Rapidly Evolving Genes and Genetic Systems

EDITED BY

Rama S. Singh
McMaster University, Canada
Jianping Xu
McMaster University, Canada
and
Rob J. Kulathinal
Temple University, USA

OXFORD UNIVERSITY PRESS
Contents

Foreword xiv
Richard Lewontin

Preface xvii

List of Contributors xviii

1 Introduction 1
Rama S. Singh, Jiaping Xu, and Rob J. Kulathinal
1.1 A gradualist history 1
1.2 Mechanisms of rapid and episodic change 2
  1.2.1 Unconstrained neutral space 2
  1.2.2 Horizontal gene transfer 3
  1.2.3 Developmental macromutations 3
  1.2.4 Evolution by gene regulation 3
  1.2.5 Coevolutionary forces 4
  1.2.6 Sexual selection and sexual arms races 4
  1.2.7 Population demography and genetic revolutions 5
  1.2.8 Adaptive radiation 5
1.3 Punctuated equilibrium within a microevolution framework 5
1.4 Tempo, mode, and the genomic landscape 6
1.5 ‘Rapidly evolving genes and genetic systems’: a brief overview 7
1.6 Future prospects 8

Part I From Theory to Experiment 13

2 Theoretical perspectives on rapid evolutionary change 13
Sarah P. Otto
2.1 Introduction 13
2.2 When is strong selection strong? 16
2.3 Does strong selection differ in kind from weak selection? 20

3 Recombination reshuffles the genotypic deck, thus accelerating the rate of evolution 23
Mihai Albu, Amir R. Kermany, and Donal A. Hickey
3.1 Introduction 23
3.2 Simulating selection on multilocus genotypes 24
3.3 Discussion 27
3.4 Conclusions 29
4 Heterogeneity in neutral divergence across genomic regions induced by sex-specific hybrid incompatibility
Seiji Kumagai and Marcy K. Uyenoyama

4.1 Introduction
4.1.1 Detecting incompatibility factors
4.1.2 Within-species polymorphisms for incompatibility factors with sex-limited transmission

4.2 Genealogical migration rate
4.2.1 Definition
4.2.2 Non-sex-specific incompatibility
4.2.3 Sex-specific incompatibility

4.3 Applications
4.3.1 Mitochondrial introgression
4.3.2 Interpreting region-specific $F_{ST}$

4.4 Conclusions

5 Rapid evolution in experimental populations of major life forms
Jianping Xu

5.1 Introduction

5.2 Features of experimental evolution

5.3 Types of experimental evolution
5.3.1 Directional selection
5.3.2 Adaptation
5.3.3 Mutation accumulation

5.4 Rapid change and divergence among mutation accumulation population lines
5.4.1 Microbial growth rate
5.4.2 Other microbial traits
5.4.3 Plants and animals

5.5 Adaptation and directional selection experiments
5.5.1 Adaptation of E. coli populations
5.5.2 Adaptation of viral populations
5.5.3 Adaptation and directional selection in fruit flies
5.5.4 Adaptation in yeast
5.5.5 Directional selection in mammals
5.5.6 Correlated changes between traits
5.5.7 Acquisition of novel phenotypes

5.6 Genomic analysis of experimental evolution populations

5.7 Conclusions and perspectives

Part II Rapidly Evolving Genetic Elements

6 Rapid evolution of low complexity sequences and single amino acid repeats across eukaryotes
Wilfried Haerty and G. Brian Golding

6.1 Introduction

6.2 Rapid evolution of low complexity sequences
6.2.1 Mutational processes
6.3 Rapid divergence of LCRs and their impact on surrounding sequences 57
   6.3.1 LCRs as indicators of regions of lowered purifying selective pressures 57
   6.3.2 Mutagenic effect of LCRs 58
6.4 Low complexity sequences under selection 59
   6.4.1 Deleterious effects of LCR size variation 59
6.4.2 DNA composition 59
6.4.3 LCR distribution 60
6.4.4 Phenotypic effects of LCR size variation 60
6.4.5 Selection for low information content 61
6.5 Perspectives 61

7 Fast rates of evolution in bacteria due to horizontal gene transfer 64
Weilong Hao
7.1 Introduction 64
7.2 Quantifying horizontal gene transfer 65
7.3 Understanding the variation of gene gain and loss 66
7.4 Horizontal gene transfer in duplicated genes 67
7.5 Pseudogenization of horizontally transferred genes 67
7.6 Mobile sequences and gene movement 68
7.7 Gene exchange goes fine-scale 69
7.8 Conclusions 69

8 Rapid evolution of animal mitochondrial DNA 73
Xuhua Xia
8.1 Introduction 73
8.2 Mitochondrial replication, strand bias, and evolutionary rates 74
8.3 The change in genetic code and evolutionary rate 77
8.4 The change in tRNA genes and evolutionary rate 79
8.5 Conclusions 81

9 Rapid evolution of centromeres and centromeric/kinetochore proteins 83
Kevin C. Roach, Benjamin D. Ross, and Harmit S. Malik
9.1 Centromeres in ‘the fast lane’ 83
9.2 Rapidly evolving centromeric histones 83
9.3 Bewildering centromeric DNA complexity and evolution 85
9.4 The ‘centromere paradox’: conflict, not coevolution 87
9.5 Support for the centromere drive model 89
9.6 Taxonomic differences in susceptibility to centromere drive 89
9.7 Rapid evolution of other centromeric proteins 90
9.8 Centromere drive and postzygotic isolation between species 91
9.9 Future directions 91

