biology, School of Biological Sciences, Ashworth Laboratories, King's Buildings, University of Edinburgh, West Mains Road, Edinburgh, Scotland EH9 3JT, UK
*Correspondence: mark.blaxter@ed.ac.uk
DOI 10.1016/j.chom.2007.09.008

While lateral transfer is the rule in the evolutionary history of bacterial and archaeal genes, events of transfer from prokaryotes to eukaryotes are rare. Germline-transmitted animal symbionts, such as *Wolbachia pipientis*, are well placed to participate in such transfers. In a recent issue of *Science*, Dunning Hotopp et al. identified instances of transfer of *Wolbachia* DNA to host genomes. It is unknown whether these transfers represent innovation in animal evolution.

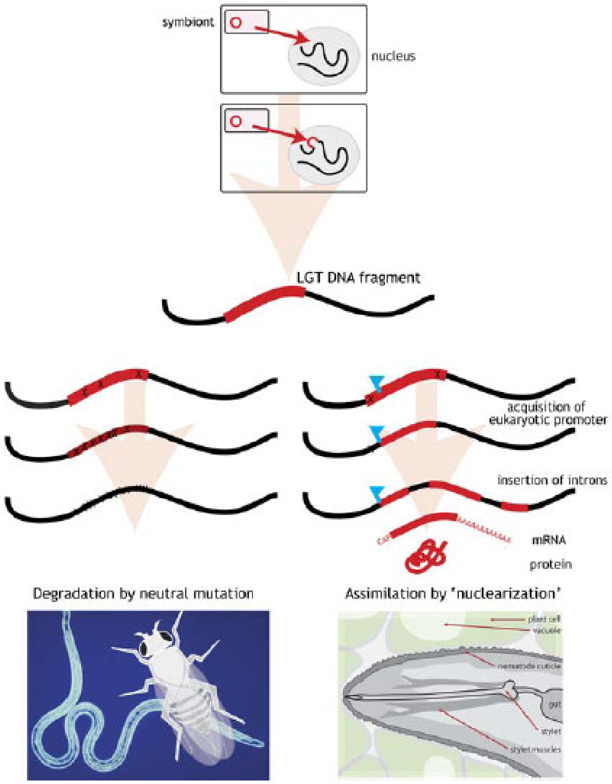


Figure 1. The Fate of Laterally Transferred Genes
A DNA fragment laterally transferred from an intracellular germline symbiont to the nucleus of an eukaryotic cell (top) can either be degraded by neutral mutation, and play no significant part in the evolution of the eukaryotic host genome (left), or become integrated into the host genome and provide a novel function (right) by acquiring eukaryotic promoter elements and RNA processing signals. The *Wolbachia* insertions thus far described fit the left-hand model, while the rhizosphere bacterial genes acquired by plant parasitic nematodes fit the right-hand model. The images below represent *Drosophila melanogaster*, the filarial nematode *Brugia malayi*, and a cartoon of a transmission electron micrograph of a *Meloidogyne* sp. nematode within a root showing the feeding stylet (in the center) used to penetrate cell walls with the aid of laterally transferred gene products.

BMC Biology

Research article

Aphids acquired symbiotic genes via lateral gene transfer

Naruo Nikoh[1] and Atsushi Nakabachi*[2]

Address: [1]Division of Natural Sciences, The Open University of Japan, Chiba, Japan and [2]Advanced Science Institute, RIKEN, Saitama, Japan

Email: Naruo Nikoh - nikoh@u-air.ac.jp; Atsushi Nakabachi* - bachi@riken.jp

* Corresponding author

Published: 10 March 2009

BMC Biology 2009, **7**:12 doi:10.1186/1741-7007-7-12

Received: 12 February 2009
Accepted: 10 March 2009

This article is available from: http://www.biomedcentral.com/1741-7007/7/12

Abstract

Background: Aphids possess bacteriocytes, which are cells specifically differentiated to harbour the obligate mutualist *Buchnera aphidicola* (γ-Proteobacteria). *Buchnera* has lost many of the genes that appear to be essential for bacterial life. From the bacteriocyte of the pea aphid *Acyrthosiphon pisum*, we previously identified two clusters of expressed sequence tags that display similarity only to bacterial genes. Southern blot analysis demonstrated that they are encoded in the aphid genome. In this study, in order to assess the possibility of lateral gene transfer, we determined the full-length sequences of these transcripts, and performed detailed structural and phylogenetic analyses. We further examined their expression levels in the bacteriocyte using real-time quantitative RT-PCR.

Lateral Transfer of Genes from Fungi Underlies Carotenoid Production in Aphids

Nancy A. Moran[1]* and Tyler Jarvik[2]

Carotenoids are colored compounds produced by plants, fungi, and microorganisms and are required in the diet of most animals for oxidation control or light detection. Pea aphids display a red-green color polymorphism, which influences their susceptibility to natural enemies, and the carotenoid torulene occurs only in red individuals. Unexpectedly, we found that the aphid genome itself encodes multiple enzymes for carotenoid biosynthesis. Phylogenetic analyses show that these aphid genes are derived from fungal genes, which have been integrated into the genome and duplicated. Red individuals have a 30-kilobase region, encoding a single carotenoid desaturase that is absent from green individuals. A mutation causing an amino acid replacement in this desaturase results in loss of torulene and of red body color. Thus, aphids are animals that make their own carotenoids.

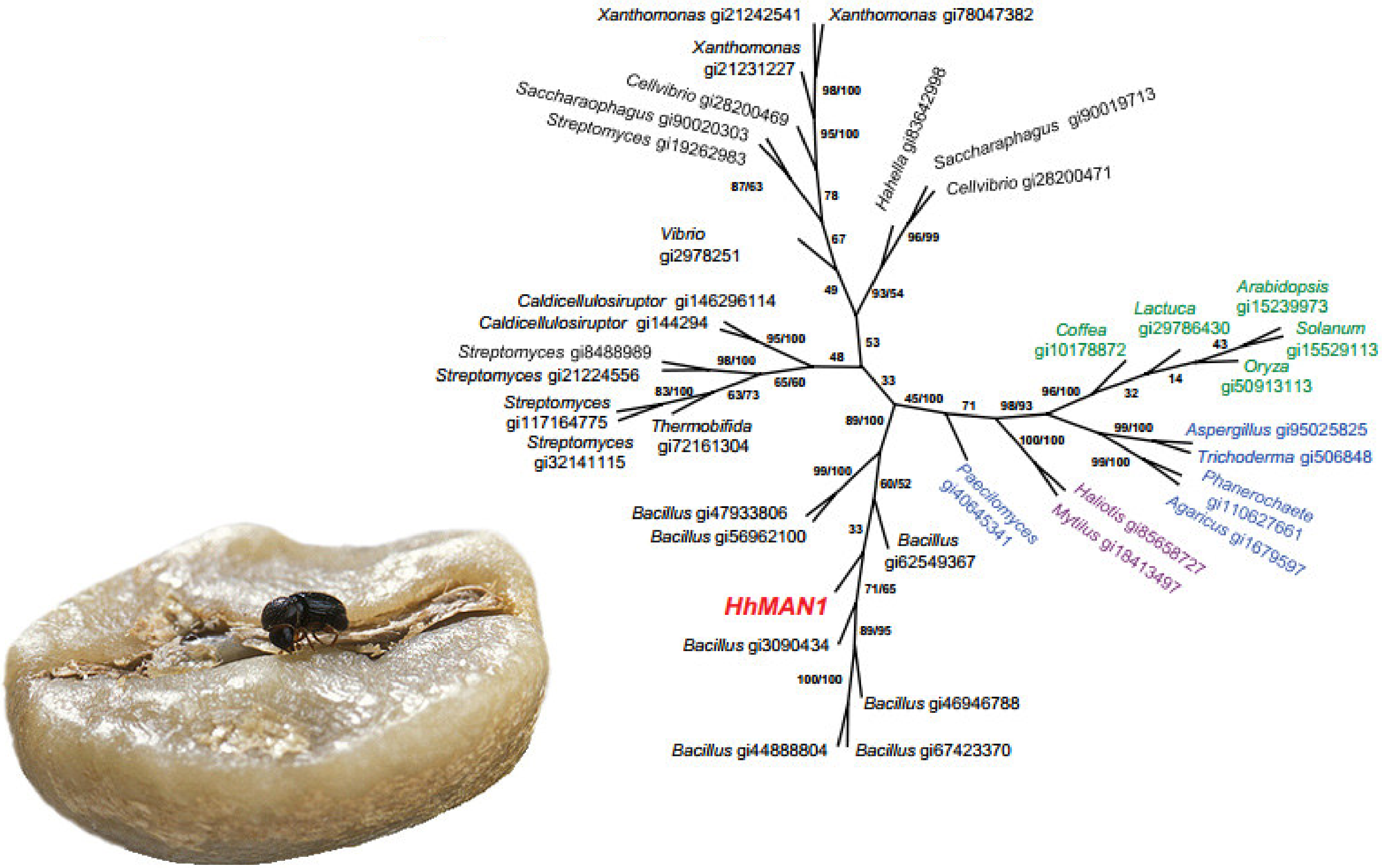
Adaptive horizontal transfer of a bacterial gene to an invasive insect pest of coffee

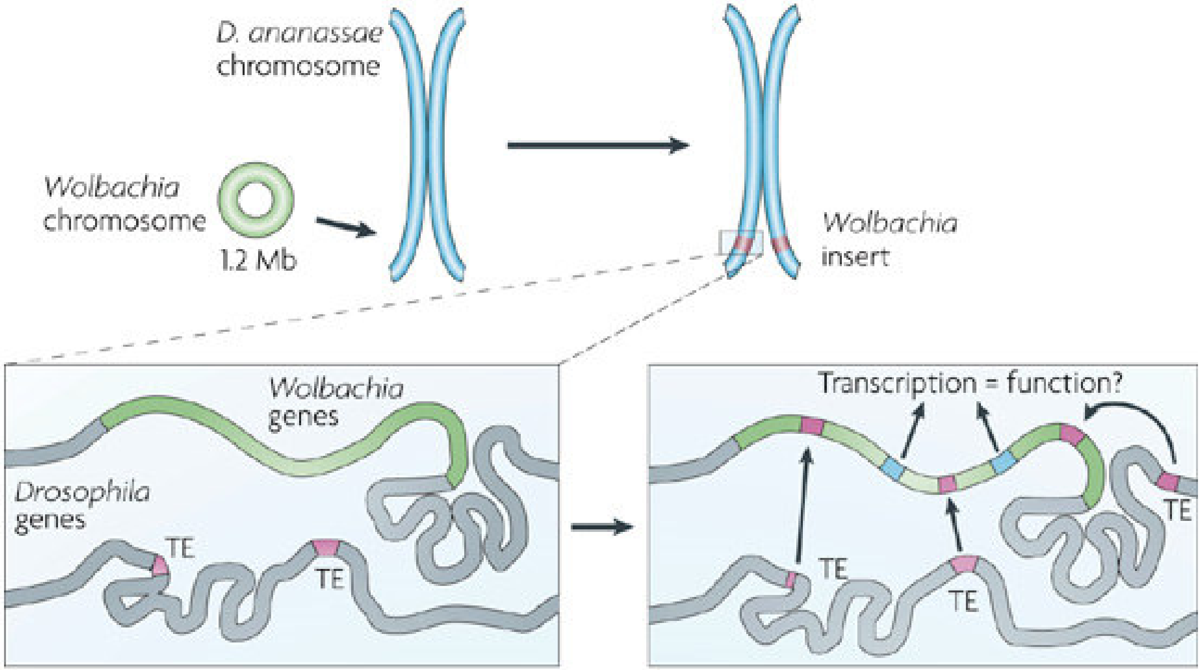
Ricardo Acuña[a,1], Beatriz E. Padilla[a,1], Claudia P. Flórez-Ramos[a], José D. Rubio[a], Juan C. Herrera[a], Pablo Benavides[a], Sang-Jik Lee[b,c], Trevor H. Yeats[b], Ashley N. Egan[b,d], Jeffrey J. Doyle[b], and Jocelyn K. C. Rose[b,2]

[a]Plant Breeding, Biotechnology, and Entomology Departments, Cenicafé, A.A. 2427 Manizales, Colombia; [b]Department of Plant Biology, Cornell University, Ithaca, NY 14853; [c]Biotechnology Institute, Nongwoo Bio Co., Ltd., Gyeonoggi 469-885, Korea; and [d]Department of Biology, East Carolina University, Greenville, NC 27858

Edited by Nancy A. Moran, Yale University, West Haven, CT, and approved February 1, 2012 (received for review December 21, 2011)

Horizontal gene transfer (HGT) involves the nonsexual transmission of genetic material across species boundaries. Although often detected in prokaryotes, examples of HGT involving animals are relatively rare, and any evolutionary advantage conferred to the recipient is typically obscure. We identified a gene (*HhMAN1*) from the coffee berry borer beetle, *Hypothenemus hampei*, a devastating pest of coffee, which shows clear evidence of HGT from bacteria. *HhMAN1* encodes a mannanase, representing a class of glycosyl hydrolases that has not previously been reported in insects. Recombinant HhMAN1 protein hydrolyzes coffee berry galactomannan, the major storage polysaccharide in this species and the presumed food of *H. hampei*. *HhMAN1* was found to be widespread in a broad biogeographic survey of *H. hampei* accessions, indicating that the HGT event occurred before radiation of the insect from West Africa to Asia and South America. However, the gene was not detected in the closely related species *H. obscurus* (the tropical nut borer or "false berry borer"), which does not colonize coffee beans. Thus, HGT of *HhMAN1* from bacteria represents a likely adaptation to a specific ecological niche and may have been promoted by intensive agricultural practices.

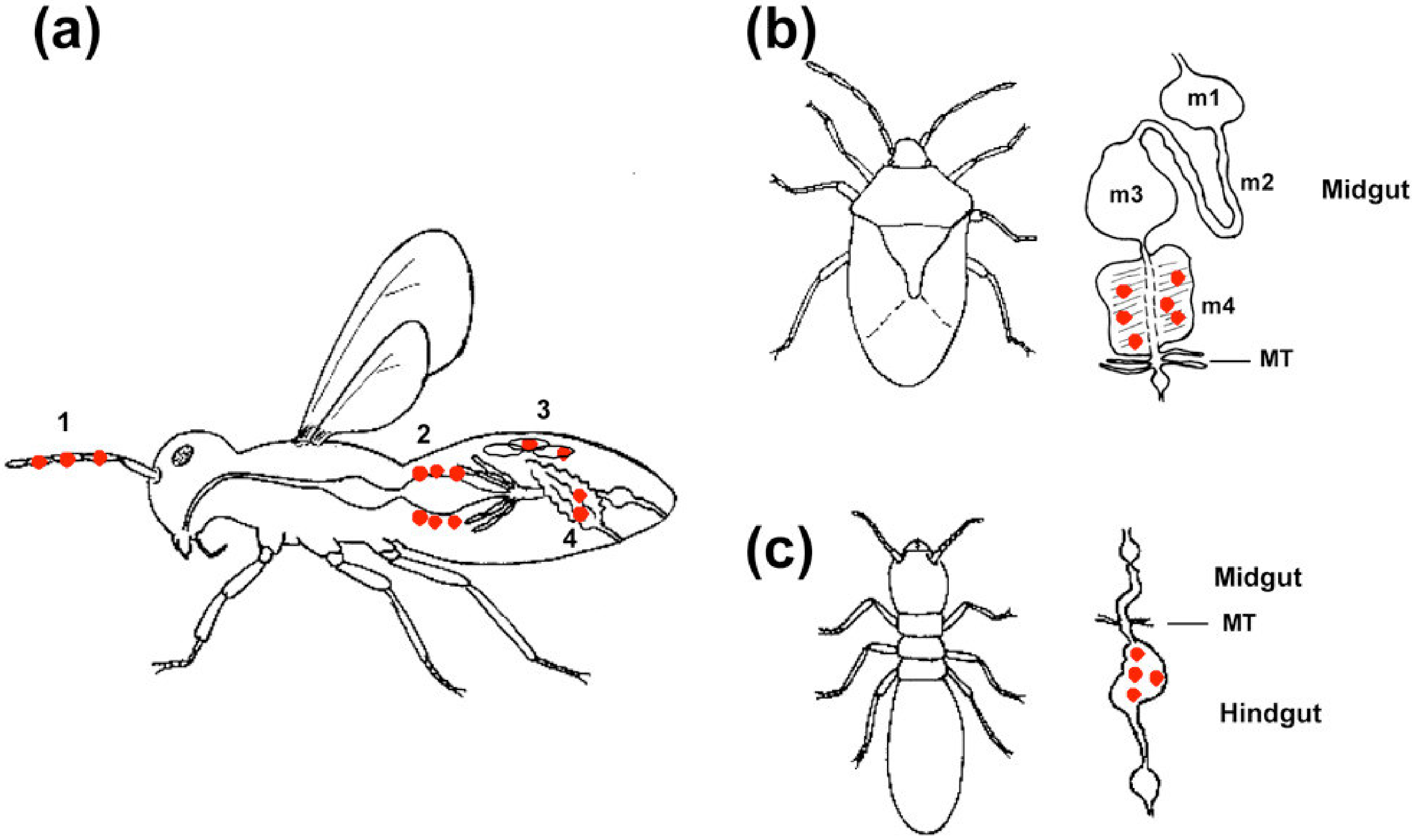

Xanthomonas gi21242541
Xanthomonas gi78047382
Xanthomonas gi21231227
Cellvibrio gi28200469
Saccharaophagus gi90020303
Streptomyces gi19262983
Hahella gi83642998
Saccharaphagus gi90019713
Cellvibrio gi28200471
98/100
95/100
87/63
78
67
96/99
Vibrio gi2978251
49
93/54
Caldicellulosiruptor gi146296114
Caldicellulosiruptor gi144294
95/100
53
48
Streptomyces gi8488989
Streptomyces gi21224556
98/100
65/60
83/100
63/73
33
Streptomyces gi117164775
Streptomyces gi32141115
Thermobifida gi72161304
Coffea gi10178872
Lactuca gi29786430
Arabidopsis gi15239973
Solanum gi15529113
Oryza gi50913113
43
14
32
96/100
45/100
71
98/93
89/100
99/100
Aspergillus gi95025825
Trichoderma gi506848
100/100
99/100
Phanerochaete gi110627661
Agaricus gi1679597
Haliotis gi85658727
Mytilus gi18413497
Paecilomyces gi40645341
99/100
60/52
Bacillus gi47933806
Bacillus gi56962100
33
Bacillus gi62549367
HhMAN1
71/65
Bacillus gi3090434
89/95
100/100
Bacillus gi46946788
Bacillus gi44888804
Bacillus gi67423370

