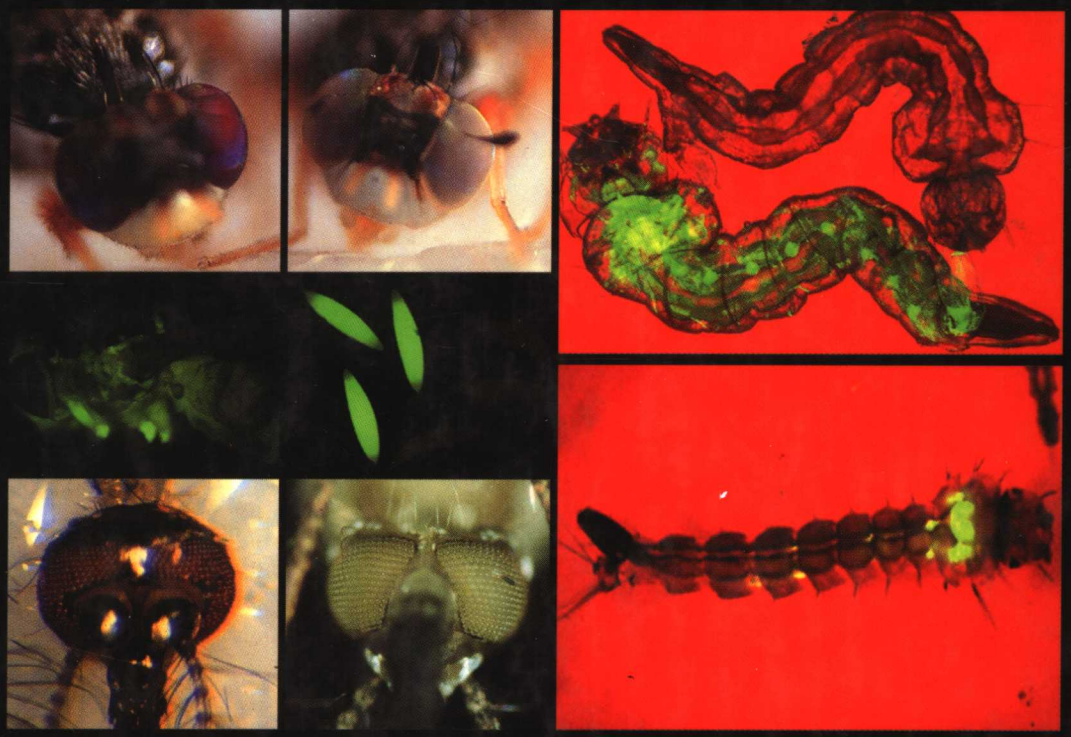
Insect Transgenesis

Methods and Applications



EDITED BY

Alfred M. Handler
Anthony A. James

INSECT TRANSGENESIS

Methods and Applications

EDITED BY

Alfred M. Handler
Anthony A. James

CRC Press
Boca Raton London New York Washington, D.C.

Library of Congress Cataloging-in-Publication Data

Insect transgenesis : methods and applications / edited by Alfred M. Handler, Anthony A. James

p. cm.

Includes bibliographical references (p.).

ISBN 0-8493-2028-3

1. Insect cell biotechnology. 2. Insects—Genetic engineering. I. Handler, Alfred M. II. James, Anthony A.

TP248.27.I56 I577 2000

638—dc21

00-023660

CIP

This work was created in the performance of a Cooperative Research and Development Agreement with the U.S. Department of Agriculture.