10 Rapid evolution via chimeric genes 94
Rebekah L. Rogers and Daniel L. Hartl
10.1 Introduction 94
10.2 Mechanisms of formation 94
10.3 Selection 96
CONTENTS

10.4 Genomic stability 96
10.5 Function 97
10.6 Non-coding DNA 98
10.7 Future directions 99

11 Evolutionary interactions between sex chromosomes and autosomes 101
Manyuan Long, Maria D. Vibranovski, and Yong E. Zhang

11.1 Introduction 101
11.2 Gene traffic between sex chromosome and autosomes 102
  11.2.1 Gene traffic in Drosophila 102
  11.2.2 Gene traffic in mammals 103
  11.2.3 The cause and consequence of gene traffic 104
11.3 The generality of gene traffic out of the X in the genus Drosophila 105
  11.3.1 Gene traffic in Drosophilidae and RNA-based and DNA-based duplication 105
  11.3.2 Independent tests of gene traffic 105
11.4 Mechanisms underlying gene traffic out of the X: the detection of meiotic sex chromosome inactivation 107
  11.4.1 Evolutionary genetic models 107
  11.4.2 Molecular mechanistic models 107
11.5 The X-recruitment of young male-biased genes and gene traffic out of the X chromosome 108
  11.5.1 Age-dependence in Drosophila 109
  11.5.2 Age-dependence in mammals 110
  11.5.3 The slow enrichment of X-linked female genes 110
11.6 Concluding remarks 111

12 Evolutionary signatures in non-coding DNA 115
Dara G. Torgerson and Ryan D. Hernandez

12.1 Introduction 115
12.2 Challenges to studying the evolution of non-coding DNA 116
  12.2.1 Identifying functional non-coding DNA 116
  12.2.2 Estimating the neutral evolutionary rate 117
  12.2.3 Limitations of identifying rapid evolution in non-coding DNA 117
12.3 Patterns of evolution in non-coding DNA 117
  12.3.1 Selection in conserved non-coding sequences? 118
  12.3.2 Detecting selection in promoters and TFBSs 120
  12.3.3 Emerging trends in microRNA binding sites 121
  12.3.4 Coding versus non-coding 121
12.4 Future prospects 122

Part III Sex- and Reproduction-Related Genetic Systems

13 Evolution of sperm–egg interaction 127
Melody R. Palmer and Willie J. Swanson

13.1 Introduction 127
13.2 Evolution at each step of sperm–egg interaction 127
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.3 Causes of rapid evolution</td>
<td>130</td>
</tr>
<tr>
<td>13.4 Methods to identify interacting proteins</td>
<td>132</td>
</tr>
<tr>
<td>13.5 Conclusions</td>
<td>132</td>
</tr>
<tr>
<td><strong>14 Rates of sea urchin bindin evolution</strong></td>
<td>136</td>
</tr>
<tr>
<td>H. A. Lessios and Kirk S. Zigler</td>
<td></td>
</tr>
<tr>
<td>14.1 Introduction</td>
<td>136</td>
</tr>
<tr>
<td>14.2 Function and structure of bindin</td>
<td>136</td>
</tr>
<tr>
<td>14.3 Rate of bindin evolution</td>
<td>137</td>
</tr>
<tr>
<td>14.4 Possible reasons for different evolutionary rates in bindin</td>
<td>139</td>
</tr>
<tr>
<td>14.5 Conclusions and future prospects</td>
<td>141</td>
</tr>
<tr>
<td><strong>15 Evolution of Drosophila seminal proteins and their networks</strong></td>
<td>144</td>
</tr>
<tr>
<td>Alex Wong and Mariana F. Wolfner</td>
<td></td>
</tr>
<tr>
<td>15.1 Introduction</td>
<td>144</td>
</tr>
<tr>
<td>15.2 Drosophila seminal fluid as a model system for rapidly evolving proteins</td>
<td>144</td>
</tr>
<tr>
<td>15.3 Extensive variation in rates of SFP evolution</td>
<td>147</td>
</tr>
<tr>
<td>15.4 Selection on a network?</td>
<td>149</td>
</tr>
<tr>
<td>15.5 Conclusions</td>
<td>150</td>
</tr>
<tr>
<td><strong>16 Evolutionary genomics of the sperm proteome</strong></td>
<td>153</td>
</tr>
<tr>
<td>Timothy L. Karr and Steve Dorus</td>
<td></td>
</tr>
<tr>
<td>16.1 Introduction</td>
<td>153</td>
</tr>
<tr>
<td>16.2 Characterization of the Drosophila sperm proteome</td>
<td>154</td>
</tr>
<tr>
<td>16.3 Molecular evolution of the Drosophila sperm proteome</td>
<td>154</td>
</tr>
<tr>
<td>16.4 Evolution of novel Drosophila sperm components</td>
<td>155</td>
</tr>
<tr>
<td>16.4.1 Novel genes in the sperm proteome</td>
<td>156</td>
</tr>
<tr>
<td>16.4.2 Expansion and diversification of S-LAP gene family</td>
<td>157</td>
</tr>
<tr>
<td>16.5 The mouse sperm proteome: intensified selection on sperm membrane and acrosome genes</td>
<td>157</td>
</tr>
<tr>
<td>16.6 Rapid evolution of immunity-related genes in mammalian sperm</td>
<td>160</td>
</tr>
<tr>
<td>16.7 Sexual selection and compartmentalized adaptation in reproductive genetic systems</td>
<td>161</td>
</tr>
<tr>
<td>16.8 Future perspectives</td>
<td>162</td>
</tr>
<tr>
<td><strong>17 Fast evolution of reproductive genes: when is selection sexual?</strong></td>
<td>165</td>
</tr>
<tr>
<td>Alberto Civetta</td>
<td></td>
</tr>
<tr>
<td>17.1 Introduction</td>
<td>165</td>
</tr>
<tr>
<td>17.2 What has been the role of selection during the evolution of male reproductive genes?</td>
<td>167</td>
</tr>
<tr>
<td>17.3 When is selection sexual? The phylogenetic approach</td>
<td>168</td>
</tr>
<tr>
<td>17.4 Testing sexual selection in the era of genomes</td>
<td>168</td>
</tr>
<tr>
<td>17.5 The need for association studies and functional assays</td>
<td>171</td>
</tr>
<tr>
<td>17.6 Conclusions</td>
<td>172</td>
</tr>
</tbody>
</table>
CONTENTS

