

Conclusion

Symbioses, Biocomplexity, and Metagenomes

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A common thread in this book is symbiosis, organisms living together in an association, the outcome of which could be neutral, positive, or negative and that might even change from time to time. Ever since de Bary (1879) popularized this term, we have seen the reductionist approach of science in vogue, an approach that has been quite productive. This approach has resulted in the development of revolutionary technologies, such as the tools of molecular biology, which have revealed a tremendous level of biocomplexity in nature. For example, Hermsmeier et al. (2001) found that more than 500 genes in *Nicotiana attenuata* respond to attack by the lepidopteran *Manduca sexta*. This mind-boggling analysis points at the need for a concerted effort aimed at understanding the biocomplexity of insect–fungal associations. We have to start fitting the parts together in a new puzzle that uses parts based on molecular studies. It is not just a matter of examining tri-trophic interactions or of examining how insects deal with plant trichomes, allelochemicals, or a thick cuticle.

Finding more than 500 plant genes responding to herbivory by just one insect is significant. We need to be aware, however, that there are organisms residing in the insect and the plant, in addition to those found on the cuticle and on the phylloplane. When we consider the myriad endophytes in the plant, including yeasts, bacteria, and fungi, and how these might be influencing the plant, and consequently the insect, the picture becomes even more complex. There is an enormous complexity of interactions involving several different trophic levels.

To assess this complexity, it will be necessary to examine metagenomes in insects, an approach that considers a particular insect species as a community in

which genomes belonging to various other organisms such as yeasts, fungi, and bacteria might be present. This concept has been used for microbial community analyses in soil (Handelsman et al. 1998; Torsvik and Øvreås 2002), hot springs (Barns et al. 1994), and marine environments (DeLong 1992; López-García et al. 2001), and it is based on various molecular techniques, including polymerase chain reaction (PCR) with primers for small subunit ribosomal RNA (rDNA) (Amann et al. 1995; Jarrell et al. 1999), gene cassette PCR (Stokes et al. 2001), and microarrays (Murray et al. 2001; Zhou 2003), among others (see Torsvik et al. 1998). These techniques allow a better understanding of the vastness of microbial community diversity, which in the past was not known simply because the microbes could not be cultured.

What will we find when we apply these or other related techniques to insects? Will insects reveal themselves to be reservoirs of unknown biodiversity, or will we find that we have been close to reality in terms of knowing what is present, and that the metagenome approach used in other systems is not applicable to insects? Using the metagenome approach, Reed and Hafner (2002) extracted total community DNA from chewing lice to study bacterial communities associated with the insect; their results revealed 35 bacterial lineages and was the first study documenting bacterial associations with chewing lice in the Trichodectidae. Ohkuma and Kudo (1996) used mixed-population DNA from the termite *Reculitermes speratus* to analyze the diversity of intestinal bacteria, revealing the presence of a wide array of previously unknown microorganisms. These results indicate that there is likely a huge knowledge gap in the breadth of insect–fungal associations that can be filled using various molecular techniques. The insect–fungal association field is likely to become much more complex.

It also would be interesting to find out how the “genomic islands” concept (*sensu* Doolittle 2002) applies to insect-associated fungi. Are there “pathogenicity islands” (large gene clusters correlated with virulence) or “symbiotic islands” (genes necessary for symbiosis)? Can these be identified and manipulated for pest control strategies?

Referring to molecular biology, the late Peter Medawar (1968) wrote: “It is simply not worth arguing with anyone so obtuse as not to realize that this complex of discoveries is the greatest achievement of science in the twentieth century” (p. 4). Are there further developments in molecular tools that can be used to advance the field? Certainly. Are there tools at present that are not being used to study insect–fungal association studies? Obviously. We need to pursue a broader approach to understanding the nature of insect–fungal associations knowing that Occam’s razor will forever be with us, but with one important caveat: From the reductionist approach that has yielded powerful tools which in turn point at the importance of more holistic studies, we must become true naturalists as our scientific ancestors once were. It is not just a matter of being well versed in molecular biology, but also in ecology, pathology, mycology, and entomology. Once we have put these various fields together as individuals or, perhaps, as teams of reductionist biologists to comprise the new naturalist of our studies, we will be able to advance at gigantic steps in our understanding of insect–fungal associations.

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Insect–Fungal Associations

Ecology and Evolution

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OXFORD
UNIVERSITY PRESS

2005

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UNIVERSITY PRESS

Oxford New York
Auckland Bangkok Buenos Aires Cape Town Chennai
Dar es Salaam Delhi Hong Kong Istanbul Karachi Kolkata
Kuala Lumpur Madrid Melbourne Mexico City Mumbai Nairobi
São Paulo Shanghai Taipei Tokyo Toronto

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198 Madison Avenue, New York, New York 10016
www.oup.com

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Library of Congress Cataloging-in-Publication Data
Insect-fungal associations : ecology and evolution /
edited by Fernando E. Vega, Meredith Blackwell.
p. cm.

Includes bibliographical references.
ISBN 0-19-516652-3

1. Fungi as biological pest control agents. 2. Insect
pests—Biological control. 3. Fungi in agriculture. 4. Insect-fungus
relationships. I. Vega, Fernando E. II. Blackwell, Meredith.
SB976.F85 I57 2004

9 8 7 6 5 4 3 2 1

Printed in the United States of America
on acid-free paper