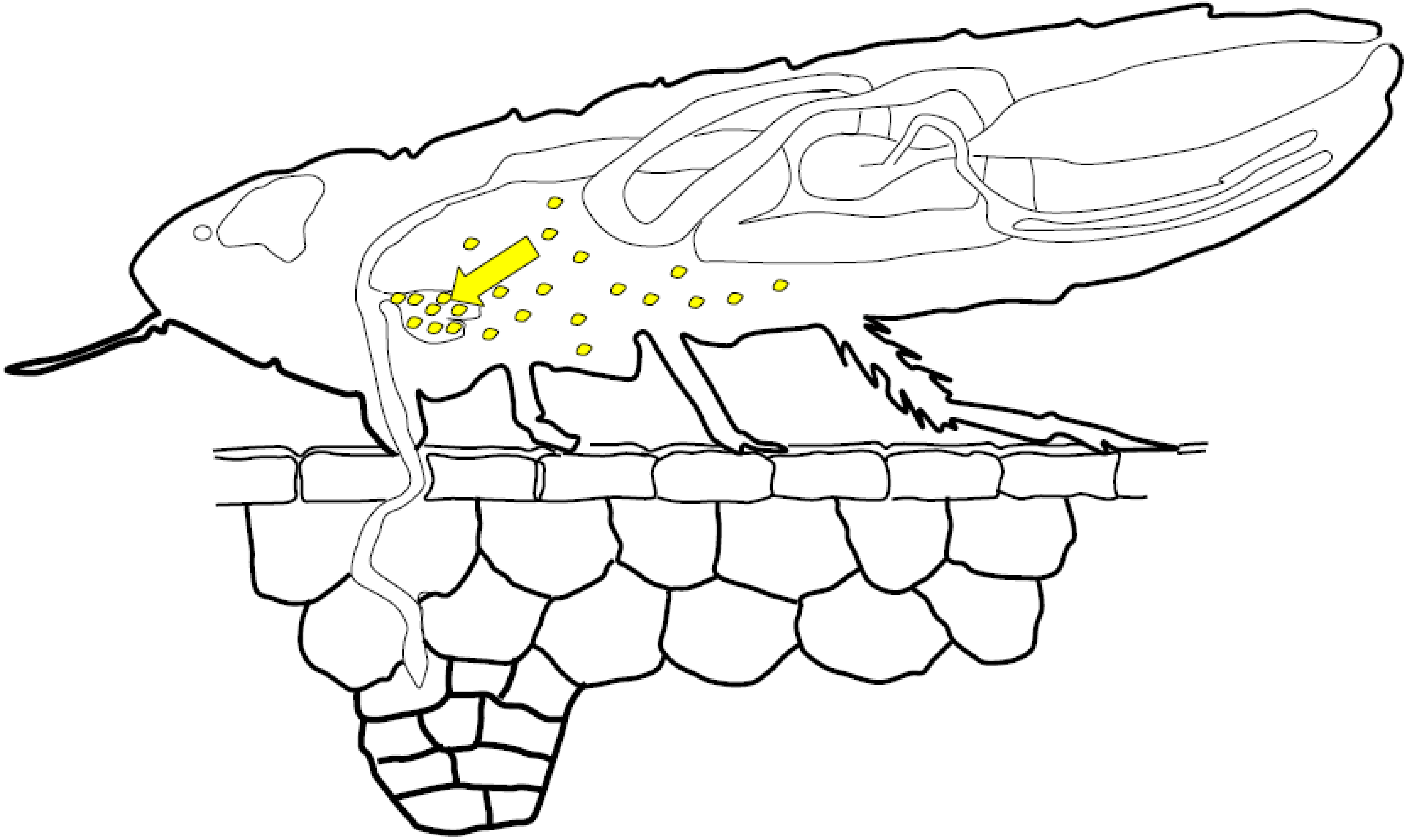


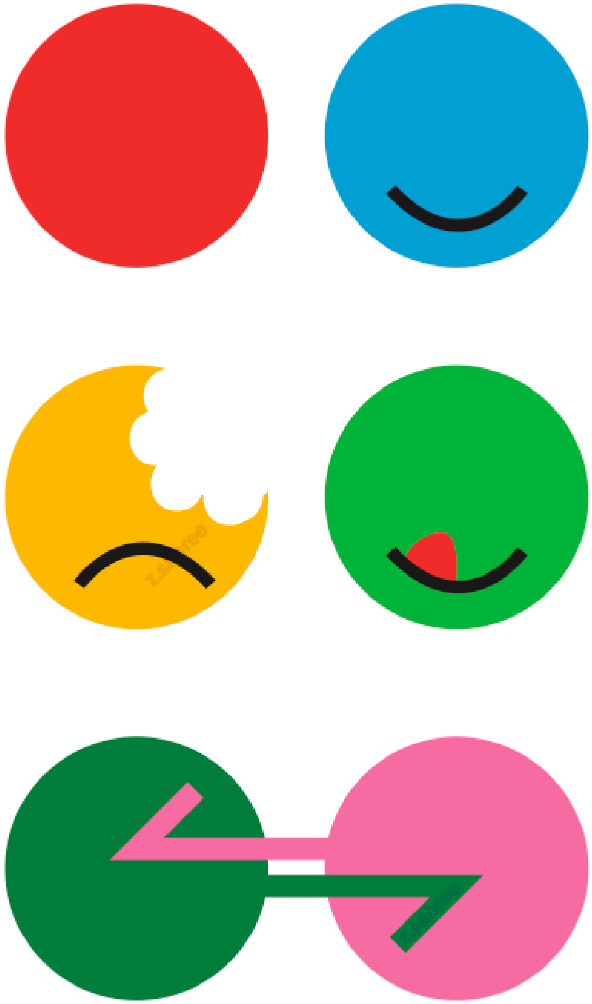
Nature Reviews | Microbiology

Almost the entire *Wolbachia* genome (green) has been transferred into the second chromosome of *Drosophila ananassae* (blue). Following this lateral gene transfer, Drosophila ananassae transposable elements (TEs) have become inserted within Wolbachia genes. At least 28 Wolbachia genes are transcribed from within the D. ananassae genome, although the functional significance of this is unknown.

Secondary Symbionts



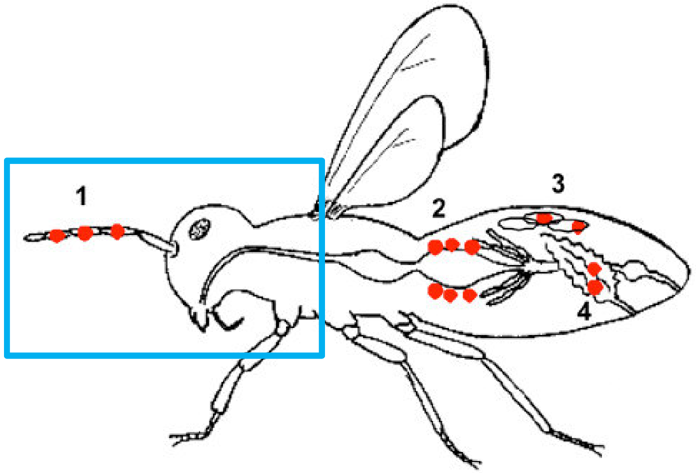




commensal

parasitic

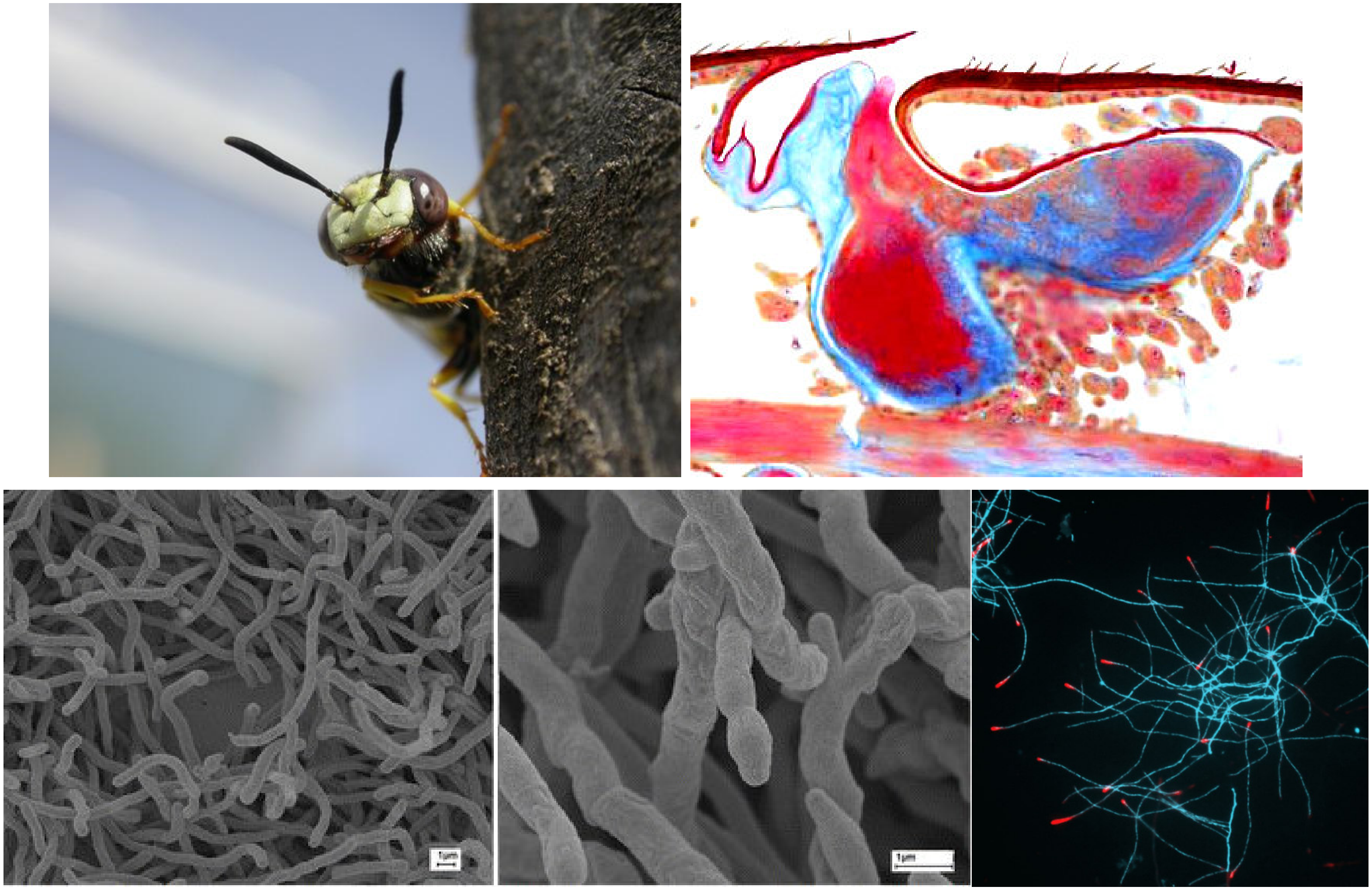
mutualistic



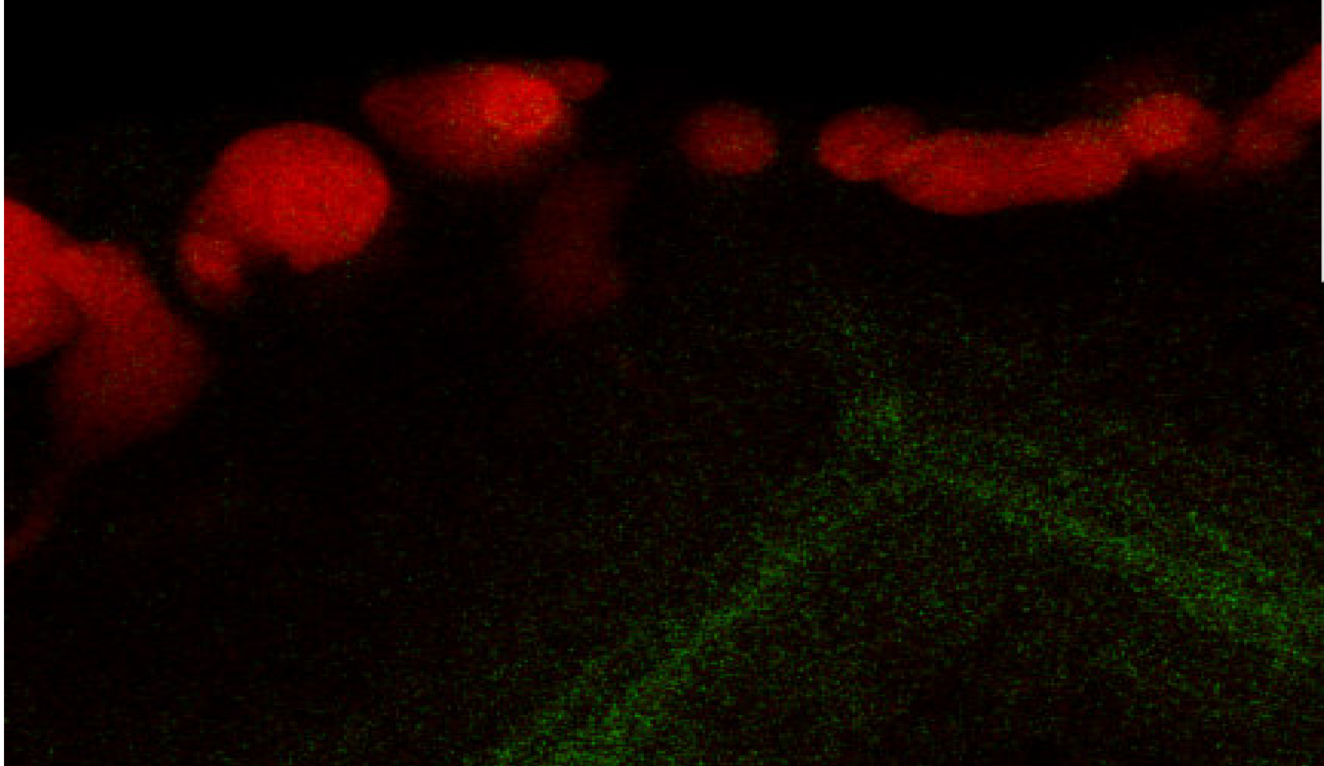
Life within insect antennae: Symbiotic bacteria protect wasp larvae against fungal infestation

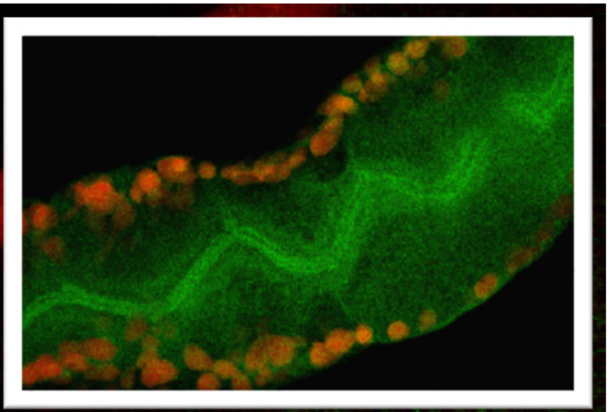
M. Kaltenpoth, (University of Würzburg, Germany);
E. Strohm, (University of Regensburg, Germany)

In most known insect-bacteria symbioses, the symbionts play an important role in the nutrition of the host. Recent studies show that symbiotic microorganisms can also be involved in the protection of the host against pathogen infestation. Here we report on a highly specialized interaction between an insect species and bacteria for the defense of the host's offspring against microorganisms. European beewolves (*Philanthus triangulum*, Hymenoptera, Crabronidae) cultivate symbiotic bacteria of the novel species '*Candidatus Streptomyces philanthi*' in the reservoirs of unique antennal glands. Females apply these bacteria to the brood cell prior to oviposition. The larva takes up the bacteria and applies them to the silk threads of its cocoon. Bioassays indicate that the streptomycetes protect the cocoon from fungal infestation and significantly enhance the survival probability of the larva during the long phase of hibernation in the soil, possibly by producing antibiotics. Behavioral observations and experiments strongly suggest a vertical transmission of the bacteria. All congeneric beewolf species investigated so far (n=27) harbor closely related ecotypes of '*Candidatus Streptomyces philanthi*' in their antennae, indicating that the association with protective bacteria is obligate for the genus *Philanthus* and might play an important role in other insects as well. This is the first report of bacteria being cultivated in insect antennae and the first case of a specialized symbiosis involving bacteria of the important antibiotic–producing genus *Streptomyces*.