18 Rapid morphological, behavioral, and ecological evolution in Drosophila: comparisons between the endemic Hawaiian Drosophila and the cactophilic repleta species group 176
Patrick M. O’Grady and Therese Ann Markow

18.1 Introduction 176
18.1.1 Ecological adaptations 177
18.1.2 Morphological adaptations 177
18.1.3 Behavioral adaptations 178
18.2 Hawaiian Drosophila radiation 179
18.2.1 Phylogenetic relationships 179
18.2.2 Sexual adaptations to morphology and behavior 179
18.2.3 Ecological adaptations to morphology and behavior 179
18.3 Cactophilic Drosophila radiation in the New World 180
18.3.1 Phylogenetic relationships 180
18.3.2 Rapid evolution of ecological adaptations 180
18.3.3 Rapid evolution of behavioral traits 182
18.4 Conclusions: adaptive radiation versus adaptive infiltration 183

19 Ancient yet fast: rapid evolution of mating genes and mating systems in fungi 187
Timothy Y. James

19.1 Introduction 187
19.2 Incompatibility systems in fungi 189
19.3 Fungal reproductive proteins show evidence for positive and balancing selection 190
19.4 Evidence for rapid evolution of fungal incompatibility genes and systems 193
19.4.1 Sequence evolution 194
19.4.2 Mating systems and loci 194
19.5 Evidence for ancient alleles and mating systems 196
19.6 Conclusions 198

Part IV Pathogens and their Hosts

20 Rapid evolution of innate immune response genes 203
Brian P. Lazzaro and Andrew G. Clark

20.1 The evolution of immunity 203
20.2 Orthology and gene family evolution in antimicrobial immunity 204
20.3 Molecular evolution of the antimicrobial immune system 205
20.4 The evolution of defense against viruses and transposable elements 206
20.5 Concluding remarks 208

21 Rapid evolution of the plague pathogen 211
Ruifu Yang, Yujun Cui, and Dongsheng Zhou

21.1 Introduction 211
21.2 Plasmid acquisition in Y. pestis 212
21.3 The impact of phages on genome structure 213
21.4 Prophages in the Y. pestis genome 213
21.5 CRISPRs diversity and the battle between phage and Y. pestis 214
21.6 Gene acquisition, loss, and inactivation 216
21.7 Rearrangements and copy number variants 217
21.8 Neutral versus adaptive evolution 219
21.9 Conclusions 220

22 Evolution of human erythrocyte-specific genes involved in malaria susceptibility 223
Wen-Ya Ko, Felicia Gomez, and Sarah A. Tishkoff
22.1 Introduction 223
22.2 Adaptive evolution in erythrocyte-specific genes 224
  22.2.1 Genetic variants causing erythrocytic structural, regulatory, or enzymatic deficiency: candidates for heterozygote advantage 224
  22.2.2 Positive selection on erythrocyte-surface receptors 226
22.3 Evolutionary response of the human genome to malaria infection 227
  22.3.1 Maintenance of deleterious mutations due to selective pressure of malaria 227
  22.3.2 Effects of population substructure on genetic variation in malaria-endemic human populations 230
  22.3.3 Effects of gene conversion between homologous sequences on genetic variation at loci associated with malarial susceptibility 232
22.4 Future perspectives 232

Part V From Gene Expression to Development to Speciation

23 The rapid evolution of gene expression 237
Carlo G. Artieri
23.1 Introduction 237
23.2 One genome harbors many transcriptomes 238
23.3 Transcriptome divergence is complex 239
23.4 Factors affecting the rate of evolution of gene expression 240
  23.4.1 Spatial heterogeneity 240
  23.4.2 Temporal heterogeneity 241
23.5 Beyond comparisons of expression levels 242
23.6 Open questions and future directions 243

24 Rate variation in the evolution of development: a phylogenetic perspective 246
Artyom Kopp
24.1 Introduction 246
24.2 Examples of rate variation in the evolution of development 247
  24.2.1 Same clade, different pathways: evolution of vulval development in rhabditid nematodes 247
  24.2.2 Same pathway, different clades: evolution of sex combs and pigmentation in Drosophila 248
24.2.3 Same clade, same pathway, different genes: evolution of embryonic development and sex determination in insects 251
24.3 Technical and conceptual challenges to quantifying the evolution of development 252
24.4 Future directions: the promise of phylogenetic approaches to the evolution of development 253