No claim to original U.S. Government works

International Standard Book Number 0-8493-2028-3

Library of Congress Card Number 00-023660

Printed in the United States of America 1 2 3 4 5 6 7 8 9 0

Printed on acid-free paper

Preface

The transfer of genetic material into the genome of insects has been a goal of geneticists and entomologists for more than 30 years, and with the successful transposon-mediated transformation of *Drosophila melanogaster* reported by Rubin and Spradling in 1982, efforts have been focused on duplicating this methodology in insects of agricultural and medical importance. The transfer of *Drosophila* transformation systems to other insects, however, has not been straightforward, and only in the past several years have notable successes been reported. These recent successes have resulted from a maturation over the past decade of all aspects of gene transfer technology. As with *Drosophila*, transformation in tephritid fruit flies, mosquitoes, and moths has been achieved with transposon-based vectors, although most have taken advantage of newly discovered elements. Viral transformation vectors are beginning to meet with success, and symbiont-mediated transformation is already finding practical application. The development of reliable marker gene systems has been important to the progress achieved in transgenesis. Genes encoding the green fluorescent protein or its variants appear to have broad utility as marker genes for screening transformants, and these genes may be used as reporter genes in transformation-based analyses of promoter function. Furthermore, genes encoding enzymes important in establishing eye color in insects continue to be useful when appropriate mutant recipient strains are available. Work continues on selectable marker genes such as those that encode enzymes involved in insecticide or antibiotic resistance, and these may be available soon for analyzing large numbers of potential transformants.

The high level of interest in insect transgenesis has resulted in two major forums for the discussion of this research: the Keystone Symposium, Toward the Genetic Manipulation of Insects, held in 1995 and 1998, and the International Workshop Series on Transgenesis of Invertebrate Organisms, held every 2 years since 1995. The idea for this book arose, in part, from discussions at these meetings that indicated that the field was at a turning point where rapid progress was being made and a wider dissemination of ideas was necessary for these results and techniques to reach the scientific mainstream. This was especially important since many of the researchers worldwide who might take greatest advantage of the methodology have remained unaware of the current state of the art. It also has become clear that despite recent breakthroughs, none of the successful systems or specific techniques will be useful for all insects. Thus, it seemed important to present the existing methodologies so that others might understand the potentials and pitfalls relevant to their species of interest (which also includes noninsect invertebrate systems). While some of the available systems might be directly utilized, it is also a hope that the information provided here will serve as a foundation and guide for independent investigation leading to new methods and strategies. Only in this way will the field continue to advance.

As insect transgenesis becomes more routine and widespread, more applications will depend on the release, if not mass release, of transgenic strains. This raises important ecological concerns that undoubtedly will be challenging, and the need for biological risk assessment to address these concerns in a rational and comprehensive manner cannot be understated. Many of the chapters in this volume address these questions relevant to particular systems, with a more in-depth consideration given in the final two chapters that discuss risk assessment from a scientific and regulatory standpoint. It is clear that these issues will differ for each release in terms of the specific transgene, the transgenic host, and the particular ecological niches into which the insects are released or which they must invade. Each investigator interested in creating transgenic strains for release must be highly aware of these issues, and take them into consideration in the planning stages of vector

development and strategies for use of the transgenic strain. We also hope that the information provided here will be a starting point for what will be an ongoing discussion.

The contributors to this book include many of the invited speakers to the Keystone Symposia and Transgenesis Workshops, all of whom are held in high regard in their fields of expertise. While we have tried to include all of the major areas of importance to insect transgensis, some existing or potential vector and marker systems may have been given only limited attention. In a rapidly developing field such as this, new systems appearing close to the time of publication may have been omitted, although we have tried as much as possible to anticipate these possibilities. In terms of strategies for the use of transgenic insects, there are numerous possibilities for both basic and applied purposes, and here we provide only a sampling of strategies for insects that are plant predators and vectors of disease. Again, the purpose of this book is to serve as a foundation and guide to this emerging field, and as with most scientific disciplines, it is important if not critical to be continually kept up to date by frequent literature reviews. Comprehensive reference lists, appendices, and Web-site listings have been included in this volume to help with this process.

In many ways, and possibly due to the many roadblocks encountered early on, the field of insect transgenesis has become a large, collaborative effort. We therefore very sincerely thank the contributors to this book who have been on the vanguard of this effort, as well as numerous other colleagues who have helped lay the foundation for this technology in past years and those who have more recently provided data and ideas that have contributed to successes. We especially acknowledge the contribution of one of our mentors, Howard A. Schneiderman, to whom this book is dedicated. Schneiderman was the founder and first director of the Developmental Biology Center (DBC) at the University of California, Irvine, where we did research as a student (AAJ) and post-doc (AMH). Schneiderman was a leading insect physiologist, who early on appreciated the power of genetics to understand all phases of insect biology, and this led him to become one of the first insect biologists to turn a large portion of his research efforts toward the use of *Drosophila* as a model system. This was not only to understand genetics, but also to understand insects. It is not surprising that some of the first attempts at *Drosophila* transformation were undertaken at the DBC. Later on in his career, Schneiderman become Vice President for Research and Development at Monsanto where he led this company's pioneering endeavors into plant transgenics. Beyond ourselves, others who were trained as *Drosophila* geneticists at the DBC have gone on to become leaders in genetic analysis and transgenesis of non-drosophilid insects and other organisms and, by doing so, are hopefully carrying forward and helping complete the circle of Schneiderman's original thoughts and inspiration.