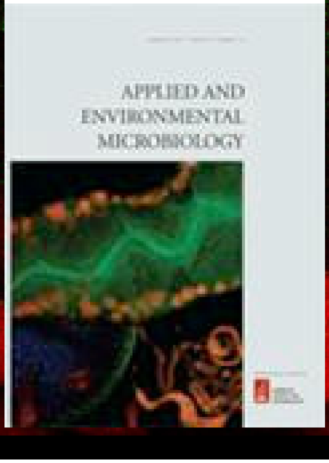

1µm
1µm

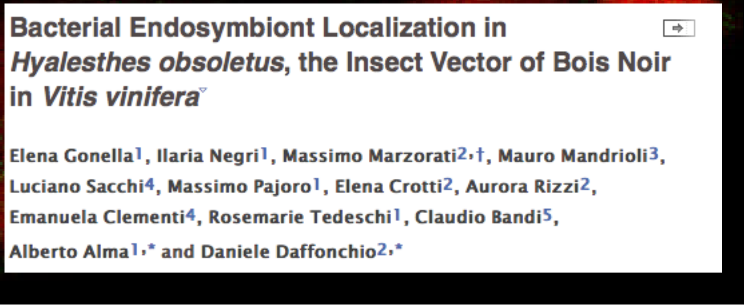
Phytoplasma in the salivary glands of *H. obsoletus.*





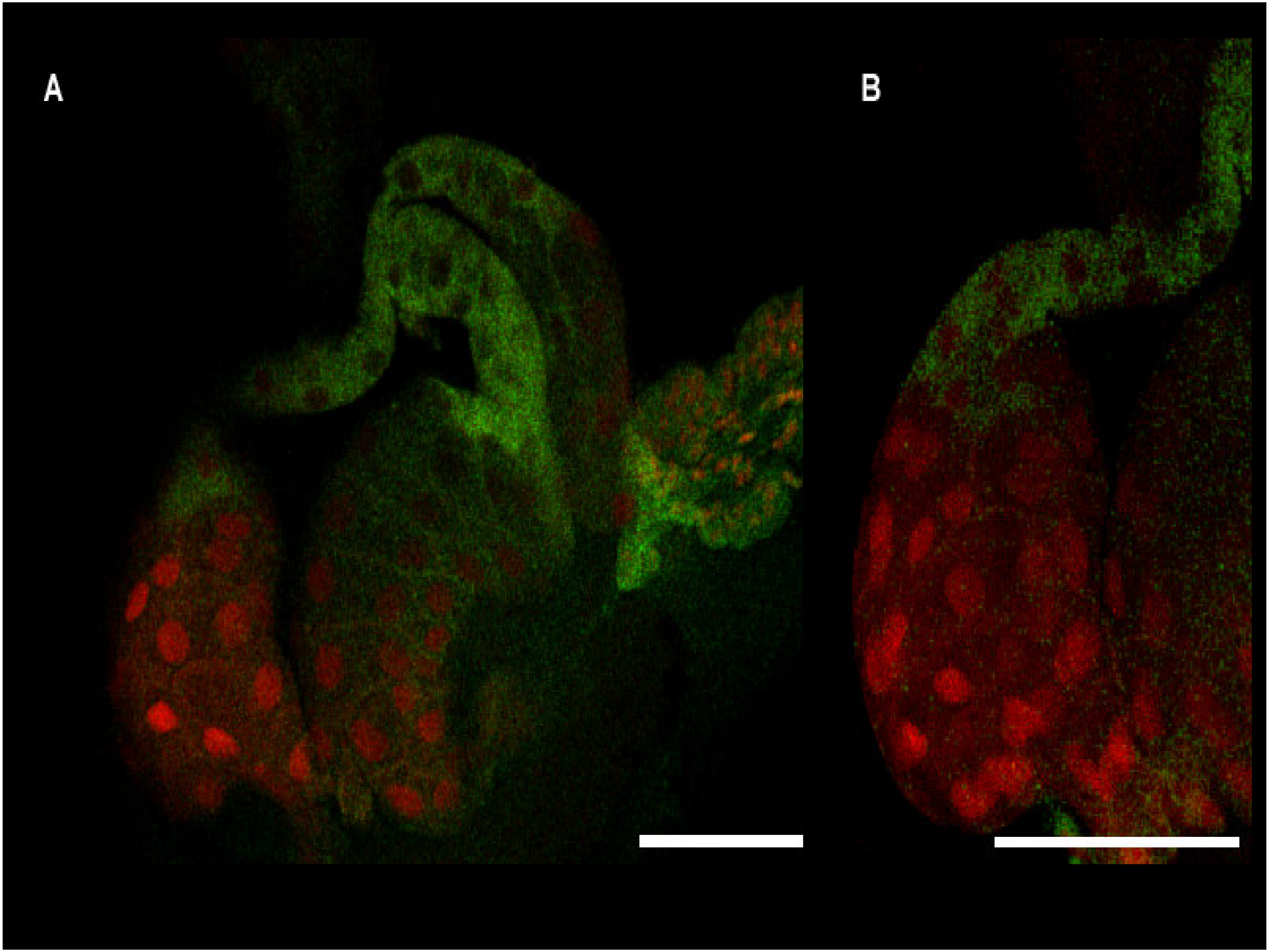
Appl. Environ. Microbiol.
February 2011 vol. 77 no. 4
1423-1435



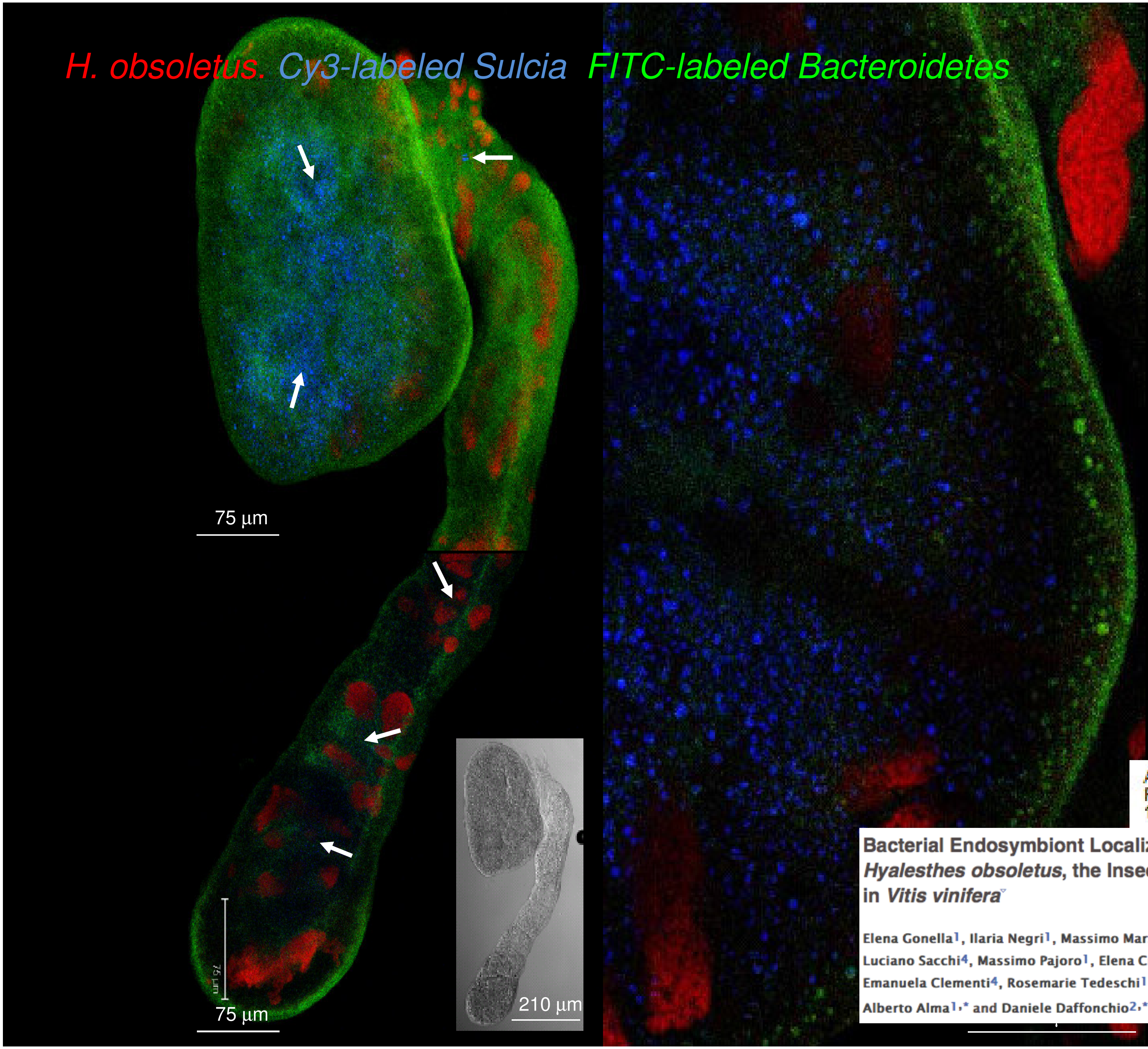


Bacterial Endosymbiont Localization in *Hyalesthes obsoletus*, the Insect Vector of Bois Noir in *Vitis vinifera*[▽]

Elena Gonella[1], Ilaria Negri[1], Massimo Marzorati[2,†], Mauro Mandrioli[3], Luciano Sacchi[4], Massimo Pajoro[1], Elena Crotti[2], Aurora Rizzi[2], Emanuela Clementi[4], Rosemarie Tedeschi[1], Claudio Bandi[5], Alberto Alma[1,*] and Daniele Daffonchio[2,*]


A
B

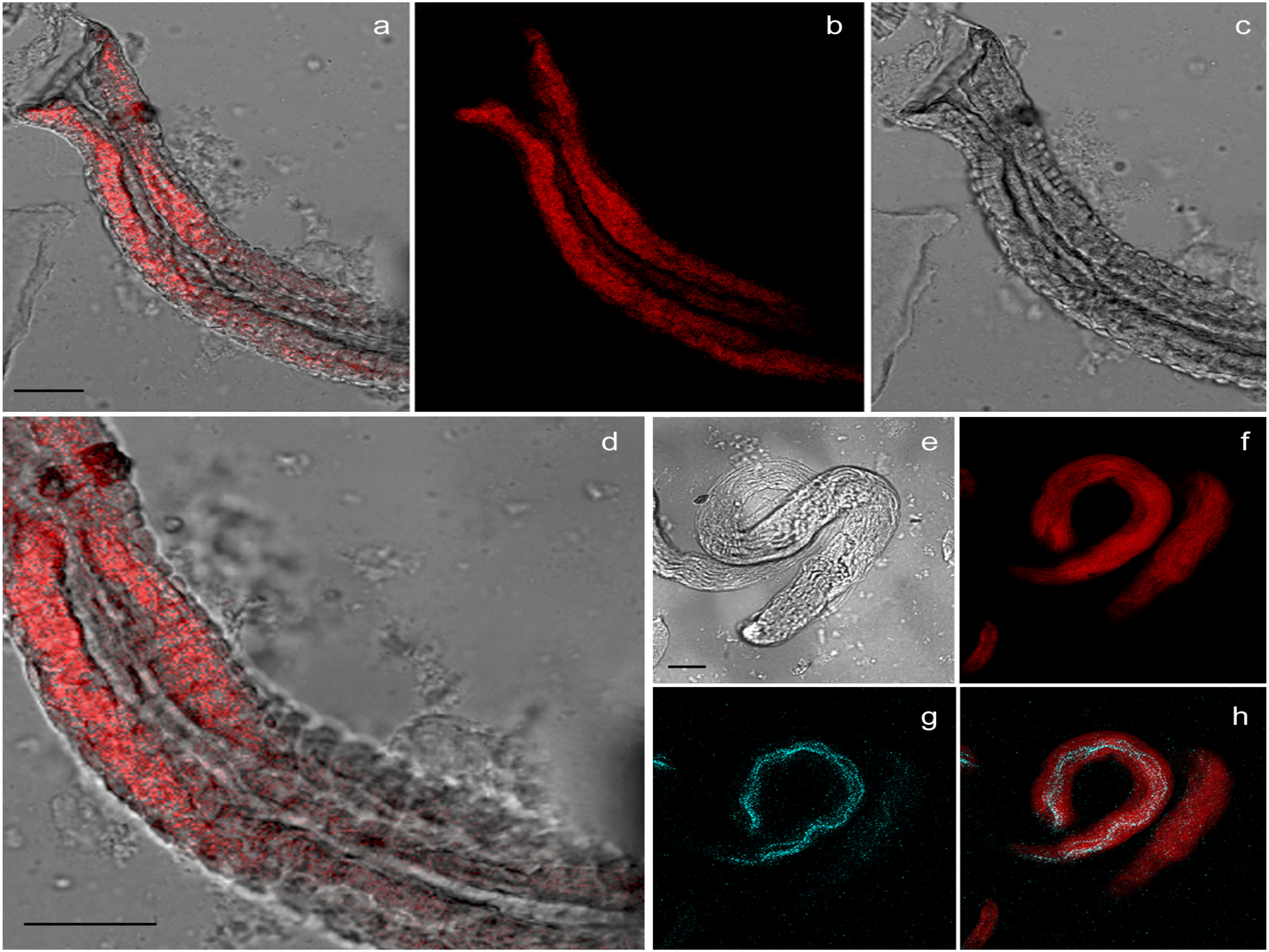
H. obsoletus. *Cy3-labeled Sulcia* *FITC-labeled Bacteroidetes*