25 Natural hybridization as a catalyst of rapid evolutionary change 256
Michael L. Arnold, Jennafer A.P. Hamlin, Amanda N. Brothers, and Evangeline S. Ballerini
25.1 Introduction 256
25.2 Adaptive trait introgression: when strange is really good
25.2.1 Adaptive trait transfer in Canis: wolves in dogs’ clothing 257
25.2.2 Adaptive trait origin in Saccharomyces cerevisiae: hybrids make the best wine 258
25.3 Hybrid speciation: when opposites attract
25.3.1 Homoploid hybrid speciation: hybrid butterflies (quickly) change their spots 259
25.3.2 Allopolyploid speciation: Tragopogon hybrid polyploids form again, and again, and again . . . in less than 100 years . . . 260
25.4 Natural hybridization and adaptive radiations: hybrid speciation on steroids
25.4.1 Hybridization and adaptive radiations of Lake Malawi cichlids: from hybrid swarm to 800 species, in one lake?! 261
25.4.2 Hybridization and adaptive radiations in Alpine lake whitefish: Swiss fish diversify after the last big thaw 262
25.4.3 Hybridization and adaptive radiations in Hawaiian silverswords: allopolyploids in an island paradise 263
25.5 Conclusions and future prospects 264

26 Rapid evolution of pollinator-mediated plant reproductive isolation 266
Annika M. Moe, Wendy L. Clement, and George D. Weiblen
26.1 Plant–insect diversification 266
26.2 Pollination and reproductive isolation 266
26.3 Ficus versus Castilleae 267
26.4 A pollinator-mediated model for fig speciation 269
26.5 Future directions: plant–pollinator interactions and rapid evolution 271

27 Sexual system genomics and speciation 274
Rob J. Kulathinal and Rama S. Singh
27.1 In the beginning: Darwin and Wallace on sexual selection and speciation 274
27.2 The Modern Synthesis and the development of speciation theory 275
27.3 A new paradigm: the genomics of sexual systems and the origin of species
27.3.1 Functional genomics: organization into sexual and non-sexual systems 277
27.3.2 Higher variation among reproductive systems 277
27.3.3 Strength of sexual selection 278
27.3.4 Sexual systems interaction, coevolution, and rapid change 279
27.3.5 Rapid breakdown of sexual systems in species hybrids 280
27.4 Towards a post-genomics synthesis of speciation 280
27.5 Future prospects: sex as a major force in evolution 281

Index 285
CHAPTER 14

Rates of sea urchin bindin evolution

H. A. Lessios and Kirk S. Zigler

14.1 Introduction

Reproduction at the level of gametic interactions involves activation and attraction of the sperm by egg compounds, induction of the acrosome reaction by the egg jelly, adhesion of the sperm to the egg, and fusion of the two membranes in order to permit the transmission of genetic material. All of these interactions are mediated by molecules. Some of these molecules, such as sea urchin speract, carry out their functions indiscriminately, even if sperm and egg belong to distantly related taxa (Vieira and Miller 2006). Others function in a species-specific or even genotype-specific manner. Selectivity between sperm gamete recognition molecules and their egg receptors is particularly important in organisms with external fertilization, because in the absence of copulation, there are few other opportunities for exercising mate choice. Consequently, such molecules are exposed to the action of selection more directly than molecules with the same function in organisms with internal fertilization. The DNA that codes for gamete recognition molecules often, but not always, evolves rapidly, displaying ratios of amino acid replacement to synonymous substitutions larger than unity, a signature of positive (diversifying) selection (Swanson and Vacquier 2002a, b; Lessios 2007, 2011; Zigler 2008; Palumbi 2009; Vacquier and Swanson 2011). In this chapter, we explore what bindin sequences from various sea urchin species reveal about the rate of evolution of this molecule. Does bindin really evolve in the fast lane?

14.2 Function and structure of bindin

Sea urchin bindin is a protein that coats the acrosome process of sperm after the acrosomal reaction occurs. It interacts with the egg bindin receptor, EBR1, a glycoprotein (Kamei and Glabe 2003), to attach the sperm to the egg’s vitelline layer and to fuse membranes of the gametes. The full-length precursor of bindin is cleaved after translation to form the mature molecule. Among the sea urchin species that have been studied to date, the length of mature bindin ranges from 193–418 amino acids (Zigler and Lessios 2003a). The single sea star in which bindin has been characterized was found to contain 793 amino acids (Patino et al. 2009). In both sea urchins and sea stars, there is a single intron separating two exons. Bindins of 11 species of sea urchins from six orders contain a conserved region in the second exon that codes for approximately 55 amino acids. Eighteen amino acids in this conserved region, thought to be involved in membrane fusion (Rocha et al. 2008), have not changed since the extant orders of Echinoidea split from each other, 250 million years ago (mya). Only one amino acid in this region has changed between sea stars and sea urchins in the 500 million years (mya).
that the two echinoderm classes have been evolving independently (Patino et al. 2009; Vacquier and Swanson 2011). The reputation of bindin as a fast-evolving protein is owed to two regions flanking the conserved core, which in some genera have accumulated many point mutations and insertions–deletions. These are the regions that most likely confer fertilization species-specificity (Lopez et al. 1993). The protein moiety of EBR1, which contains 3713–4595 amino acids, has only been sequenced in two species of Strongylocentrotus (Kamei and Glabe 2003).

