

**ECOLOGICAL ASSOCIATIONS AND EVOLUTIONARY BIOLOGY OF  
THE MARINE LUMINOUS BACTERIUM, *VIBRIO FISCHERI*.**

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The population biology of *V. fischeri* was studied in two environments: the light organ of the Hawaiian bobtail squid, *Euprymna scolopes*; the estuarine environment of Plum Island Sound, Massachusetts. The evolution of dark variants from bioluminescent progenitors during *in vitro* laboratory culture in both seawater and rich liquid medium was studied with *Vibrionaceae* isolates from New Zealand.

Phenotypic, physiological, and genetic methods demonstrated that *V. fischeri* exhibit an oligoclonal population structure (2-5 major strains) in adult *E. scolopes* light organs. Laboratory initiation experiments of aposymbiotic, juvenile *E. scolopes* with isogenic GFP- and RFP-labeled *V. fischeri* were used to calculate an initiating number of approximately one cell per light organ crypt upon horizontal acquisition of symbionts from seawater. The biogeography of *V. fischeri* appeared to be similar to host biogeography on Hawaii – two host populations (from Maunalua and Kaneohe Bays) were found to harbor *V. fischeri* strains at different relative abundances. Phylogenetic reconstructions were created using a novel multi-locus sequence analysis scheme. These reconstructions showed that one monophyletic clade of strains (“Group A”) dominated light-organ populations in adult Maunalua Bay hosts; Group A strains outcompeted sympatric *V. fischeri* strains in initiation experiments in

Maunalua Bay juveniles. Group A strains also lost culturability in unfiltered Maunalua Bay seawater more rapidly than sympatric strains.

The majority of *V. fischeri* collected from Plum Island Sound Estuary were found to lack the entire ~10 kb *lux* region; this region contains all genes known to be necessary and sufficient for bacterial bioluminescence. These strains were phenotypically dark and unresponsive to both autoinducer and decanal. Phylogenetic reconstructions of *V. fischeri* suggested that dark strains are non-monophyletic within the species. These natural, dark *V. fischeri* exhibited both a persistence and competition defect during laboratory initiation experiments in *E. scolopes*.

Bioluminescent *Vibrionaceae* isolates from New Zealand were cultured in filtered seawater and rich medium for extended periods of time. Both genus- and species-specific differences in the genesis of dark variants from these cultures were observed. Dark variants were found to be adaptive in the rich-medium environment after 10-day competition experiments with bright progenitors.

## DEDICATION

to the cat and to Ned

who, over the last few years, helped me to better  
appreciate a little bit of sugar.

The honeybees that fetch the nectar  
From the flowers to the comb  
Never tire of ever buzzing to and fro  
Because they take a little nip  
From every flower that they sip  
And hence...  
They find...  
Their task is not a grind.

A spoonful of sugar helps the medicine go down,  
The medicine go down,  
The medicine go down.  
Just a spoonful of sugar helps the medicine go down  
In a most delightful way.

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## TABLE OF CONTENTS

<b>Abstract</b>	<b>i</b>
<b>Dedication</b>	<b>iii</b>
<b>Acknowledgements</b>	<b>iv</b>
<b>Table of Contents</b>	<b>vii</b>
<b>List of Figure Headings</b>	<b>xi</b>
<b>List of Table Titles</b>	<b>xiii</b>
<b>Chapter 1. Introduction and Thesis Outline</b>	<b>1</b>
The Diversity of Microbial Life.....	3
The Symbiotic Habit.....	13
<i>V. fischeri</i> (History) and Ecology.....	24
The Squid/ <i>Vibrio</i> Symbiosis.....	38
Thesis Outline.....	52
Works Cited.....	54
<b>Chapter 2. Population Structure of <i>Vibrio fischeri</i> Within the Light Organs of <i>Euprymna scolopes</i> Squids from Two Oahu (Hawaii) Populations.</b>	<b>74</b>
Authors' Contributions.....	75
Summary.....	76
Introduction.....	77
Materials and Methods.....	82
Results.....	105

	viii
Discussion.....	117
Conclusions.....	124
Acknowledgements.....	124
Works Cited.....	125
<b>Chapter 3. Phylogeny and Fitness of <i>Vibrio fischeri</i> Within the Light Organs of <i>Euprymna scolopes</i> Squids from Two Oahu (Hawaii) Populations.</b>	<b>135</b>
Authors' Contributions.....	136
Summary.....	138
Introduction.....	138
Materials and Methods.....	140
Results.....	164
Discussion.....	184
Conclusions.....	189
Acknowledgements.....	189
Works Cited.....	190
<b>Chapter 4. Polyphyly of Non-Bioluminescent <i>Vibrio fischeri</i> Sharing a <i>lux</i>-Locus Deletion.</b>	<b>199</b>
Authors' Contributions.....	200
Summary.....	201
Introduction.....	202
Materials and Methods.....	205
Results.....	219

	ix
Discussion.....	242
Conclusions.....	249
Acknowledgements.....	249
Works Cited.....	250
<b>Chapter 5. Experimental and Taxonomic Study of Bioluminescence Variation in Natural <i>Vibrionaceae</i> Isolates from New Zealand.</b>	<b>256</b>
Authors' Contributions.....	257
Summary.....	258
Introduction.....	259
Materials and Methods.....	262
Results.....	270
Discussion.....	285
Conclusions.....	291
Acknowledgements.....	291
Works Cited.....	292
<b>Chapter 6. Synthesis and Future Directions</b>	<b>297</b>
Thesis Synthesis.....	298
Future Directions.....	308
Works Cited.....	312
<b>Appendix A. Population Structure of <i>V. fischeri</i> in Lab-Kept Adult <i>E. scolopes</i> Over a Month.</b>	<b>315</b>
Introduction	316

	x
Methods	318
Results and Discussion	320
Works Cited	324

## LIST OF TABLE TITLES

**Chapter 2. Population Structure of *Vibrio fischeri* Within the Light Organs of *Euprymna scolopes* Squids from Two Oahu (Hawaii) Populations.**