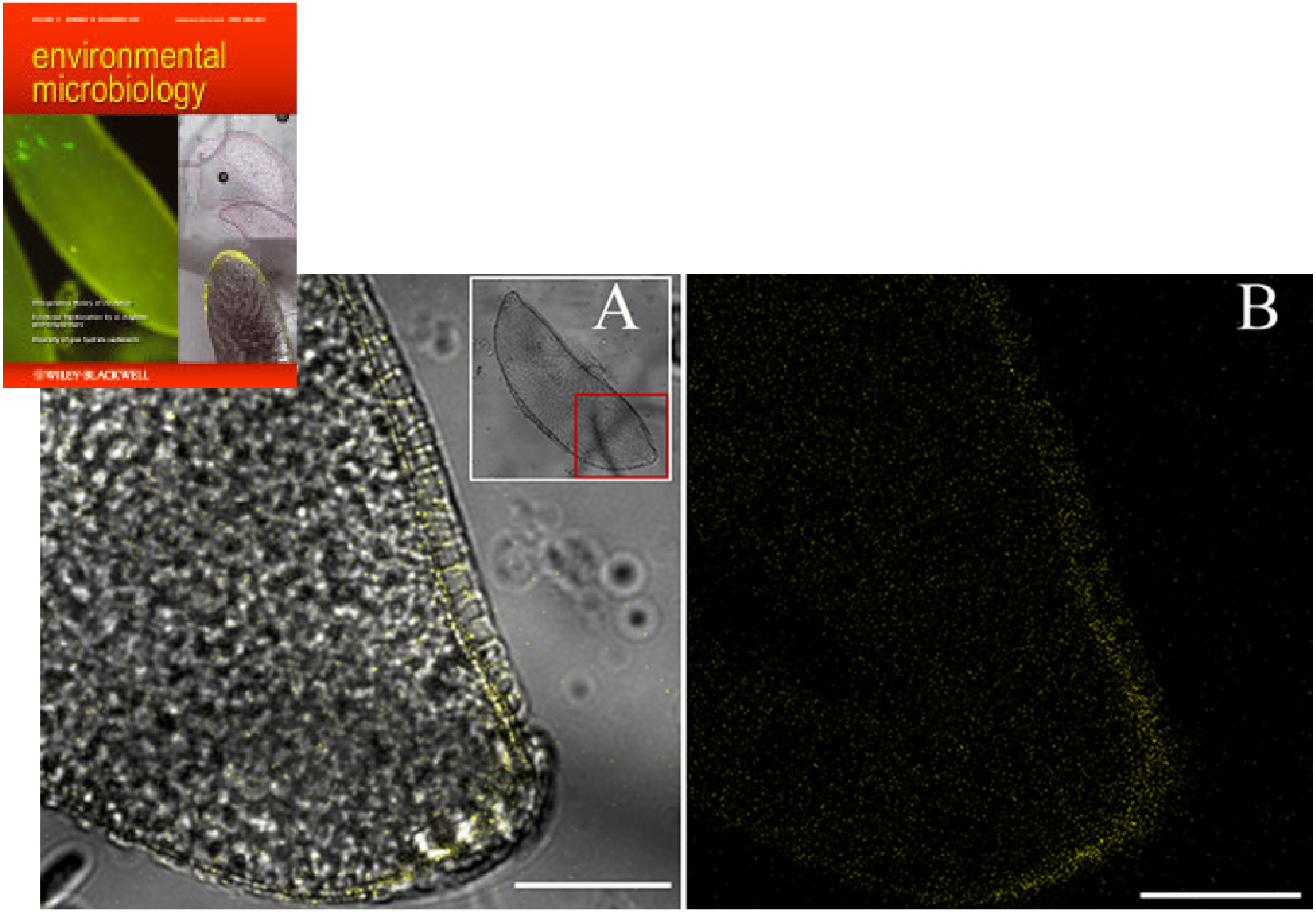


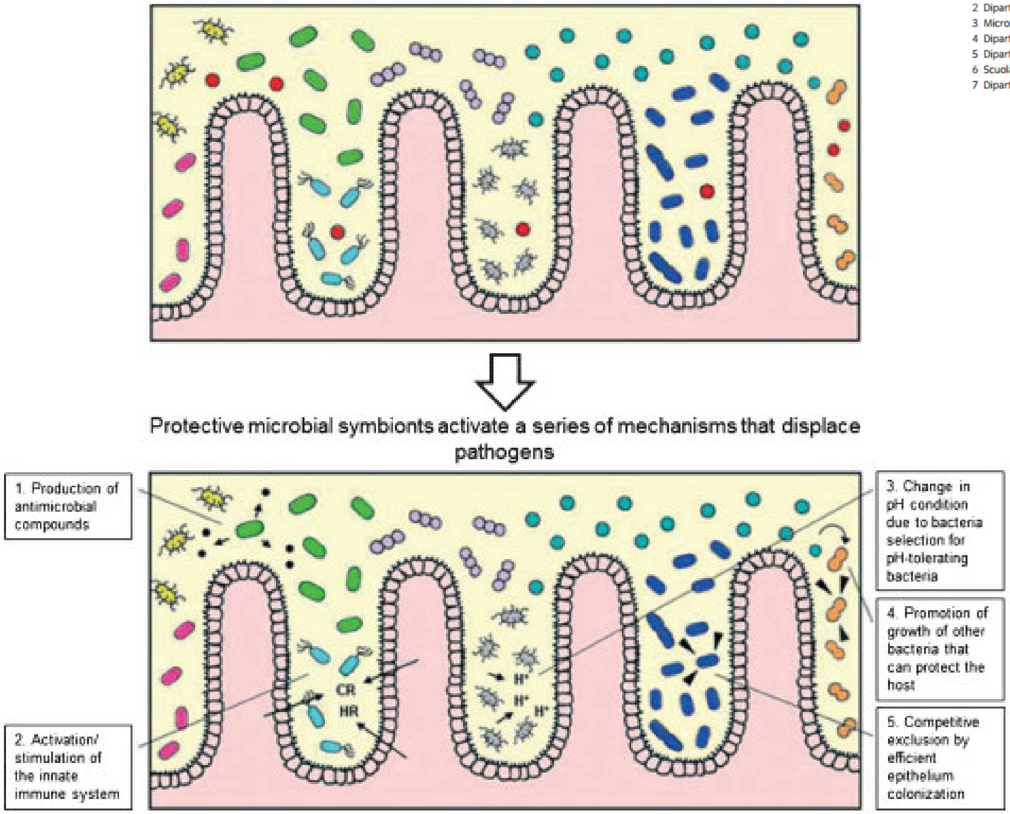
Appl. Environ. Microbiol.
February 2011 vol. 77 no. 4
1423-1435

Bacterial Endosymbiont Localization in *Hyalesthes obsoletus*, the Insect Vector of Bois Noir in *Vitis vinifera*

Elena Gonella[1], Ilaria Negri[1], Massimo Marzorati[2,†], Mauro Mandrioli[3], Luciano Sacchi[4], Massimo Pajoro[1], Elena Crotti[2], Aurora Rizzi[2], Emanuela Clementi[4], Rosemarie Tedeschi[1], Claudio Bandi[5], Alberto Alma[1,*] and Daniele Daffonchio[2,*]


a
b
c
d
e
f
g
h


environmental
microbiology
WILEY-BLACKWELL
A
B



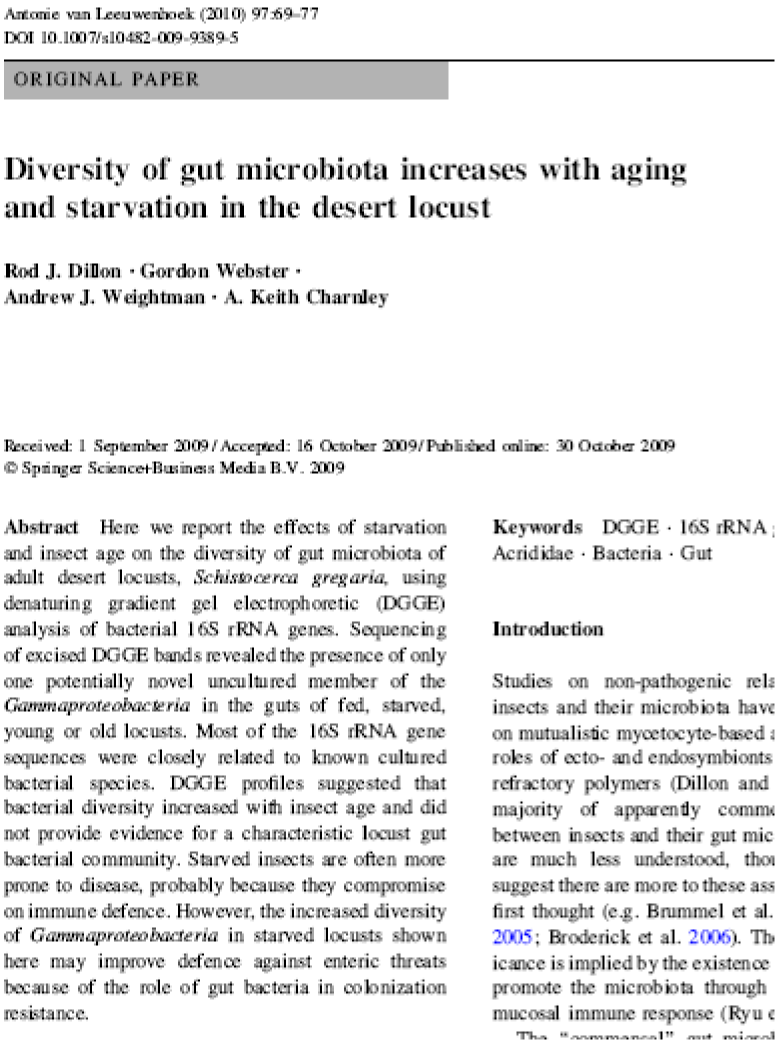
JOURNAL OF APPLIED ENTOMOLOGY
J. Appl. Entomol.

REVIEW ARTICLE

Gut microbiome dysbiosis and honeybee health

C. Hamdi[1,†], A. Balloi[2,3,†], J. Essanaa[1], E. Crotti[2], E. Gonella[4], N. Raddadi[5], I. Ricci[6], A. Boudabous[1], S. Borin[2], A. Manino[4], C. Bandi[7], A. Alma[4], D. Daffonchio[2] & A. Cherif[1]

1 Laboratoire des Microrganismes et Biomolecules Actives (LMBA), Université de Tunis 'El Manar', Tunis, Tunisia
2 Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche (DiSTAM), Università degli Studi di Milano, Milan, Italy
3 Micro$_4$yoU s.r.l., Milan, Italy
4 Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali (DIVAPRA), Università degli Studi di Torino, Grugliasco, Italy
5 Dipartimento di Ingegneria Civile, Ambientale e dei Materiali (DICASM), Università di Bologna, Bologna, Italy
6 Scuola di Bioscienze e Biotecnologie, Università degli Studi di Camerino, Camerino, Italy
7 Dipartimento di Patologia Animale, Igiene e Sanità Pubblica Veterinaria (DiPAV), Università degli Studi di Milano, Milan, Italy



Antonie van Leeuwenhoek (2010) 97:69–77
DOI 10.1007/s10482-009-9389-5

ORIGINAL PAPER

Diversity of gut microbiota increases with aging and starvation in the desert locust

Rod J. Dillon · Gordon Webster ·
Andrew J. Weightman · A. Keith Charnley

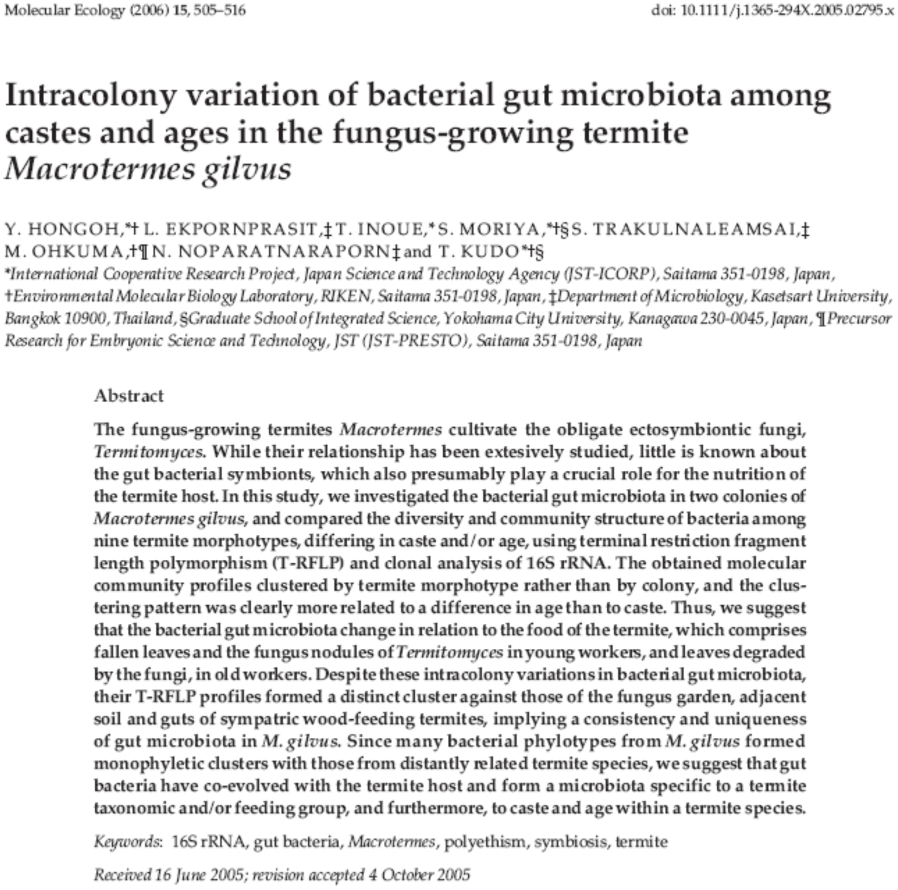
Received: 1 September 2009 / Accepted: 16 October 2009 / Published online: 30 October 2009

Abstract Here we report the effects of starvation and insect age on the diversity of gut microbiota of adult desert locusts, *Schistocerca gregaria*, using denaturing gradient gel electrophoretic (DGGE) analysis of bacterial 16S rRNA genes. Sequencing of excised DGGE bands revealed the presence of only one potentially novel uncultured member of the *Gammaproteobacteria* in the guts of fed, starved, young or old locusts. Most of the 16S rRNA gene sequences were closely related to known cultured bacterial species. DGGE profiles suggested that bacterial diversity increased with insect age and did not provide evidence for a characteristic locust gut bacterial community. Starved insects are often more prone to disease, probably because they compromise on immune defence. However, the increased diversity of *Gammaproteobacteria* in starved locusts shown here may improve defence against enteric threats because of the role of gut bacteria in colonization resistance.

Keywords DGGE · 16S rRNA
Acrididae · Bacteria · Gut

Introduction

Studies on non-pathogenic rel
insects and their microbiota have
on mutualistic mycetocyte-based
roles of ecto- and endosymbionts
refractory polymers (Dillon and
majority of apparently comm
between insects and their gut mic
are much less understood, tho
suggest there are more to these ass
first thought (e.g. Brummel et al.
2005; Broderick et al. 2006). Th
icance is implied by the existence
promote the microbiota through
mucosal immune response (Ryu
The “commensal” gut micro



Molecular Ecology (2006) 15, 505–516 doi: 10.1111/j.1365-294X.2005.02795.x

Intracolony variation of bacterial gut microbiota among castes and ages in the fungus-growing termite *Macrotermes gilvus*

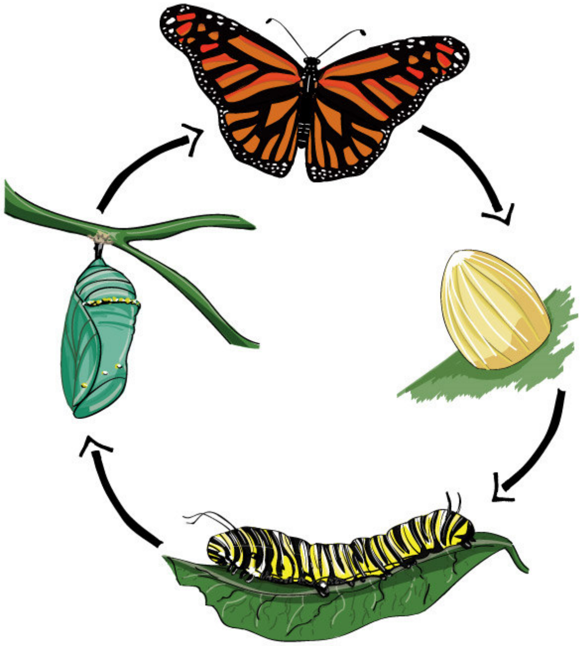
Y. HONGOH,*† L. EKPORNPRASIT,‡ T. INOUE,* S. MORIYA,*†§ S. TRAKULNALEAMSAI,‡
M. OHKUMA,†¶ N. NOPARATNARAPORN‡ and T. KUDO*†§
**International Cooperative Research Project, Japan Science and Technology Agency (JST-ICORP), Saitama 351-0198, Japan, †Environmental Molecular Biology Laboratory, RIKEN, Saitama 351-0198, Japan, ‡Department of Microbiology, Kasetsart University, Bangkok 10900, Thailand, §Graduate School of Integrated Science, Yokohama City University, Kanagawa 230-0045, Japan, ¶Precursor Research for Embryonic Science and Technology, JST (JST-PRESTO), Saitama 351-0198, Japan*

Abstract

The fungus-growing termites *Macrotermes* cultivate the obligate ectosymbiontic fungi, *Termitomyces*. While their relationship has been extesively studied, little is known about the gut bacterial symbionts, which also presumably play a crucial role for the nutrition of the termite host. In this study, we investigated the bacterial gut microbiota in two colonies of *Macrotermes gilvus*, and compared the diversity and community structure of bacteria among nine termite morphotypes, differing in caste and/or age, using terminal restriction fragment length polymorphism (T-RFLP) and clonal analysis of 16S rRNA. The obtained molecular community profiles clustered by termite morphotype rather than by colony, and the clustering pattern was clearly more related to a difference in age than to caste. Thus, we suggest that the bacterial gut microbiota change in relation to the food of the termite, which comprises fallen leaves and the fungus nodules of *Termitomyces* in young workers, and leaves degraded by the fungi, in old workers. Despite these intracolony variations in bacterial gut microbiota, their T-RFLP profiles formed a distinct cluster against those of the fungus garden, adjacent soil and guts of sympatric wood-feeding termites, implying a consistency and uniqueness of gut microbiota in *M. gilvus*. Since many bacterial phylotypes from *M. gilvus* formed monophyletic clusters with those from distantly related termite species, we suggest that gut bacteria have co-evolved with the termite host and form a microbiota specific to a termite taxonomic and/or feeding group, and furthermore, to caste and age within a termite species.

Keywords: 16S rRNA, gut bacteria, *Macrotermes*, polyethism, symbiosis, termite

Received 16 June 2005; revision accepted 4 October 2005

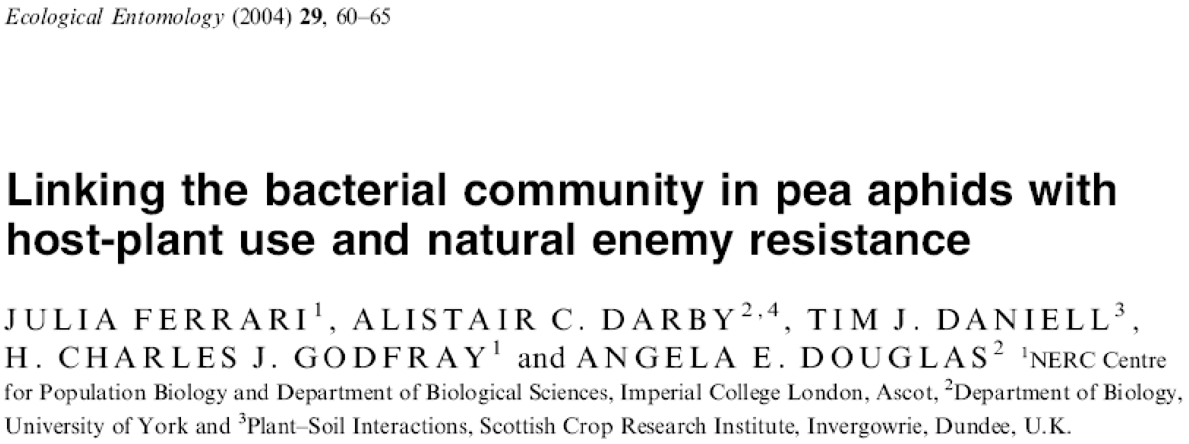


Rev. Biol. Trop. 50(2): 547-560, 2002
www.ucr.ac.cr www.ots.ac.cr www.ots.duke.edu

Does a polyphagous caterpillar have the same gut microbiota when feeding on different species of food plants?