### 14.3 Rate of bindin evolution

Bindin has been sequenced in 11 genera of sea urchins, but intrageneric variation, which permits insights in the evolution of the molecule, has been studied in only seven: Echinometra (Metz and Palumbi 1996; McCartney and Lessios 2004), Strongylocentrotus (Biermann 1998), Arbacia (Metz et al. 1998a), Tripneustes (Zigler and Lessios 2003b), Heliocidaris (Zigler et al. 2003), Lytechinus (Zigler and Lessios 2004), and Paracentrotus (Calderon et al. 2009, 2010). Selection on bindin in all of these genera has been studied as the ratio of amino acid replacement to silent substitutions (ω = dN/dS). By this criterion, there is evidence of positive selection (ω > 1) in Echinometra, Strongylocentrotus, Heliocidaris, and Paracentrotus, but not in Arbacia, Tripneustes, and Lytechinus. In addition to being an indication of selection at the nucleotide level, the ω ratio would also be a good measure of relative rates of adaptive evolution if silent sites evolved at the same rate in all genera. This, however, is not the case in bindin. Bindins with higher rates of nonsynonymous substitution also have higher rates of synonymous substitution (Zigler and Lessios 2003b). This correlation has also been observed in other molecules such as alcohol dehydrogenase, ATP synthetase, cyclophilin 1, or enolase (e.g. Dunn et al. 2001), and there are a number of hypotheses as to its cause. While it is typically thought to arise from some form of codon bias, codon usage in sea urchin bindin is very equitable (Zigler and Lessios 2003a). Thus, due to different codon biases, comparing ω ratios between bindins of different genera may lead to erroneous conclusions regarding evolutionary rates. To compare the absolute rate of evolution between genera we need to determine the number of nonsynonymous substitutions per nonsynonymous site that accumulate per unit time. Such a calculation requires evidence of dates of divergence. In this chapter, we will use the interspecific divergence of cytochrome oxidase I (COI) as a proxy for the time since speciation. Calibrated by the rise of the Isthmus of Panama, approximately 3 mya, COI of sea urchins diverges at an average rate of 3.6% per my (Lessios 2008).

Gauged by divergence in COI, average rates of adaptive divergence of bindin within a genus vary between 2.80 × 10⁻³ nonsynonymous substitutions per nonsynonymous site per my (dN/dS = 1) in Arbacia and 22.4 × 10⁻³ dN/dS = 1 in Strongylocentrotus (Table 14.1). As one might expect, genera in which bindin evolves under positive selection, show amino acid divergence rates almost four times higher than genera in which bindin appears to be under purifying selection: the average substitution rate in Strongylocentrotus, Echinometra, and Heliocidaris is 20.4 × 10⁻³ dN/dS = 1 whereas in Arbacia, Tripneustes, Lytechinus, Pseudoboletia, and Diadema, it is 5.96 × 10⁻³ dN/dS = 1. The question we would like to answer is how these rates of adaptive evolution compare with those of other proteins, both of those that have been deemed to evolve rapidly in other taxa, and those that carry out other functions in sea urchins.