We are grateful to those who provided enormous assistance throughout the development and production of this book including Lynn Olson at the University of California, Irvine, and members of CRC Press editorial and production staff including Christine Andreasen, Pat Roberson, and John Sulzycki.

Alfred M. Handler
Gainesville, Florida

Anthony A. James
Irvine, California

Editors

Alfred M. Handler, Ph.D., is a Research Geneticist at the USDA, ARS Center for Medical, Agricultural and Veterinary Entomology in Gainesville, Florida.

Dr. Handler received his B.S. in biology from the State University of New York at Stony Brook in 1972, and his Ph.D. in biology (developmental genetics) from the University of Oregon in 1977. He held a postdoctoral fellowship in genetics at the Division of Biology, California Institute of Technology, Pasadena, from 1977 to 1979, and then joined the Developmental Biology Center at the University of California, Irvine as a research biologist from 1979 to 1985. During this time he was a visiting research associate at the Zoological Institute, University of Zürich, Switzerland and the Technische Hochschule, Darmstadt, Germany. In 1985 he joined the Agricultural Research Service in Gainesville, Florida, and in 1995 he was a visiting scientist at the Laboratory of Comparative Pathology, University of Montpellier II, France.

Dr. Handler is the U.S. scientific coordinator for a cooperative scientific program between the USDA–ARS and the French Centre National Recherche Scientifiques (CNRS) on "Transgenesis of Invertebrate Organisms of Economic and Medical Importance." As part of this role he has served as conference organizer for the "International Workshops on Transgenesis of Invertebrate Organisms." Dr. Handler has served as a consultant and expert panel member for several international organizations in the field of insect genetics and transgenesis.

Dr. Handler's research at the USDA–ARS has centered on the use of transgenic insects for biological control programs. Most of his efforts have focused on the development of efficient gene transfer vector and marker systems.

Anthony A. James, Ph.D., is Professor of Molecular Biology and Biochemistry at the University of California, Irvine (UCI).

Dr. James received his B.S. in biology from UCI in 1973, and his Ph.D. in developmental biology from UCI in 1979. He held postdoctoral positions in the Department of Biological Chemistry, Harvard Medical School, and the Department of Biology, Brandeis University, before joining the faculty of the Department of Tropical Public Health at the Harvard School of Public Health in 1985. Dr. James returned to his alma mater in 1989, where he has remained.

Dr. James was a principal investigator with the Network on the Biology of Parasite Vectors funded by the John D. and Catherine T. MacArthur Foundation and was a recipient of the Molecular Parasitology Award from the Burroughs–Wellcome Fund. He is a fellow of the American Association for the Advancement of Science and the Royal Entomological Society of London. He is a founding editor of *Insect Molecular Biology*, and is on the editorial board of *Experimental Parasitology*.

Dr. James' areas of interest include vector–parasite interactions, mosquito molecular biology, and other problems in insect developmental biology. His current work focuses on using genetic and molecular genetic tools to interrupt parasite transmission by mosquitoes.

Contributors

Boris Afanasiev
Arthropod-Borne Infectious Disease Lab
Department of Microbiology
Colorado State University
Fort Collins, CO 80523

Peter W. Atkinson
Department of Entomology
University of California, Riverside
Riverside, CA 92521

Charles B. Beard
Division of Parasitic Diseases
Centers for Disease Control and Prevention
4770 Buford Highway
Chamblee, GA 30341

Mark Q. Benedict
Division of Parasitic Diseases
Centers for Disease Control and Prevention
4770 Buford Highway
Chamblee, GA 30341

Alain Bucheton
Institut de Génétique Humaine
141 rue de la Cardonille
34396 Montpellier
France

Jane C. Burns
Department of Pediatrics
School of Medicine
University of California, San Diego
9500 Gilman Drive
La Jolla, CA 92093

Jonathan Carlson
Arthropod-Borne Infectious Disease Lab
Department of Microbiology
Colorado State University
Fort Collins, CO 80523