Ana Sittenfeld[1, 3], Lorena Uribe-Lorío[1], Marielos Mora[1], Vanesa Nielsen[1], Glen Arrieta[1] and Daniel H. Janzen[2]

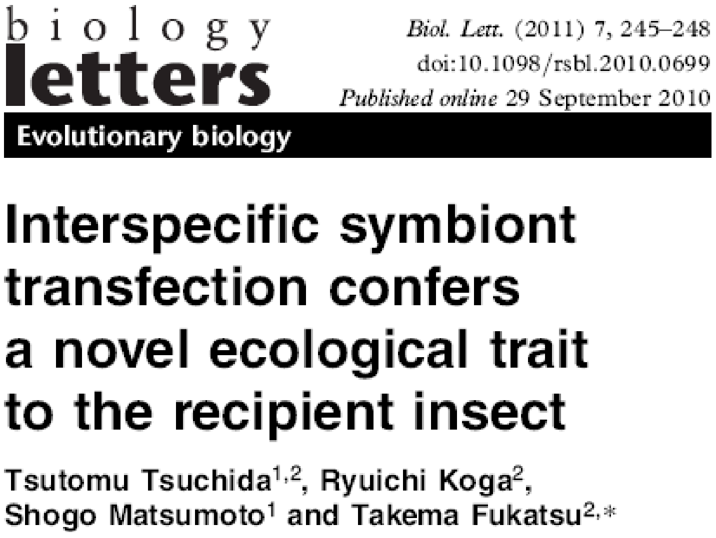
1 Centro de Investigación en Biología Celular y Molecular (CIBCM), Universidad de Costa Rica, Ciudad Universitaria Rodrigo Facio, San José, Costa Rica.
2 Department of Biology, University of Pennsylvania, Philadelphia, PA 19104; djanzen@sas.upenn.edu
3 Corresponding autor. Tel.: +506 207 3204; Fax +506 207 3190; sitten@racsa.co.cr



Ecological Entomology (2004) **29**, 60–65

Linking the bacterial community in pea aphids with host-plant use and natural enemy resistance

JULIA FERRARI[1], ALISTAIR C. DARBY[2,4], TIM J. DANIELL[3], H. CHARLES J. GODFRAY[1] and ANGELA E. DOUGLAS[2] [1]NERC Centre for Population Biology and Department of Biological Sciences, Imperial College London, Ascot, [2]Department of Biology, University of York and [3]Plant–Soil Interactions, Scottish Crop Research Institute, Invergowrie, Dundee, U.K.



biology letters

Biol. Lett. (2011) 7, 245–248
doi:10.1098/rsbl.2010.0699

Evolutionary biology

Interspecific symbiont transfection confers a novel ecological trait to the recipient insect

Tsutomu Tsuchida[1,2], Ryuichi Koga[2], Shogo Matsumoto[1] and Takema Fukatsu[2,*]

www.sciencemag.org SCIENCE VOL 303 26 MARCH 2004

Host Plant Specialization Governed by Facultative Symbiont

Tsutomu Tsuchida, Ryuichi Koga, Takema Fukatsu*

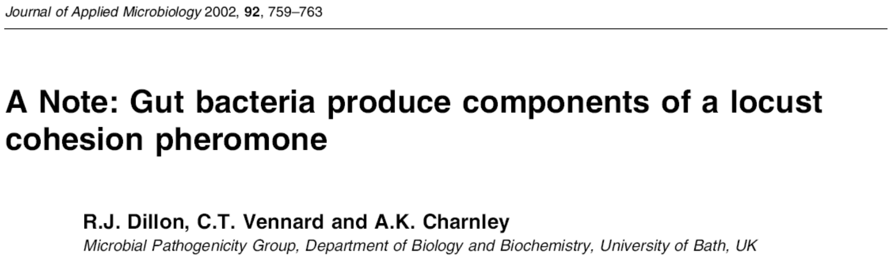
Research in Microbiology 153 (2002) 503–509
Research in Microbiology
www.elsevier.com/locate/resmic

Mini-review

Mutualism between the desert locust *Schistocerca gregaria* and its gut microbiota

Rod Dillon, Keith Charnley *
Department of Biology and Biochemistry, University of Bath, Claverton Down, Bath, BA2 7AY, UK

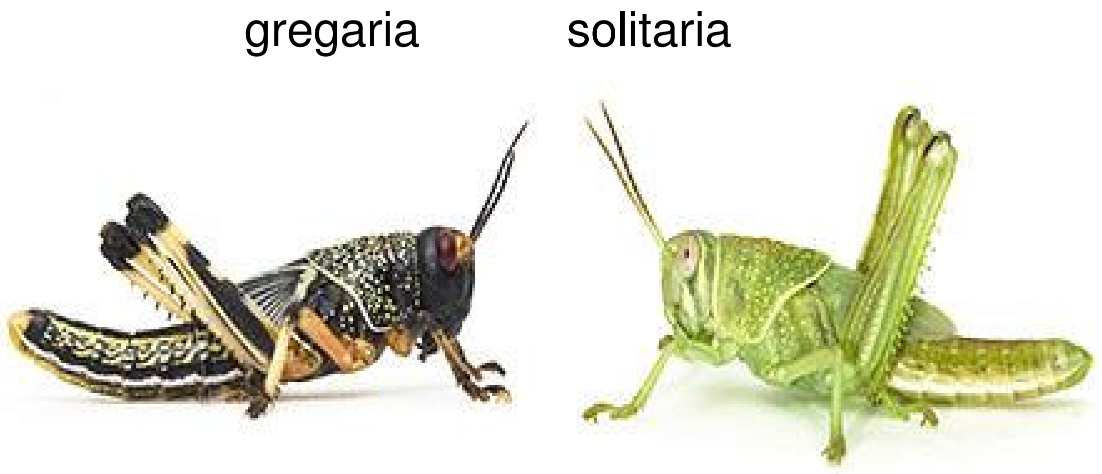
Received 18 March 2002; accepted 12 June 2002



Journal of Applied Microbiology 2002, **92**, 759–763

A Note: Gut bacteria produce components of a locust cohesion pheromone

R.J. Dillon, C.T. Vennard and A.K. Charnley
Microbial Pathogenicity Group, Department of Biology and Biochemistry, University of Bath, UK



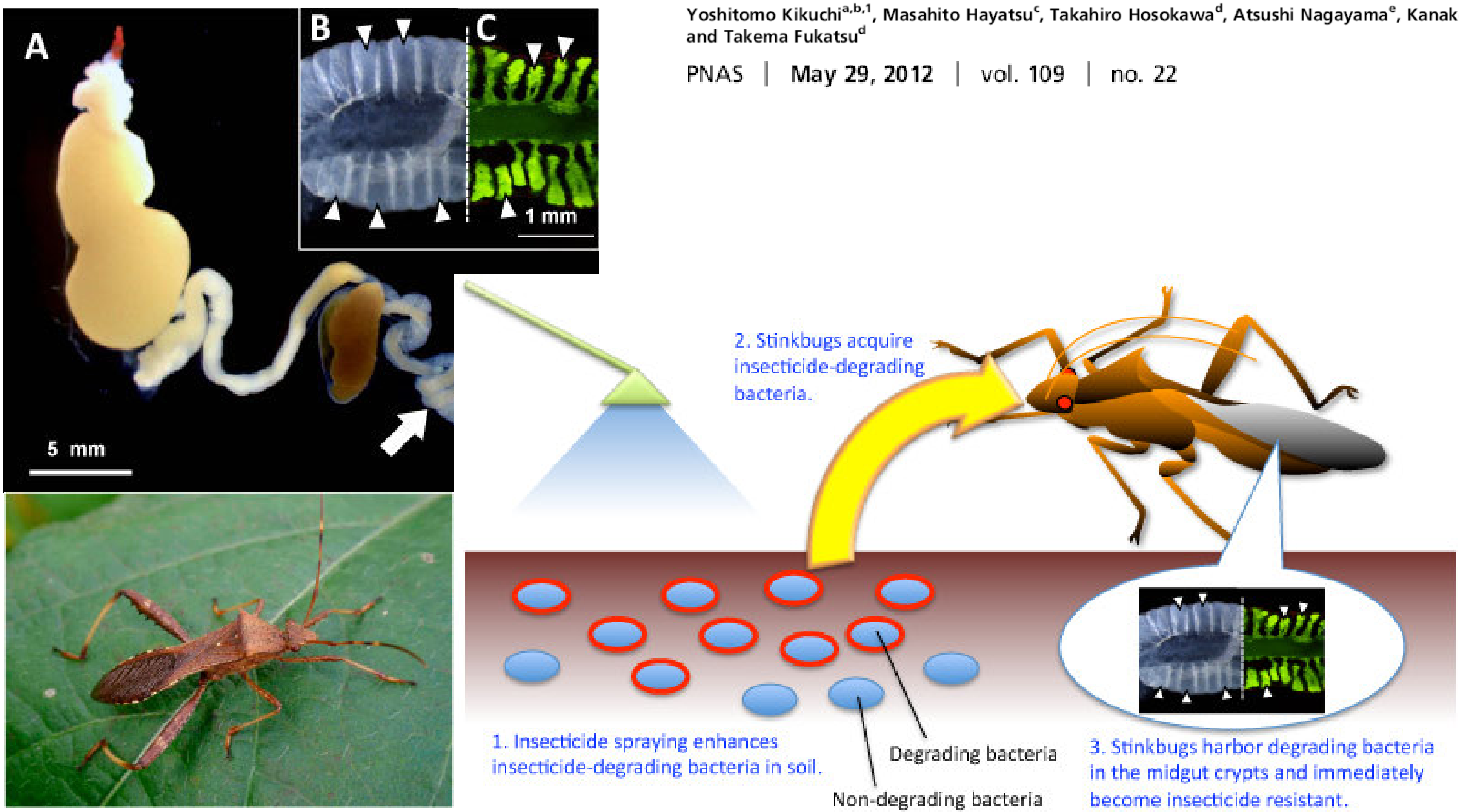
Symbionts provide pesticide detoxification

John H. Werren[1]
Department of Biology, University of Rochester, Rochester, NY 14627

Symbiont-mediated insecticide resistance

Yoshitomo Kikuchi[a,b,1], Masahito Hayatsu[c], Takahiro Hosokawa[d], Atsushi Nagayama[e], Kanako Tago[c], and Takema Fukatsu[d]

PNAS | May 29, 2012 | vol. 109 | no. 22



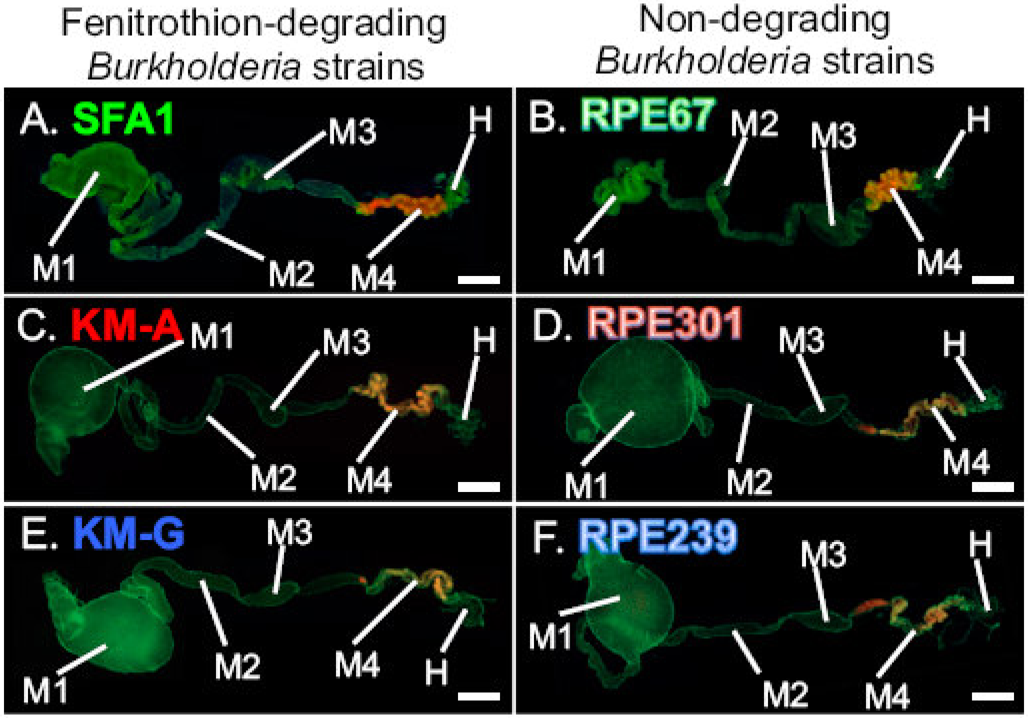
Riptortus pedestris

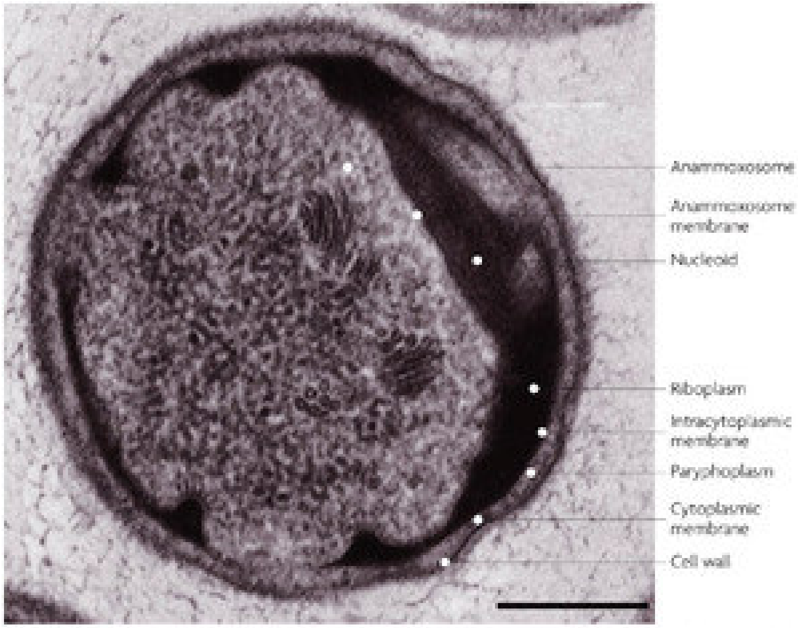
1. Insecticide spraying enhances insecticide-degrading bacteria in soil.

2. Stinkbugs acquire insecticide-degrading bacteria.

3. Stinkbugs harbor degrading bacteria in the midgut crypts and immediately become insecticide resistant.

Development of insecticide resistance has been a serious concern worldwide, whose mechanisms have been attributed to evolutionary changes in pest insect genomes such as alteration of drug target sites, up-regulation of degrading enzymes, and enhancement of drug excretion. Here, we report a previously unknown mechanism of insecticide resistance: Infection with an insecticide-degrading bacterial symbiont immediately establishes insecticide resistance in pest insects. The bean bug *Riptortus pedestris* and allied stinkbugs harbor mutualistic gut symbiotic bacteria of the genus *Burkholderia*, which are acquired by nymphal insects from environmental soil every generation. In agricultural fields, fenitrothion-degrading *Burkolderia* strains are present at very low densities. We demonstrated that the fenitrothion-degrading *Burkholderia* strains establish a specific and beneficial symbiosis with the stinkbugs and confer a resistance of the host insects against fenitrothion. Experimental applications of fenitrothion to field soils drastically enriched fenitrothion-degrading bacteria from undetectable levels to >80% of total culturable bacterial counts in the field soils, and >90% of stinkbugs reared with the enriched soil established symbiosis with fenitrothion-degrading *Burkholderia*. In a Japanese island where fenitrothion has been constantly applied to sugarcane fields, we identified a stinkbug population wherein the insects live on sugarcane and ≈8% of them host fenitrothion-degrading *Burkholderia*. Our finding suggests the possibility that the symbiont-mediated insecticide resistance may develop even in the absence of pest insects, quickly establish within a single insect generation, and potentially move around horizontally between different pest insects and other organisms.