Fig. 14.1 presents a comparison of the rates of adaptive evolution of bindin to seven other classes of reproductive proteins from five groups of organisms. These are all proteins that are generally considered as fast-evolving. Because COI in different taxa evolves at different rates, it is necessary to apply taxon-specific calibrations to calculate divergence rates. To estimate absolute rates of protein evolution, we have assumed that COI evolves at an average rate of 3.6% per my in sea urchins (Lessios 2008), 2.7% per MY in gastropods (Lessios 2008), 2.3% per my in insects (Papadopoulou et al. 2010), and 1.6% per my in hominids (Kumar et al. 2005). Estimated in this manner, the evolutionary rates of bindins in different genera of sea urchins, even those found to be under selection, are slower than that of reproductive proteins of gastropods or insects. They are more comparable to those of
Table 14.1  Pairwise divergence in bindin and in cytochrome oxidase I (COI) of selected species of sea urchin genera in which bindin variation has been studied. K2P: Kimura two-parameter distance; dN: amino acid substitutions per non-synonymous site; dS: synonymous substitutions per synonymous site; MY: million years. Estimated rates of divergence of bindin are based on the assumption that COI in sea urchins diverges at a rate of 0.036 per site per MY.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
<th>Species</th>
<th>Bindin dN</th>
<th>COI K2P</th>
<th>Bindin dS</th>
<th>COI K2P</th>
<th>MY</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arbacia</td>
<td>lixula</td>
<td>punctulata</td>
<td>0.007</td>
<td>0.069</td>
<td>0.090</td>
<td>0.072</td>
<td>0.764</td>
<td>0.0026  Metz et al. 1998a</td>
</tr>
<tr>
<td>Arbacia</td>
<td>lixula</td>
<td>stellata-+incisa</td>
<td>0.007</td>
<td>0.096</td>
<td>0.134</td>
<td>0.053</td>
<td>0.716</td>
<td>0.0019</td>
</tr>
<tr>
<td>Arbacia</td>
<td>lixula</td>
<td>dufres nei</td>
<td>0.016</td>
<td>0.071</td>
<td>0.124</td>
<td>0.129</td>
<td>0.570</td>
<td>0.0046</td>
</tr>
<tr>
<td>Arbacia</td>
<td>punctulata</td>
<td>stellata-+incisa</td>
<td>0.003</td>
<td>0.088</td>
<td>0.139</td>
<td>0.022</td>
<td>0.635</td>
<td>0.0008</td>
</tr>
<tr>
<td>Arbacia</td>
<td>punctulata</td>
<td>dufres nei</td>
<td>0.011</td>
<td>0.059</td>
<td>0.124</td>
<td>0.085</td>
<td>0.477</td>
<td>0.0031</td>
</tr>
<tr>
<td>Arbacia</td>
<td>stellata-+incisa</td>
<td>dufres nei</td>
<td>0.013</td>
<td>0.071</td>
<td>0.119</td>
<td>0.105</td>
<td>0.597</td>
<td>0.0038</td>
</tr>
<tr>
<td>Heliocidaris</td>
<td>erythrogramma</td>
<td>tuberculata</td>
<td>0.069</td>
<td>0.149</td>
<td>0.147</td>
<td>0.469</td>
<td>1.014</td>
<td>0.0169  Zigler et al. 2003</td>
</tr>
<tr>
<td>Tripneustes</td>
<td>ventricus</td>
<td>grallia-+depres sus</td>
<td>0.016</td>
<td>0.026</td>
<td>0.087</td>
<td>0.187</td>
<td>0.293</td>
<td>0.0067  Zigler and Lessios 2003</td>
</tr>
<tr>
<td>Echinometra</td>
<td>oblonga</td>
<td>mathaei</td>
<td>0.021</td>
<td>0.054</td>
<td>0.023</td>
<td>0.905</td>
<td>2.328</td>
<td>0.0326  Metz and Palumbi 1996</td>
</tr>
<tr>
<td>Echinometra</td>
<td>oblonga</td>
<td>type A</td>
<td>0.024</td>
<td>0.076</td>
<td>0.032</td>
<td>0.757</td>
<td>2.371</td>
<td>0.0273</td>
</tr>
<tr>
<td>Echinometra</td>
<td>mathaei</td>
<td>type A</td>
<td>0.028</td>
<td>0.051</td>
<td>0.024</td>
<td>1.169</td>
<td>2.107</td>
<td>0.0421</td>
</tr>
<tr>
<td>Echinometra</td>
<td>lytechinus</td>
<td>vidalis</td>
<td>0.022</td>
<td>0.047</td>
<td>0.050</td>
<td>0.440</td>
<td>0.940</td>
<td>0.0158  McCartney and Lessios 2004</td>
</tr>
<tr>
<td>Echinometra</td>
<td>lytechinus</td>
<td>vanbrunti</td>
<td>0.026</td>
<td>0.046</td>
<td>0.102</td>
<td>0.255</td>
<td>0.451</td>
<td>0.0092</td>
</tr>
<tr>
<td>Echinometra</td>
<td>vidalis</td>
<td>vanbrunti</td>
<td>0.014</td>
<td>0.083</td>
<td>0.126</td>
<td>0.111</td>
<td>0.659</td>
<td>0.0040</td>
</tr>
<tr>
<td>Lytechinus</td>
<td>pectus</td>
<td>vaniegatus</td>
<td>0.013</td>
<td>0.105</td>
<td>0.135</td>
<td>0.096</td>
<td>0.778</td>
<td>0.0035  Zigler and Lessios 2004</td>
</tr>
<tr>
<td>Lytechinus</td>
<td>vaniegatus</td>
<td>williamsi</td>
<td>0.006</td>
<td>0.022</td>
<td>0.017</td>
<td>0.353</td>
<td>1.294</td>
<td>0.0127</td>
</tr>
<tr>
<td>Lytechinus</td>
<td>semituberculatus</td>
<td>pectus</td>
<td>0.025</td>
<td>0.073</td>
<td>0.114</td>
<td>0.219</td>
<td>0.640</td>
<td>0.0079</td>
</tr>
<tr>
<td>Lytechinus</td>
<td>euerces</td>
<td>Sphaer ethics granulatus</td>
<td>0.019</td>
<td>0.100</td>
<td>0.089</td>
<td>0.213</td>
<td>1.124</td>
<td>0.0077</td>
</tr>
<tr>
<td>Pseudotheleia</td>
<td>in rhiana</td>
<td>maculata</td>
<td>0.006</td>
<td>0.024</td>
<td>0.073</td>
<td>0.082</td>
<td>0.329</td>
<td>0.0030  Zigler et al. (in press)</td>
</tr>
<tr>
<td>Strongylocentrotus</td>
<td>purpuratus</td>
<td>purpuratus</td>
<td>0.021</td>
<td>0.062</td>
<td>0.072</td>
<td>0.287</td>
<td>0.863</td>
<td>0.0103  Biermann 1998</td>
</tr>
<tr>
<td>Strongylocentrotus</td>
<td>purpuratus</td>
<td>purpuratus</td>
<td>0.031</td>
<td>0.086</td>
<td>0.075</td>
<td>0.418</td>
<td>1.148</td>
<td>0.0190</td>
</tr>
<tr>
<td>Strongylocentrotus</td>
<td>purpuratus</td>
<td>H. pulcherinus</td>
<td>0.073</td>
<td>0.158</td>
<td>0.104</td>
<td>0.704</td>
<td>1.514</td>
<td>0.0253</td>
</tr>
<tr>
<td>Strongylocentrotus</td>
<td>purpuratus</td>
<td>H. pulcherinus</td>
<td>0.025</td>
<td>0.036</td>
<td>0.055</td>
<td>0.715</td>
<td>1.011</td>
<td>0.0227</td>
</tr>
<tr>
<td>Strongylocentrotus</td>
<td>purpuratus</td>
<td>H. pulcherinus</td>
<td>0.066</td>
<td>0.119</td>
<td>0.070</td>
<td>0.841</td>
<td>1.696</td>
<td>0.0339</td>
</tr>
<tr>
<td>Strongylocentrotus</td>
<td>purpuratus</td>
<td>H. pulcherinus</td>
<td>0.063</td>
<td>0.139</td>
<td>0.094</td>
<td>0.672</td>
<td>1.481</td>
<td>0.0242</td>
</tr>
</tbody>
</table>
Figure 14.1 Bindin evolution relative to known fast-evolving reproductive proteins from other taxa. Non-synonymous substitutions per non-synonymous site (dN) per million years, between congeneric species (except in hominids, in which they are within the same family) in sea urchin bindin (B) (data from references in Table 14.1), abalone lysin (HL) and 18 kD protein (H18) (data from Metz et al. 1998b), Tegula lysin (TL), and the mature region of TMAP protein (TMAP) (data from Hellberg and Vacquier 1999; Hellberg et al. 2000), Drosophila Acp26Aa and Acp36DE (Acps) (data from Tsaur and Wu 1997), hominid protamine 1 and 2 (P), ZP2, ZP3 and oviductal glycoprotein (ZP/OGP) (data from Wyckoff et al. 2000).

