

# TABLE OF CONTENTS

## Insect Histology: Practical Laboratory Techniques

Preface

Acknowledgements

Introduction

### **1 Problems of sclerotized chitin: Softening insect cuticle 1**

1.1 Introduction 1

1.2 General Methods 3

1.3 Preparations of insect eggs 14

1.4 Double Embedding Techniques 16

References 19

### **2 Fixation 21**

2.1 Introduction 21

2.2 Aldehyde based fixatives 21

2.3 Protein denaturing 30

2.4 Picric acid based 33

2.5 Mercuric chloride based 37

2.6 SEM/TEM 40

2.7 Other 46

References 51

### **3 Dehydrating, clearing, and embedding 54**

3.1 Dehydration 54

3.2 Clearing 60

3.3 Embedding General 65

3.4 Embedding – Ester Wax 73

3.5 Embedding – Methacrylate 74

References 77

## **4 Staining 79**

4.1 Single-contrast staining – Carmines 81

4.2 Single contrast staining – Nuclear Stains 83

4.3 Single contrast staining – General Stains 86

4.4 Single contrast staining – Golgi 89

4.5 Single contrast staining – Eggs 89

4.6 Single contrast staining – Silver Stains 90

4.7 Polychrome staining techniques – General 92

4.8 Polychrome staining – Brain/Nerve 102

4.9 Polychrome staining – blood 103

4.10 Single contrast procedures for chitinous material 105

4.11 Polychrome staining procedures for chitinous material 106

4.12 Polychrome staining for chitinous material – KOH 110

4.13 Polychrome staining for chitinous material – Differential staining of Individual Organs 111

4.14 Staining of specific tissues 113

4.15 Two dye combinations 114

References 117

## **5 Immunohistochemical techniques 119**

5.1 Introduction 119

5.2 General immunostaining techniques 127

5.3 Immunolabeling of samples for Transmission Electron Microscopy (TEM) 135

5.4 Proliferation assays 140

5.5 Methods to detect specific proteins 142

References 144

## **6 Use of genetic markers in insect histology 146**

6.1 Introduction 146

6.2 Inducible genetic markers 149

6.3 Mosaic gene expression 156

6.4 Fluorescent markers for live imaging and kinetic microscopy 165

References 169

## **7 Fluorescence 171**

7.1 Introduction 171

References 192

## **8 Mounting 194**

8.1 Introduction 194

References 206

## **9 Preparation of whole mounts 208**

9.1 Introduction 208

References 229

## **10 Preparation of whole mounts for staining 231**

10.1 Introduction 231

10.2 Detection of NAPDHd 237

10.3 SEM 238

10.4 In situ hybridization 240

References 244

**11 Preparation of genitalia, mouthparts and other body parts 246**

References 256

**12 Preparation of chromosomes 258**

References 288

**13 Preparation of other specific insect organs and tissues 290**

13.1 Introduction 290

References 323

Appendix Dissecting fluids and saline solutions 325

Index 333.