Nature Reviews | Microbiology

Acyrthosiphon pisum secondary endosymbiont virus (APSE-1)

J Mol Hist
DOI 10.1007/s10735-011-9382-y

ORIGINAL PAPER

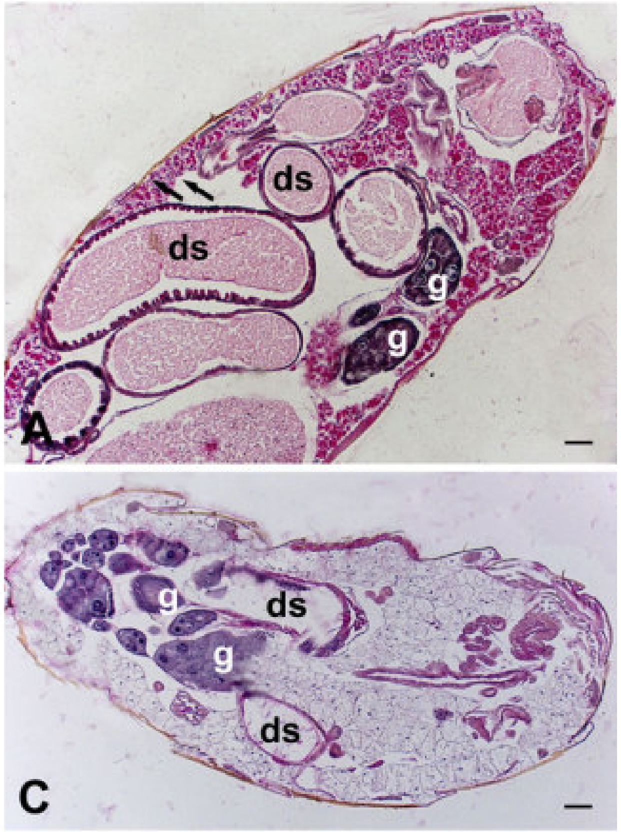
Morpho-functional changes of fat body in bacteria fed *Drosophila melanogaster* strains

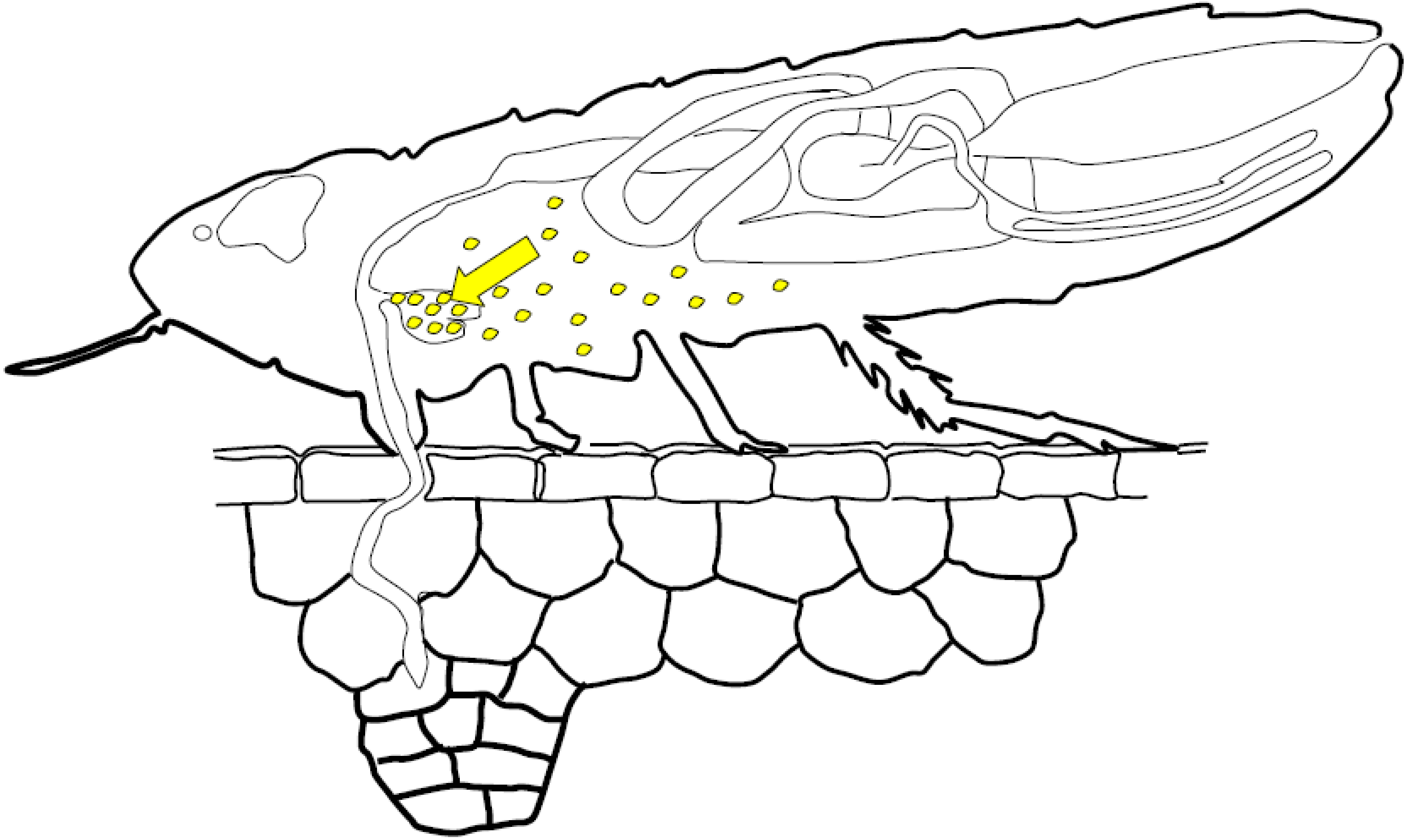
**Antonella Franchini · Mauro Mandrioli ·
Claudio Franceschi · Enzo Ottaviani**

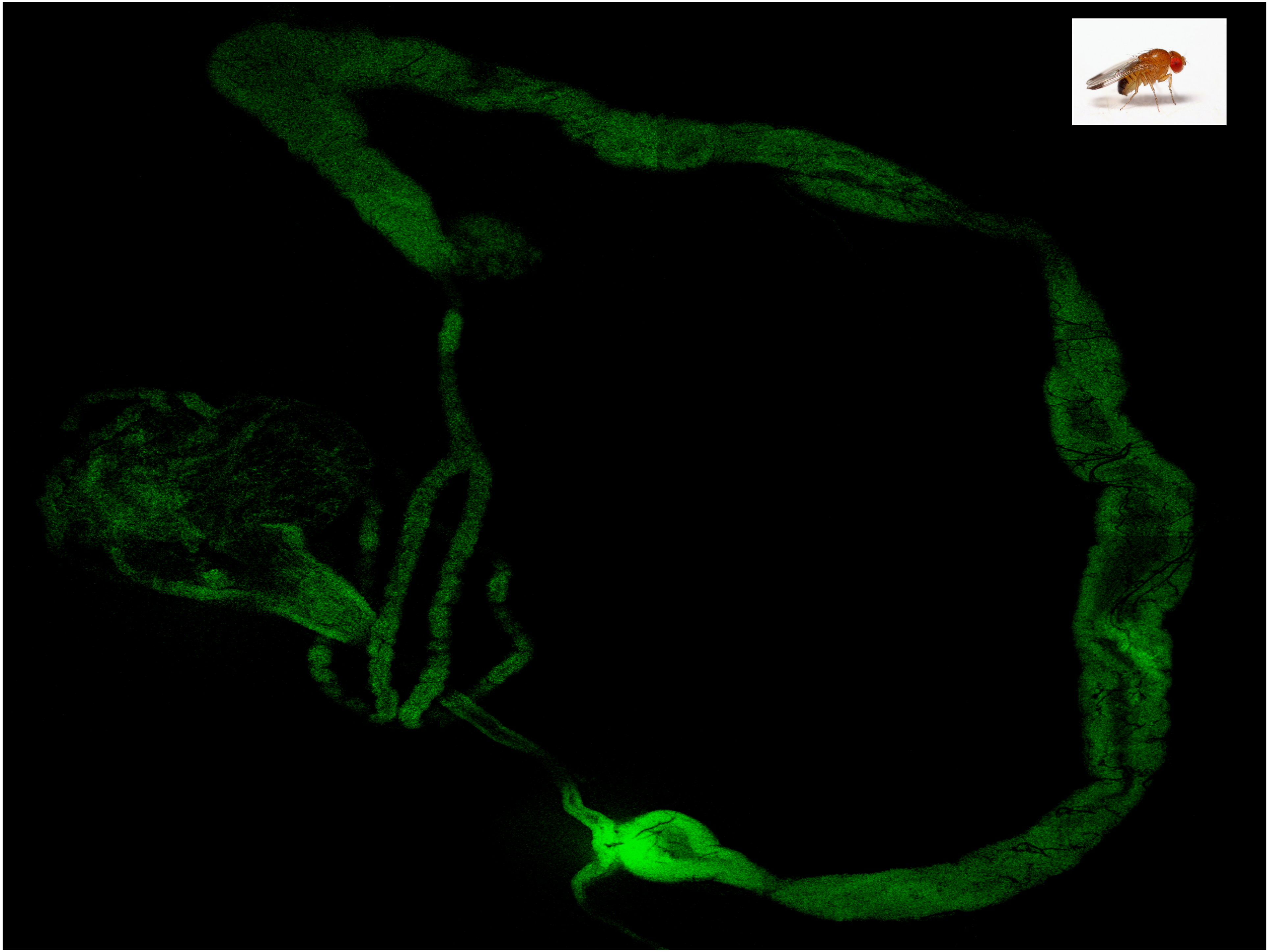
Received: 13 June 2011 / Accepted: 4 December 2011

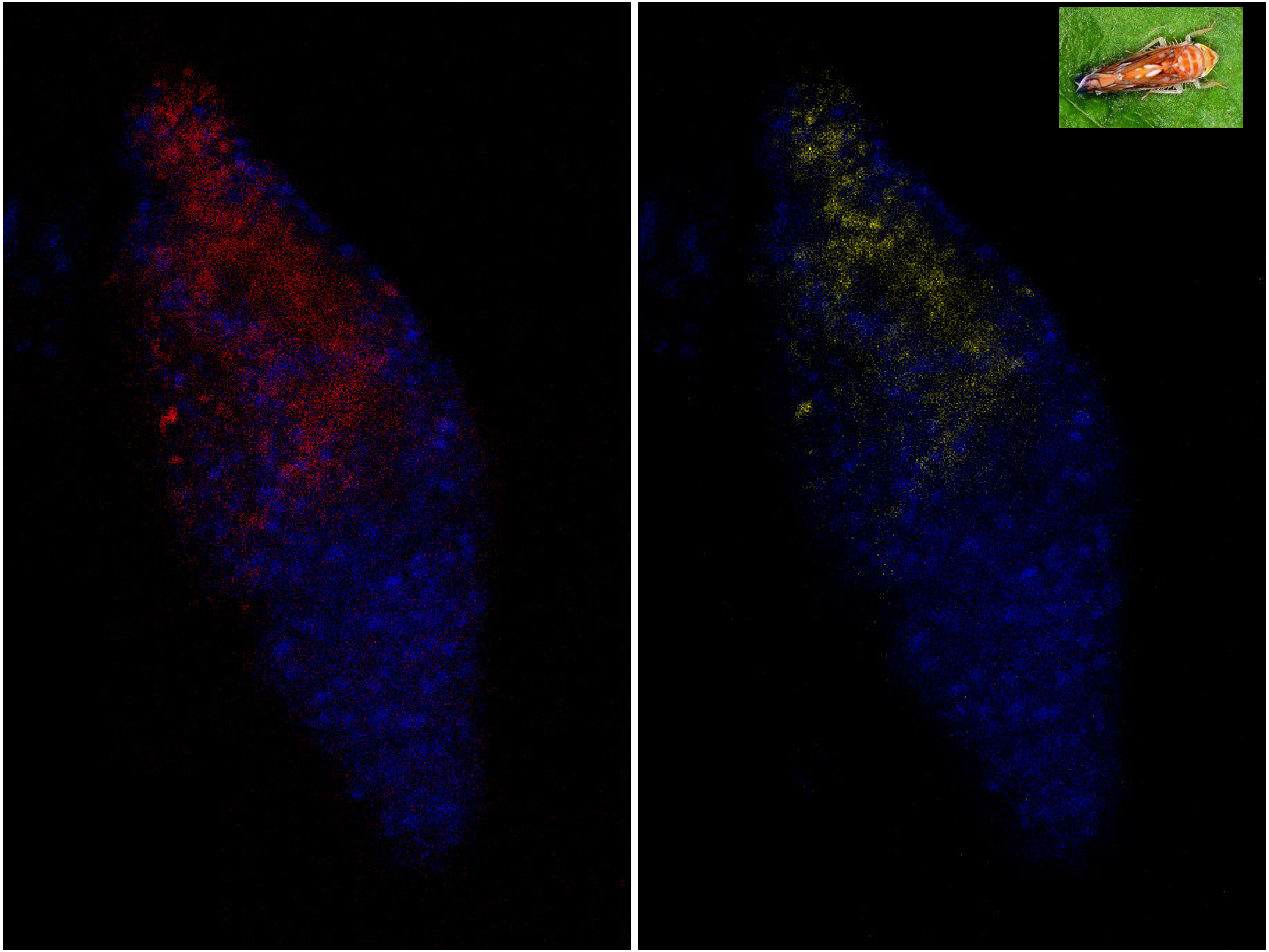
Abstract We have examined the addition of *Escherichia coli* to the diet at day 0 of adult life of females from two Oregon R *Drosophila melanogaster* strains, selected for different longevities: a short-life with an average adult life span of 10 days and a long-life standard R strain with an average adult life span of 50 days. The addition of bacteria to the diet significantly prolonged the fly longevity in both strains and affected the structure and histochemical reactivity of the fat body. The increased survival was characterized by great amount of glycogen accumulated in fat body cells from both strains. In aged control animals, fed with standard diet, lipid droplets were seen to be stored in fat body of short-lived, but not long-lived, flies. On the whole, our data indicate that exogenous bacteria are able to extend the survival of *Drosophila* females, and suggest that such a beneficial effect can be mediated, at least in part, by the fat body cells that likely play a role in modulating the accumulation and mobilization of reserve stores to ensure lifelong energy homeostasis.

Keywords *Escherichia coli* · *Drosophila melanogaster* · Longevity · Fat body · Morphology · Histochemistry









From bacterial bleaching to the hologenome theory of evolution

E. Rosenberg[1] **and I. Zilber-Rosenberg**[2]

1) Dept. Mol. Microbio.l and Biotechnol., Tel Aviv University, Ramat Aviv, Israel 69978
2) Open University of Israel, Raanana, Israel 43107

Abstract. Extensive bleaching of the coral *Oculina patagonica* in the eastern Mediterranean Sea occurs every summer when the seawater temperature exceeds 25°C. The infection by *Vibrio shiloi* and subsequent bleaching occurred only above 25°C because several of the bacterial virulence factors, superoxide dismutase, adhesion and toxin P, are only expressed above 25°C. Starting in 2002, the corals developed resistance to *V. shiloi*. Attempts to explain the resistance led to the Coral Probiotic Hypothesis, which posits that corals can adapt to their environment, including resistance to pathogens, by changing their symbiotic bacteria. The hologenome theory considers the holobiont a unit of natural selection. The hologenome is defined as the sum of the genetic information of the host and its microbiota. The theory is based on four well documented generalizations: (1) All animals and plants establish symbiotic relationships with microorganisms. (2) Symbiotic microorganisms are transmitted between generations. (3) The association between host and symbiont affects the fitness of the holobiont. (4) Under environmental stress, the symbiotic microbial community can change rapidly. These points taken together suggest that the genetic wealth of diverse microbial symbionts can play an important role both in adaptation and in evolution of higher organisms.

Keywords: Coral bleaching; *Vibrio*; holobiont, hologenome, evolution

environmental microbiology
Environmental Microbiology (2009) 11(12), 2959–2962
doi:10.1111/j.1462-2920.2009.01995.x

Opinion

The hologenome theory of evolution contains Lamarckian aspects within a Darwinian framework

Eugene Rosenberg,[1]* Gil Sharon[1] and
Ilana Zilber-Rosenberg[†]
[1]*Department of Molecular Microbiology and Biotechnology, Tel Aviv University, Ramat Aviv, Israel.*

Summary

The hologenome theory of evolution emphasizes the role of microorganisms in the evolution of animals and plants. The theory posits that the holobiont (host plus all of its symbiont microbiota) is a unit of selection in evolution. Genetic variation in the holobiont that can occur either in the host and/or in the microbial symbiont genomes (together termed hologenome) can then be transmitted to offspring. In addition to the known modes of variation, i.e. sexual recombination, chromosomal rearrangement and mutation, variation in the holobiont can occur also via two mechanisms that are specific to the hologenome theory: amplification of existing microorganisms and acquisition of novel strains from the environment. These mechanisms are Lamarckian in that (i) they are regulated by 'use and disuse' (of microbes) and (ii) the variations in the hologenome are transmitted to offspring, thus satisfying also the Lamarckian principle of 'inheritance of acquired characteristics'. Accordingly, the hologenome theory incorporates Lamarckian aspects within a Darwinian framework, accentuating both cooperation and competition within the holobiont and with other holobionts.