Protoamines, zona pellucida proteins, and oviductal glycoprotein in hominids. Adjustments to the assumed rate of COI evolution, or even an assumption of a universal COI clock, would not change this conclusion. Thus, by the standard of other fast-evolving reproductive proteins from other invertebrates, bindin evolves only at moderate rates.

How do rates of bindin evolution compare to rates of evolution among other sea urchin proteins? To answer this question, we compared all protein coding DNA sequences of Lytechinus variegatus in GenBank to their closest matches in the Strongylocentrotus purpuratus complete genome. With the exception of S. purpuratus, more genes have been sequenced from Lytechinus variegatus than any other species of sea urchin. Lytechinus and Strongylocentrotus diverged approximately 60 mya. Sequences were available for 90 L. variegatus genes. The protein sequence of each gene was compared between the two species via protein-protein BLAST to GenBank’s ‘non-redundant (nr) protein sequences’ database. The closest match to a S. purpuratus protein was noted, and the two protein sequences were aligned using Clustal in MEGA (v. 4.0). We then used MEGA to calculate the p-distance between the aligned protein sequences. We identified matches for 85 of the 90 Lytechinus genes. The five genes that did not have a match may be: (1) missing from the annotated Strongylocentrotus genome; (2) lost in the Strongylocentrotus lineage; or (3) mis-annotated in their original Lytechinus entry. The set of genes that we compared contained proteins with various functions, including many involved in reproduction, and also in development, cytoskeleton formation, cell attachment, and stress responses. After ranking the divergences of the 85 proteins, that of bindin was the sixth largest, with a p-distance of 0.326 for the full-length molecule and 0.314 for the mature portion. Of the five proteins with divergence values higher than bindin, vitellogenin and SFE-1 also carry out functions related to reproduction, whereas the other three were involved in development. Considering the inevitable bias of proteins available for comparison, the conclusion from this comparison is that bindin evolves at moderately fast rates in relation to other sea urchin proteins.

14.4 Possible reasons for different evolutionary rates in bindin

Why does bindin in four sea urchin genera evolve more rapidly under strong positive selection, than in three other genera in which it is subject to purifying selection? In the absence of data regarding variation in its egg receptor, the answer can only be speculative. Possible reasons for this lack of pattern have been thoroughly reviewed (Lessios 2007, 2011; Zigler 2008; Palumbi 2009). Here we present a summary of the hypotheses that have been proposed so far.

One possibility is that positive selection of bindin arises from the need for species recognition when two closely related species are in danger of hybridizing with each other. We will call this the ‘reinforcement hypothesis.’ This name does not imply that speciation by reinforcement has actually taken place, but rather that bindin alleles resembling those of a sympatric species—and thus allowing gamete wastage in inferior hybrids—have been selected against. A broad-brush picture of comparisons between genera is consistent with this hypothesis. When bindin rates of divergence of species that are entirely allopatric with respect to congeneres are compared to those of species that may have a higher probability of hybridization, those of the for-
mer are clustered around lower values than those of the latter (Fig. 14.2). Genera with many sympatric species, such as *Strongylocentrotus*, and *Echinometra* tend to have the highest rates of interspecific bindin divergence. Not all the data, however, are consistent with the reinforcement hypothesis. Contrary to what is expected from selection for species recognition, bindin is polymorphic and shows the signature of positive selection not just between species but also between alleles of the same species (Metz and Palumbi 1996; Lessios 2007, 2011). A pattern of character displacement is present in one species of Pacific *Echinometra* (Geyer and Palumbi 2003) in partial geographic overlap with its sister species but not in an Atlantic species of the same genus that also needs to contend with the challenge of a sister species existing over part of its range (Geyer and Lessios 2009). Given the present evidence, the hypothesis that reinforcement in sympatry accelerates bindin divergence is as likely as the hypothesis that divergence in bindin, due to other causes, allows for sympatric coexistence.