Frank H. Collins
Department of Biological Sciences
University of Notre Dame
Notre Dame, IN 46556

Ravi V. Durvasula
Department of Internal Medicine
Yale University School of Medicine
60 College Street
New Haven, CT 06520

Paul Eggleston
School of Life Sciences
Keele University
Huxley Building
Staffordshire ST5 5BG
United Kingdom

Richard H. ffrench-Constant
Department of Biology and Biochemistry
University of Bath
Bath BA2 7AY
United Kingdom

Arnold S. Foudin
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
4700 River Road
Riverdale, MD 20737

Gerald Franz
IAEA Laboratories
Agriculture and Biotechnology Laboratory
Entomology Unit
A-2444 Seibersdorf
Austria

Malcolm J. Fraser, Jr.
Department of Biological Sciences
University of Notre Dame
Notre Dame, IN 46556

Kent G. Golic
Department of Biology
University of Utah
Salt Lake City, UT 84112

Alfred M. Handler
Center for Medical, Agricultural,
and Veterinary Entomology
USDA/ARS
1700 SW 23rd Drive
Gainesville, FL 32608

Stephen Higgs
Department of Pathology
University of Texas Medical Branch
301 University Boulevard
Galveston, TX 77550

Marjorie A. Hoy
Department of Entomology and Nematology
P. O. Box 110620
University of Florida
Gainesville, FL 32611

Shirley P. Ingebritsen
Animal and Plant Health Inspection Service
U. S. Department of Agriculture
4700 River Road
Riverdale, MD 20737

Anthony A. James
Department of Molecular Biology-
Biochemistry
3205 Bio Sci II
University of California, Irvine
Irvine, CA 92697

David J. Lampe
Department of Biological Sciences
Duquesne University
913 Bluff Street
Pittsburgh, PA 15219

David L. Lewis
Laboratory of Molecular Biology
University of Wisconsin
1525 Linden Drive
Madison, WI 53706

David A. O'Brochta
Center for Agricultural Biotechnology
University of Maryland Biotechnology Institute
College Park, MD 20742

Kenneth E. Olson
Arthropod-Borne Infectious Disease Lab
Department of Microbiology
Foothills Campus
Colorado State University
Fort Collins, CO 80523

Scott L. O'Neill
Department of Epidemiology and Public Health
Yale University School of Medicine
60 College Street
New Haven, CT 06520

Alain Pélisson
Institut de Génétique Humaine
141 rue de la Cardonille
34396 Montpellier
France

Frank F. Richards
Department of Internal Medicine
Yale University School of Medicine
60 College Street
New Haven, CT 06520

Hugh M. Robertson
Department of Entomology
University of Illinois
505 S. Goodwin Avenue
Urbana, IL 61801

Alan S. Robinson
IAEA Laboratories
Agriculture and Biotechnology Laboratory
Entomology Unit
A-2444 Seibersdorf
Austria

Yikang S. Rong
Department of Biology
University of Utah
Salt Lake City, UT 84112

Abhimanyu Sarkar
Department of Biological Sciences
University of Notre Dame
Notre Dame, IN 46556

John M. Sherwood
Department of Entomology
University of Illinois
505 S. Goodwin Avenue
Urbana, IL 61801

Steven P. Sinkins
Liverpool School of Tropical Medicine
Pembroke Place
Liverpool L3 5QA
United Kingdom

Erica Suchman
Arthropod-Borne Infectious Disease Lab
Department of Microbiology
Colorado State University
Fort Collins, CO 80523

Christophe Terzian
Institut de Génétique Humaine
141 rue de la Cardonille
34396 Montpellier
France

Kimberly K. O. Walden
Department of Entomology
University of Illinois
505 S. Goodwin Avenue
Urbana, IL 61801

Bruce A. Webb
Department of Entomology
University of Kentucky
Lexington, KY 40546

Orrey P. Young
Animal and Plant Health Inspection Service
U. S. Department of Agriculture
4700 River Road
Riverdale, MD 20737

Yuguang Zhao
NERC Institute of Virology
and Environmental Microbiology
Mansfield Road
Oxford OX1 3SR
United Kingdom