VOLUME 87, NO. 4 *THE QUARTERLY REVIEW OF BIOLOGY* DECEMBER 2012

A SYMBIOTIC VIEW OF LIFE: WE HAVE NEVER BEEN INDIVIDUALS

SCOTT F. GILBERT
Department of Biology, Swarthmore College
Swarthmore, Pennsylvania 19081 USA
Biotechnology Institute, University of Helsinki
00014 Helsinki, Finland
E-MAIL: SGILBER1@SWARTHMORE.EDU

JAN SAPP
Department of Biology, York University
Toronto, Ontario M3J 1P3 Canada
E-MAIL: JSAPP@YORKU.CA

ALFRED I. TAUBER
Department of Philosophy, Boston University
Boston, Massachusetts 02215 USA
E-MAIL: AIT@BU.EDU

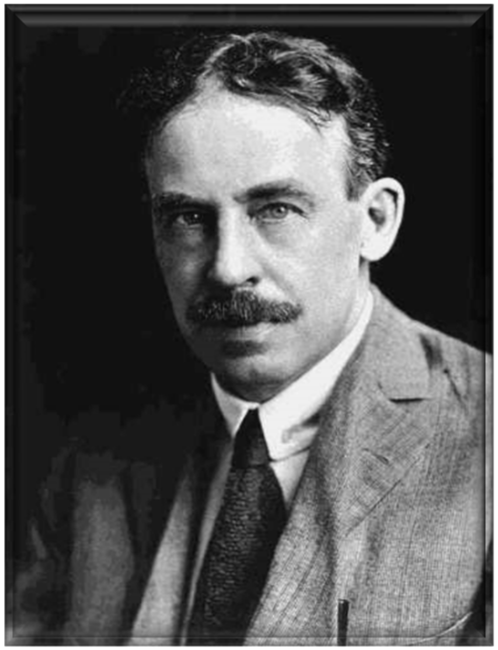
KEYWORDS
symbionts, symbiosis, individuality, evolution, holobiont

ABSTRACT

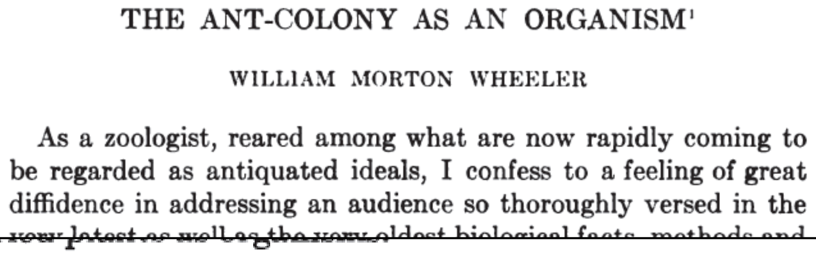
The notion of the "biological individual" is crucial to studies of genetics, immunology, evolution, development, anatomy, and physiology. Each of these biological subdisciplines has a specific conception of individuality, which has historically provided conceptual contexts for integrating newly acquired data. During the past decade, nucleic acid analysis, especially genomic sequencing and high-throughput RNA techniques, has challenged each of these disciplinary definitions by finding significant interactions of animals and plants with symbiotic microorganisms that disrupt the boundaries that heretofore had characterized the biological individual. Animals cannot be considered individuals by anatomical or physiological criteria because a diversity of symbionts are both present and functional in completing metabolic pathways and serving other physiological functions. Similarly, these new studies have shown that animal development is incomplete without symbionts. Symbionts also constitute a second mode of genetic inheritance, providing selectable genetic variation for natural selection. The immune system also develops, in part, in dialogue with symbionts and

The Quarterly Review of Biology, December 2012, Vol. 87, No. 4

0033-5770/2012/8704-0003$15.00



William Morton Wheeler
(1865 -1937)



THE ANT-COLONY AS AN ORGANISM[1]

WILLIAM MORTON WHEELER

As a zoologist, reared among what are now rapidly coming to be regarded as antiquated ideals, I confess to a feeling of great diffidence in addressing an audience so thoroughly versed in the

«un individuo non è né una cosa, né un concetto, ma un continuo flusso di processi in continuo cambiamento e pertanto mai definitivamente completato. Un organismo è quindi un complesso coordinato e individualizzato di attività che sono primariamente dirette ad ottenere ed assimilare sostanze dall'ambiente per produrre altri sistemi simili (la prole) e proteggere il sistema stesso da eventuali alterazioni dovute all'ambiente».

Grazie per l'attenzione!


microbial
biotechnology

society for applied microbiology

Microbial Biotechnology (2012) 5(3), 307–317

doi:10.1111/j.1751-7915.2011.00312.x

Review

Microbial symbionts: a resource for the management of insect-related problems


Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

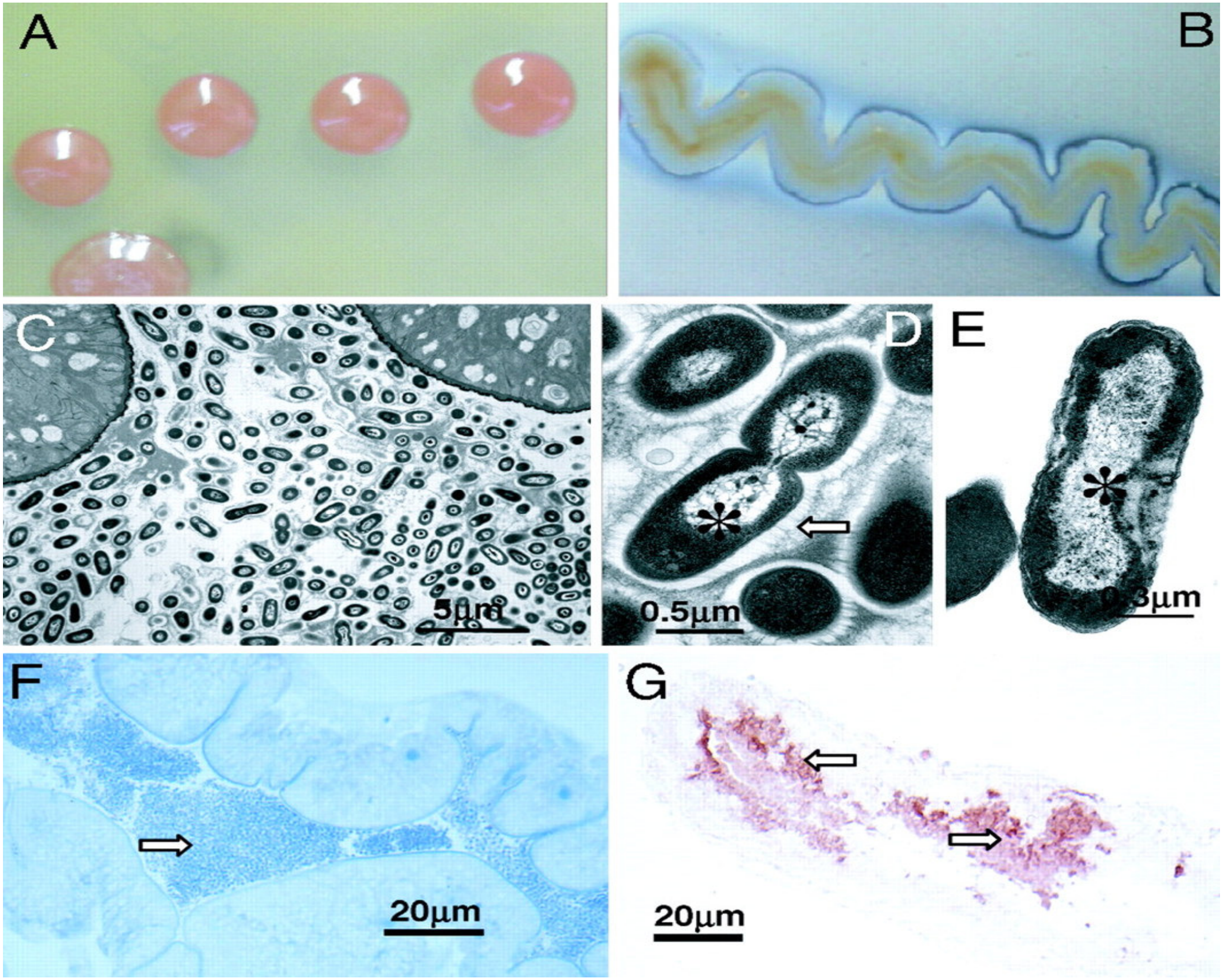
ELSEVIER

PARASITOLOGY INTERNATIONAL

Review Parasitology International 59 (2010) 1–8

Transgenesis and paratransgenesis to control insect-borne diseases: Current status and future challenges

Iliano V. Coutinho-Abreu [a], Kun Yan Zhu [b], Marcelo Ramalho-Ortigao [a,*]


A
B
C
5µm
D
0.5µm
E
0.3µm
F
20µm
G
20µm

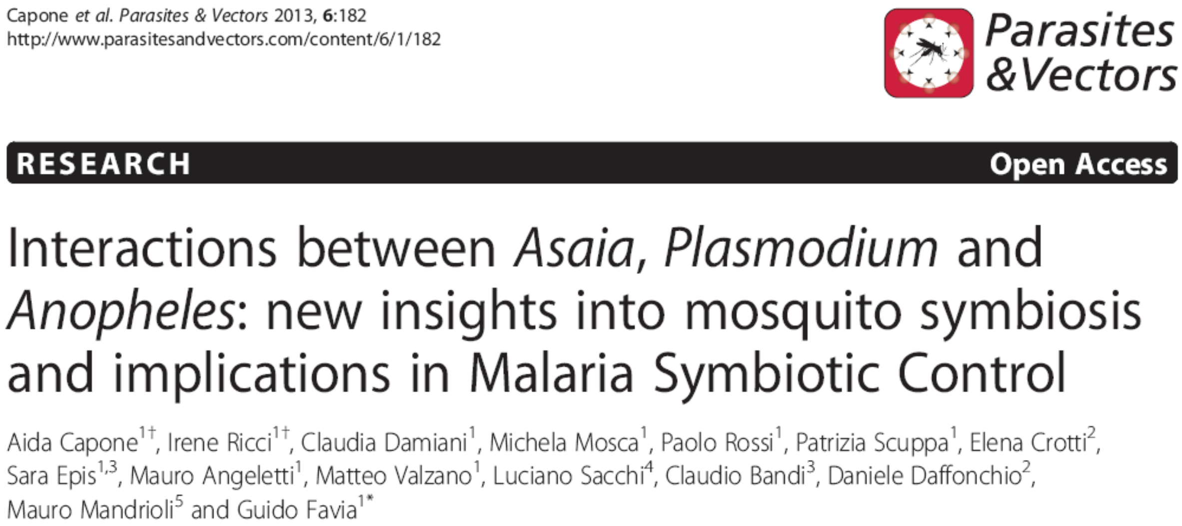
PNAS

Bacteria of the genus *Asaia* stably associate with *Anopheles stephensi*, an Asian malarial mosquito vector

Guido Favia[*,†], Irene Ricci[*], Claudia Damiani[*], Noura Raddadi[‡], Elena Crotti[‡], Massimo Marzorati[‡], Aurora Rizzi[‡], Roberta Urso[‡], Lorenzo Brusetti[‡], Sara Borin[‡], Diego Mora[‡], Patrizia Scuppa[*], Luciano Pasqualini[*], Emanuela Clementi[§], Marco Genchi[§], Silvia Corona[§], Ilaria Negri[¶], Giulio Grandi[‖], Alberto Alma[¶], Laura Kramer[‖], Fulvio Esposito[*], Claudio Bandi[**], Luciano Sacchi[§], and Daniele Daffonchio[†‡]

[*]Dipartimento di Medicina Sperimentale e Sanità Pubblica, Università degli Studi di Camerino, 62032 Camerino, Italy; Dipartimenti di [‡]Scienze e Tecnologie Alimentari e Microbiologiche and [**]Patologia Animale, Igiene e Sanità Pubblica Veterinaria, Università degli Studi di Milano, 20133 Milan, Italy; [§]Dipartimento di Biologia Animale, Università degli Studi di Pavia, 27100 Pavia, Italy; [¶]Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, Università degli Studi di Torino, 10095 Torino, Italy; and [‖]Dipartimento di Produzioni Animali, Facoltà di Medicina Veterinaria, Università degli Studi di Parma, 43100 Parma, Italy

Edited by Anthony A. James, University of California, Irvine, CA, and approved March 11, 2007 (received for review November 25, 2006)



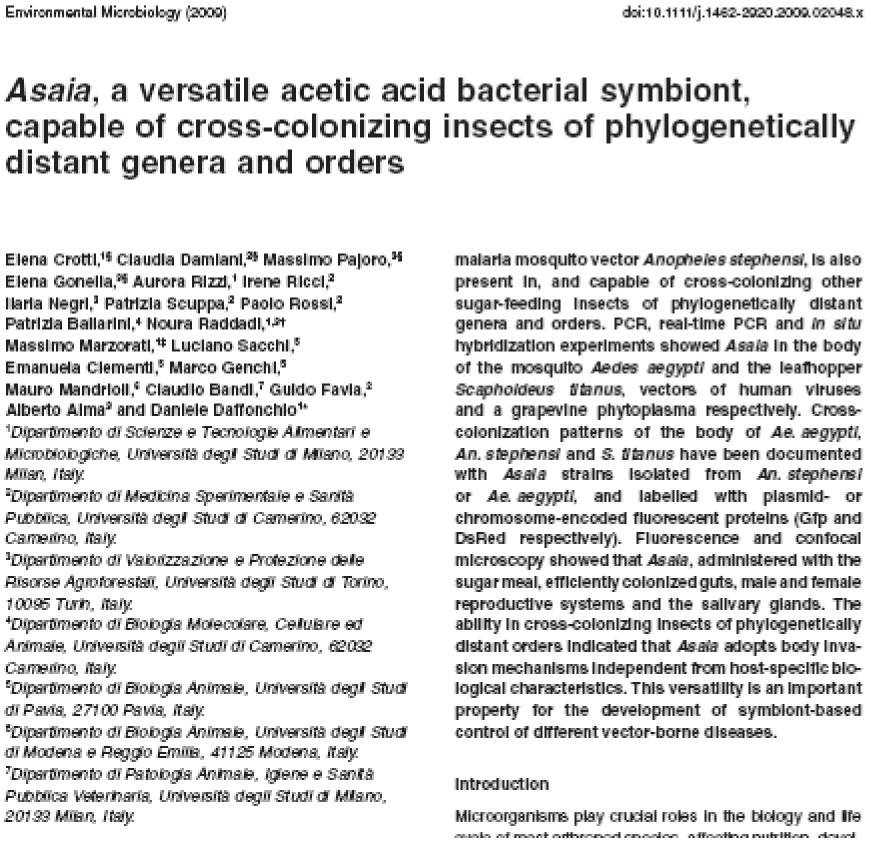
Capone *et al. Parasites & Vectors* 2013, **6**:182
http://www.parasitesandvectors.com/content/6/1/182

Parasites & Vectors

RESEARCH **Open Access**

Interactions between *Asaia*, *Plasmodium* and *Anopheles*: new insights into mosquito symbiosis and implications in Malaria Symbiotic Control

Aida Capone[1†], Irene Ricci[1†], Claudia Damiani[1], Michela Mosca[1], Paolo Rossi[1], Patrizia Scuppa[1], Elena Crotti[2], Sara Epis[1,3], Mauro Angeletti[1], Matteo Valzano[1], Luciano Sacchi[4], Claudio Bandi[3], Daniele Daffonchio[2], Mauro Mandrioli[5] and Guido Favia[1*]



Environmental Microbiology (2009) doi:10.1111/j.1462-2920.2009.02048.x

Asaia, a versatile acetic acid bacterial symbiont, capable of cross-colonizing insects of phylogenetically distant genera and orders

Elena Crotti,[1§] Claudia Damiani,[2§] Massimo Pajoro,[3§]
Elena Gonella,[3§] Aurora Rizzi,[1] Irene Ricci,[2]
Ilaria Negri,[3] Patrizia Scuppa,[2] Paolo Rossi,[2]
Patrizia Ballarini,[4] Noura Raddadi,[1,2†]
Massimo Marzorati,[1‡] Luciano Sacchi,[5]
Emanuela Clementi,[5] Marco Genchi,[5]
Mauro Mandrioli,[6] Claudio Bandi,[7] Guido Favia,[2]
Alberto Alma[3] and Daniele Daffonchio[1*]
[1]*Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano, 20133 Milan, Italy.*
[2]*Dipartimento di Medicina Sperimentale e Sanità Pubblica, Università degli Studi di Camerino, 62032 Camerino, Italy.*
[3]*Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, Università degli Studi di Torino, 10095 Turin, Italy.*
[4]*Dipartimento di Biologia Molecolare, Cellulare ed Animale, Università degli Studi di Camerino, 62032 Camerino, Italy.*
[5]*Dipartimento di Biologia Animale, Università degli Studi di Pavia, 27100 Pavia, Italy.*
[6]*Dipartimento di Biologia Animale, Università degli Studi di Modena e Reggio Emilia, 41125 Modena, Italy.*
[7]*Dipartimento di Patologia Animale, Igiene e Sanità Pubblica Veterinaria, Università degli Studi di Milano, 20133 Milan, Italy.*

malaria mosquito vector *Anopheles stephensi*, is also present in, and capable of cross-colonizing other sugar-feeding insects of phylogenetically distant genera and orders. PCR, real-time PCR and *in situ* hybridization experiments showed *Asaia* in the body of the mosquito *Aedes aegypti* and the leafhopper *Scaphoideus titanus*, vectors of human viruses and a grapevine phytoplasma respectively. Cross-colonization patterns of the body of *Ae. aegypti*, *An. stephensi* and *S. titanus* have been documented with *Asaia* strains isolated from *An. stephensi* or *Ae. aegypti*, and labelled with plasmid- or chromosome-encoded fluorescent proteins (Gfp and DsRed respectively). Fluorescence and confocal microscopy showed that *Asaia*, administered with the sugar meal, efficiently colonized guts, male and female reproductive systems and the salivary glands. The ability in cross-colonizing insects of phylogenetically distant orders indicated that *Asaia* adopts body invasion mechanisms independent from host-specific biological characteristics. This versatility is an important property for the development of symbiont-based control of different vector-borne diseases.