Another possibility for the differences in rates of bindin evolution could be that they are correlated to the relative age of species in different sea urchin genera. If, as Civetta and Singh (1998) have suggested, episodes of divergence in reproductive molecules are concentrated at the time of speciation, and if selection on these molecules is subsequently relaxed, younger species would show higher rates of bindin differentiation than older ones. This hypothesis is not supported by the data. Sea urchins tend to conform to 'Jordan’s rule' (Jordan 1905). Young sister species tend to be distributed on either side of a geographic barrier, and only older species become sympatric with the passage of time (Lessios 2010). Thus, allopatric species are, in general, younger than sympatric ones, and if bindin divergence were accelerated during speciation then slowed down, they should show more differences in this molecule per unit time than sympatric ones. The opposite is true (Fig. 14.2).

The most credible hypothesis to date for differences in the rates of bindin evolution is that they are caused by differences in the intensity of sexual selection and sexual conflict. Using variation in bindin genotypes of females as a proxy for variation in the bindin receptor (with which bindin is expected to show linkage disequilibrium), Palumbi (1999) has found that sexual selection exists in *Echinometra mathaei*. Eggs are fertilized at higher rates by sperm carrying the same bindin allele. Using the same proxy, Levitan and Farrell (2006) and Levitan and Stapper (2010) showed in *Strongylocentrotus franciscanus* and *S. purpuratus* that sperm density and the danger of polyspermy establish different selective regimes for various bindin alleles. At low sperm densities, most offspring are produced by the union of sperm and egg possessing bindin alleles that are most common in the population. At high sperm densities, rare alleles leave behind the most offspring, because common alleles, causing fast fertilization, result in polyspermic zygotes, which fail to develop. Thus, there is always selection on males to effect fast fertilization, but females in high sperm densities benefit from having alleles that retard fertilization: a typical sexual conflict situation. Depending on ecological conditions, sexual conflict can occur in some populations but not others, thus resulting in different rates of bindin evolution.

![Figure 14.2](image-url) Comparison of interspecific rates of bindin divergence between genera. Amino acid replacement substitutions (dN) per replacement site in bindin divided by Kimura-two-parameter distance in cytochrome oxidase I (COI K2P) in allopatric and sympatric species of eight genera of sea urchins. A species is considered as ‘allopatric’ if its range does not overlap with that of another member of the same genus. Genera in which bindin has been shown to be under selection are marked in the legend with S.
14.5 Conclusions and future prospects

In comparison to other invertebrate reproductive proteins, bindin evolves moderately rapidly in some genera and slowly in others. Selective reasons for the differences that cause these dissimilarities in rates are still the subject of speculation, but they may well be related to fertilization environments and intraspecific processes. Interspecific processes, such as reinforcement, can also not be ruled out. There may well be no universal explanation for the presence or absence of positive selection in different sea urchin taxa. Gametic proteins are often brought up as examples of rapid evolution. Fast evolution is certainly true for each of these proteins in the particular genus in which they have been studied. However, in a great many of the documented cases of fast molecular evolution, the evidence comes only from a small fraction of taxa. Data on sea urchin bindin, though far from covering the entire echinoid class, derive from multiple genera. This broader taxonomic coverage alone may explain why more diversity in the mode of evolution of this molecule has been documented than has been found in other invertebrate reproductive proteins.

Future laboratory studies linking the structure of different bindin alleles with the specificity of fertilization would be of great benefit in understanding the evolution of this molecule. We already know which amino acids evolve under selection, but we will need to determine the functional reasons for such selection. Additional understanding of the sources of natural selection on this molecule and the rate of its evolution would come from comparative studies that link fertilization ecology in nature with the success of particular bindin alleles. Simply characterizing species as sympatric or allopatric on the basis of their geographic distribution is not adequate for determining the role of reinforcement or other interspecific processes in bindin evolution. Ultimately, interest in the evolution of bindin and similar molecules stems from our desire to understand the process of speciation and the role of sexual selection in the evolution of reproductive isolation. In that respect, assessing the importance of bindin as a reproductive isolation barrier between species relies on studies that are not aimed directly at this molecule alone. Whether bindin is involved in speciation depends not just on the species-specificity of its interactions with its receptor but on the probability that gametes of two closely related sea urchin species will encounter each other in nature. Even when gametic interactions are, in fact, species-specific, it is still necessary to determine whether bindin or some other molecule, acting earlier in the sequence of fertilization, is responsible. Thus, information on habitat separation, reproductive timing, and pre-spawning chemical communication as well as on the role of other reproductive molecules is important in understanding whether intra- or interspecific interactions mold the evolution of the bindin. Most of all, we will need to link variation of bindin to variation in its egg receptor. The study of EBRI has been retarded by its enormous size. Recent advances in techniques for massive DNA sequencing have made it practical to gather data on individual variation in large stretches of genetic material, and will no doubt soon be applied to this problem.

Acknowledgments

We thank Laura Geyer and Santosh Jagadeesha for comments on the manuscript.

References


