

Contents

Preface	ix
Acknowledgements	xi
Introduction	xiii
About the companion website	xiv
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 NAPDH _d	237
10.3	SEM	238
10.4	<i>In situ</i> 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