Introduction

Microorganisms play crucial roles in the biology and life cycle of most arthropod species, affecting nutrition, devel-

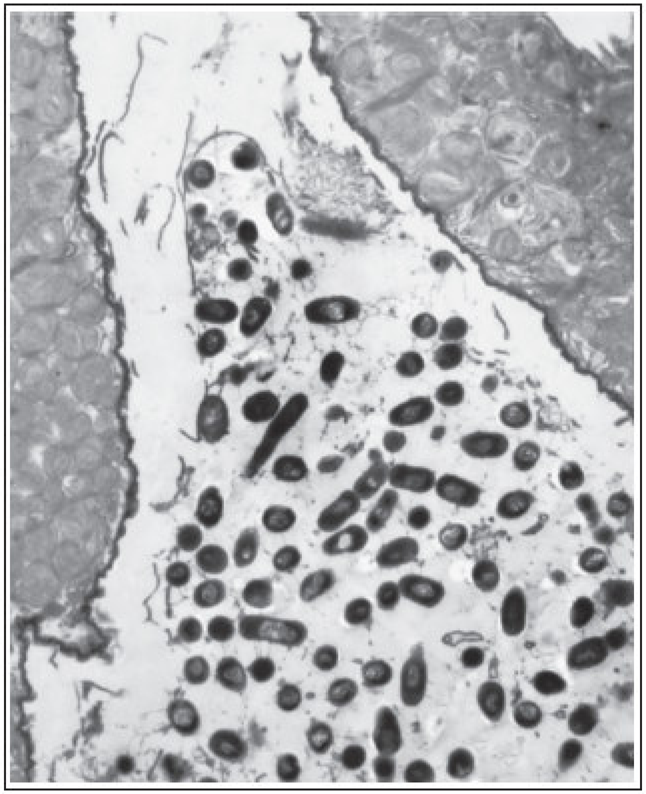


Figure 1. TEM micrograph of an An. stephensi adult male deferent duct showing high numbers of bacteria of the genus Asaia.

OPEN ACCESS Freely available online

PLoS PATHOGENS

Implication of the Mosquito Midgut Microbiota in the Defense against Malaria Parasites

Yuemei Dong, Fabio Manfredini[¤]**, George Dimopoulos***

W. Harry Feinstone Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland, United States of America

Abstract

Malaria-transmitting mosquitoes are continuously exposed to microbes, including their midgut microbiota. This naturally acquired microbial flora can modulate the mosquito's vectorial capacity by inhibiting the development of *Plasmodium* and other human pathogens through an unknown mechanism. We have undertaken a comprehensive functional genomic approach to elucidate the molecular interplay between the bacterial co-infection and the development of the human malaria parasite *Plasmodium falciparum* in its natural vector *Anopheles gambiae*. Global transcription profiling of septic and aseptic mosquitoes identified a significant subset of immune genes that were mostly up-regulated by the mosquito's microbial flora, including several anti-*Plasmodium* factors. Microbe-free aseptic mosquitoes displayed an increased susceptibility to *Plasmodium* infection while co-feeding mosquitoes with bacteria and *P. falciparum* gametocytes resulted in lower than normal infection levels. Infection analyses suggest the bacteria-mediated anti-*Plasmodium* effect is mediated by the mosquitoes' antimicrobial immune responses, plausibly through activation of basal immunity. We show that the microbiota can modulate the anti-*Plasmodium* effects of some immune genes. In sum, the microbiota plays an essential role in modulating the mosquito's capacity to sustain *Plasmodium* infection.

Citation: Dong Y, Manfredini F, Dimopoulos G (2009) Implication of the Mosquito Midgut Microbiota in the Defense against Malaria Parasites. PLoS Pathog 5(5): e1000423. doi:10.1371/journal.ppat.1000423

Editor: David S. Schneider, Stanford University, United States of America

Received January 16, 2009; **Accepted** April 9, 2009; **Published** May 8, 2009

Funding: This work has been supported by the National Institutes of Health/National Institute of Allergy and Infectious Disease 1R01AI061576-01A1, the Ellison Medical Foundation, the World Health Organization/TDR, Johns Hopkins School of Public Health, and the Johns Hopkins Malaria Research Institute. FM was supported by a PAR grant to Dr. R. Dallai from the University of Siena, Italy.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: gdimopou@jhsph.edu

¤ Current address: Department of Evolutionary Biology, University of Siena, Siena, Italy

Insect Molecular Biology

Insect Molecular Biology (2013) doi: 10.1111/imb.12066

INVITED REVIEW

Transinfection: a method to investigate *Wolbachia*–host interactions and control arthropod-borne disease

G. L. Hughes*† and J. L. Rasgon*†

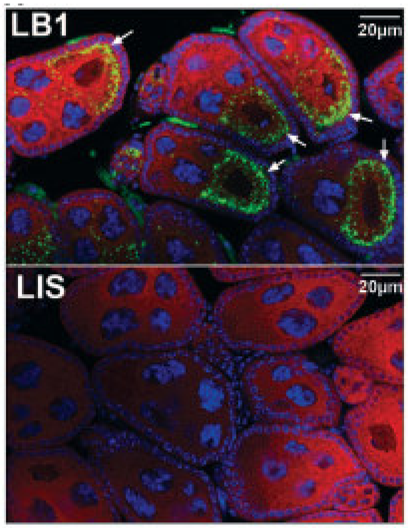
methods an

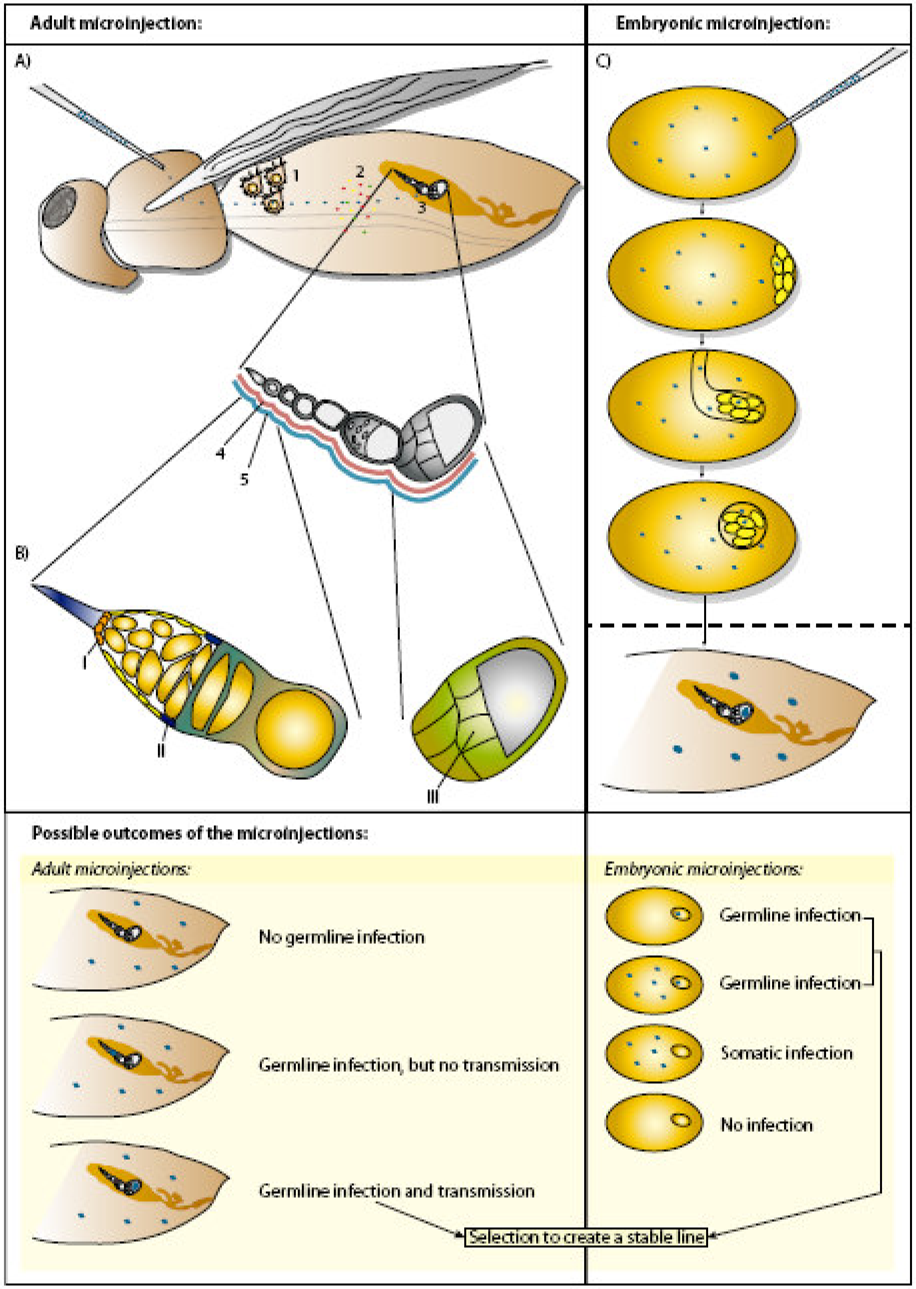
Wolbachia Invades *Anopheles stephensi* Populations and Induces Refractoriness to *Plasmodium* Infection

Guowu Bian,[1,2] Deepak Joshi,[1] Yuemei Dong,[3] Peng Lu,[1] Guoli Zhou,[1] Xiaoling Pan,[1]
Yao Xu,[1] George Dimopoulos,[3] Zhiyong Xi[1,4]*

Wolbachia is a maternally transmitted symbiotic bacterium of insects that has been proposed as a potential agent for the control of insect-transmitted diseases. One of the major limitations preventing the development of *Wolbachia* for malaria control has been the inability to establish inherited infections of *Wolbachia* in anopheline mosquitoes. Here, we report the establishment of a stable *Wolbachia* infection in an important malaria vector, *Anopheles stephensi*. In *A. stephensi*, *Wolbachia* strain *w*AlbB displays both perfect maternal transmission and the ability to induce high levels of cytoplasmic incompatibility. Seeding of naturally uninfected *A. stephensi* populations with infected females repeatedly resulted in *Wolbachia* invasion of laboratory mosquito populations. Furthermore, *w*AlbB conferred resistance in the mosquito to the human malaria parasite *Plasmodium falciparum*.

10 MAY 2013 VOL 340 SCIENCE www.sciencemag.org





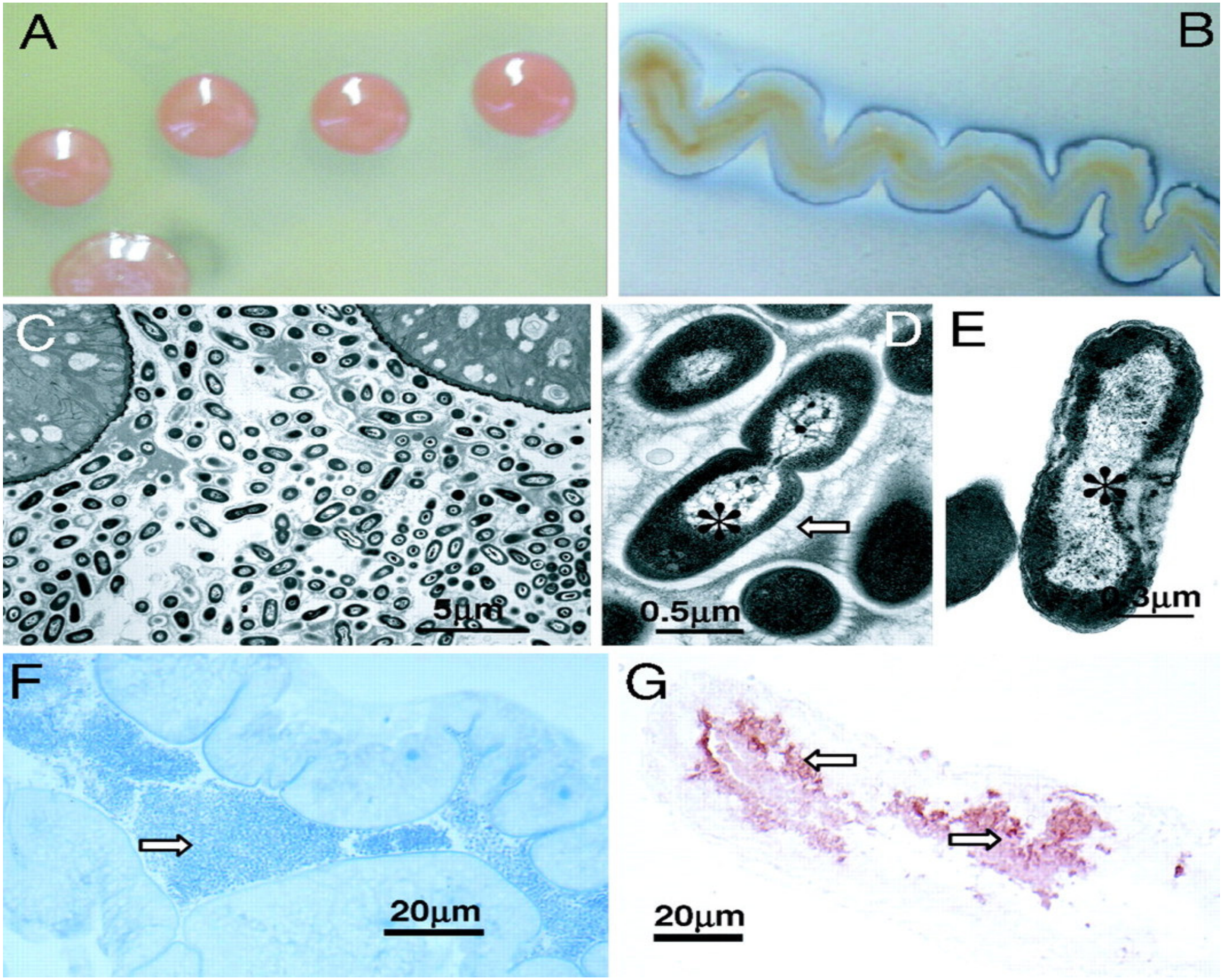
Insect Molecular Biology

Insect Molecular Biology (2013) doi: 10.1111/imb.12066

INVITED REVIEW

Transinfection: a method to investigate *Wolbachia*–host interactions and control arthropod-borne disease

G. L. Hughes*† and J. L. Rasgon*†


A
B
C
D
E
F
G
5µm
0.5µm
0.3µm
20µm
20µm

Contents lists available at ScienceDirect

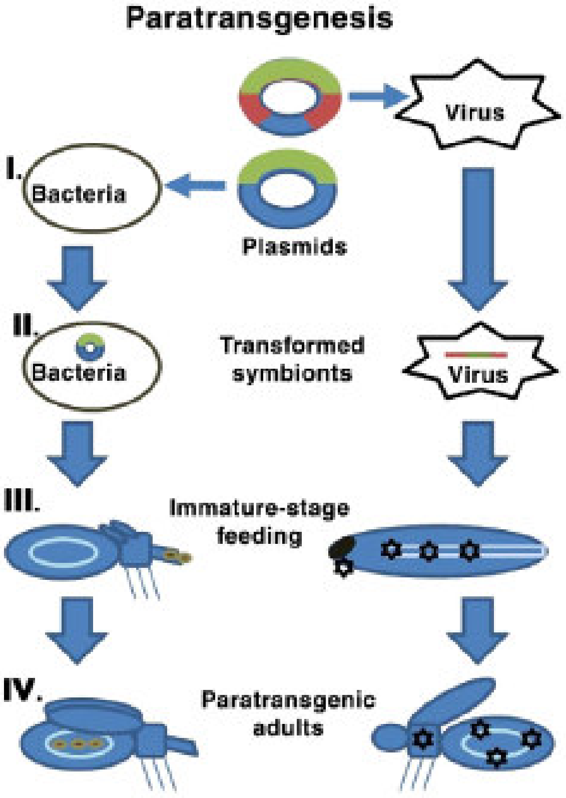
Parasitology International

journal homepage: www.elsevier.com/locate/parint

Review

Transgenesis and paratransgenesis to control insect-borne diseases: Current status and future challenges

Iliano V. Coutinho-Abreu [a], Kun Yan Zhu [b], Marcelo Ramalho-Ortigao [a,*]



“A microbe to proper express anti-parasite molecules in mosquitoes should have the following characteristics:

- Dominance within the insect-associated microflora;

- Culturability in cell-free media (and cryogenic preservability);

- Easy transformability with foreign DNA and stability;

- Wide distribution in the larvae and adult insect body;

- Co-localization in the insect body districts (i.e.: gut, salivary glands, fat bodies...) with the pathogenic microbe”.