Dedicated to the memory of
Howard A. Schneiderman

Contents

Section I

Introduction

1 An Introduction to the History and Methodology of Insect Gene Transfer

Alfred M. Handler

CONTENTS

1.1 HISTORICAL PERSPECTIVE ON INSECT GENE TRANSFER

The use of genetic material as recombinant DNA and the ability to integrate it into a host genome has proved to be a powerful method for genetic analysis and manipulation, providing a major new era in the field of genetics. Procaryotic gene transformation was actually realized early on, and in fact the pivotal bacterial transformation studies by Avery et al. (1944) gave definitive proof to DNA being the inherited genetic material. Continued procaryotic genetic transformation studies, indeed,

helped lay the foundation for modern molecular genetics. It is thus not surprising that geneticists attempted to duplicate this methodology in eucaryotes as well, long before eucaryotic DNA could be isolated as recombinant molecules and analyzed in a meaningful way.

The genetic transformation of insects was first attempted in *Ephestia* nearly 35 years ago, when mutant larvae were injected with wild-type DNA, with some developing into adults with wild-type wing scales (Caspari and Nawa, 1965). In subsequent studies with *Ephestia* (Nawa and Yamada, 1968) and *Bombyx mori* (Nawa et al., 1971), complementation of eye color mutations was observed after treatment with wild-type DNA. While these experiments yielded wild-type adults and at least limited non-Mendelian inheritance of the normal phenotype, it is likely that these initial insect transformations were somatic with inheritance occurring extrachromosomally. Shortly after the initial studies in moths, transformation of *Drosophila melanogaster* was similarly attempted, although delivery of wild-type DNA was achieved by soaking embryos in genomic DNA within ringers or sucrose solutions. As with the moth studies, somatic mosaics resulted, but inheritance of the reverted phenotyes was not clearly Mendelian and it was concluded that genetic transformation had occurred extrachromosomally, with episomal transmission and not chromosomal integration (Fox and Yoon, 1966; 1970; Fox et al., 1970).

More recent approaches to insect transformation began with studies in *Drosophila* that relied on the direct injection of wild-type DNA into embryos. These attempts to revert the *vermilion* (*v*) mutant line met with some success (Germeraad, 1976), although integrations were not verified beyond the genetic mapping of the complementing gene outside of the *v* locus (suggesting that a direct *v* reversion had not occurred), but the transformed lines were subsequently lost without further genetic or biochemical verification.

1.1.1 *P*-Element Transformation

Concurrent with the *vermilion* studies, the role of P factors in *Drosophila* hybrid dysgenesis was being eludicated (Kidwell et al., 1977), culminating in the identification and isolation of the *P* transposable element as the responsible agent. In now classical experiments by Rubin et al. (1982) and Rubin and Spradling (1982), *P* was first isolated from a *P*-induced mutation of *white* in *D. melanogaster,* and then developed into the first transposon-based system to transform the germline of *D. melanogaster* efficiently and stably (see Engels, 1989, for a comprehensive review of the discovery and early analysis of *P*).

P was found to be 2.9 kb in length with 31 bp inverted terminal repeats (O'Hare and Rubin, 1983), similar in general structure to *Activator*, the first transposable element to be discovered in maize by Barbara McClintock (see Federoff, 1989). Both of these elements, as well as all the subsequently discovered transposons used for insect germline transformation, belong to a general group of transposable elements known as Class II short inverted terminal repeat transposons (see Finnegan, 1989). These elements transpose via a DNA-intermediate and generally utilize a cut-and-paste mechanism that creates a duplication of the insertion site. Within the terminal repeats of these elements is a transcriptional unit that encodes a transposase molecule that acts at or near the termini to catalyze excision and transposition of the complete element. As first described by Rubin and Spradling (1982), the ability of the transposase to act in *trans* has allowed the development of binary vector-helper systems (Figure 1.1). Typically the vector plasmid includes the mobile terminal repeats of the element and requisite proximal internal sequences that surround a marker gene. The vector is made nonautonomous by having the transposase gene either deleted or disrupted by insertion of the marker gene, and thus it is unable to move by itself. The transposase is provided on a separate helper plasmid, and, after introduction into germ cell nuclei, the helper mediates transposition of the vector into the genome. The original helper was an autonomous *P* element (pπ25.1) that had the ability to integrate as well, and its presence could cause instability of the vector in subsequent generations (if not earlier). This problem was ameliorated somewhat by having much higher vector-to-helper ratios, but was solved more