2-1 – Bacteria used in this study .....	83-85
2-2 – Physiological and genetic data for 65 representative <i>V. fischeri</i> isolates....	96-97
2-3 – Statistical analyses of association.....	110
2-4 – Statistical analyses ... from different host populations .....	111
2-5 – Statistical analyses ... from the same host population.....	112
2-6 – Compilation of juvenile, single strain competition experiments.....	115

**Chapter 3. Phylogeny and Fitness of *Vibrio fischeri* Within the Light Organs of *Euprymna scolopes* Squids from Two Oahu (Hawaii) Populations.**

3-1 – <i>Vibrionaceae</i> strains used in this study.....	143-145
3-2 – <i>Vibrionaceae</i> strains' GenBank accession numbers.....	145-146
3-3 – Loci and primers utilized in <i>V. fischeri</i> MLST scheme.....	147
3-4 – Sequence characteristics and ML...for use in the <i>V. fischeri</i> MLST scheme	151-152
3-5 – Results of ILD and SH tests for topological incongruence.....	153
3-6 – Selection and population-growth test statistics of <i>V. fischeri</i> strains.....	154-156
3-7 – Nucleotide polymorphism mutation, recombination parameters.....	157

## **Chapter 4. Polyphyly of Non-Bioluminescent *Vibrio fischeri* Sharing a *lux*-Locus**

### **Deletion.**

4-1 – <i>Vibrionaceae</i> strains used in this study.....	206-207
4-2 – Phenotypic and genotypic bioluminescence properties of PIE <i>V. fischeri</i> ..	224-225
4-3 – Evolutionary scenarios for <i>lux</i> -region homoplasy among <i>V. fischeri</i> .....	243

## **Chapter 5. Experimental and Taxonomic Study of Bioluminescence Variation in**

### **Natural *Vibrionaceae* Isolates from New Zealand.**

5-1 – Bacteria used in this study.....	268-269
5-2 – Growth and bioluminescence of New Zealand strains.....	271
5-3 – Bioluminescence variation in selected New Zealand and <i>Vibrionaceae</i> .....	276-277
5-4 – Summary of growth and bioluminescence variation in NZ.....	279
5-5 – Medium composition affects bioluminescence variation.....	280-281

## **Chapter 6. Synthesis and Future Directions**

6-1 – Estimated number of initiating cells in three mutualism models.....	300
---	-----

## **Appendix A. Population Structure of *V. fischeri* in Lab-Kept Adult *E. scolopes* Over a**

### **Month.**

A-1 – Temporal study observations and data for three adult <i>E. scolopes</i> .....	321
---	-----

## LIST OF FIGURE HEADINGS

**Chapter 1. Introduction and Thesis Outline**

1-1 – The “species-scape” circa 1992.....	5
1-2 – <i>V. fischeri lux</i> -region organization and bioluminescence biochemistry.....	28
1-3 – Evolutionary relationships among <i>Euprymna</i> and <i>Sepiola</i> light-organ symbionts	42

**Chapter 2. Population Structure of *Vibrio fischeri* Within the Light Organs of *Euprymna scolopes* Squids from Two Oahu (Hawaii) Populations**

2-1 – Morphological comparison of juvenile and adult <i>E. scolopes</i> and their light organs.....	78
2-2 – Location of <i>E. scolopes</i> sampling sites on Oahu, Hawaii.....	80
2-3 – Genetic analysis of <i>V. fischeri</i> symbionts.....	88
2-4 – Major bands from VfRep PCR fingerprinting of ES114 and ES213.....	90
2-5 – VfRep PCR fingerprints exhibit <i>in vitro</i> clonal and temporal stability.....	91
2-6 – VfRep PCR fingerprints exhibit <i>in squido</i> clonal and temporal stability....	92
2-7 – Agglomerative hierarchical cluster analysis.....	95
2-8 – Rarefaction analyses of VfRep-types.....	108
2-9 – Confocal micrograph of fluorescently labeled <i>V. fischeri</i> cells.....	114
2-10 – Graphical analysis of the relationship between either inoculum .....	117

**Chapter 3. Phylogeny and Fitness of *Vibrio fischeri* Within the Light Organs of *Euprymna scolopes* Squids from Two Oahu (Hawaii) Populations.**

3-1 – Geographic distribution of <i>V. fischeri</i> isolates used in this study.....	142
3-2 – ML gene phylograms of individual MLST frags. under best-fit models.....	165-166
3-3 –Three 50% majority-rule consensus Bayesian phylograms.....	172-175
3-4 – Three consensus networks produced with Clonal Frame.....	176-177
3-5 – Dual-isolate juvenile <i>E. scolopes</i> experiments competing.....	179-180
3-6 – Single-strain <i>V. fischeri</i> inoculations of juvenile squid.....	181
3-7 – Single-isolate <i>V. fischeri</i> growth experiments in seawater microcosms.....	182

**Chapter 4. Polyphyly of Non-Bioluminescent *Vibrio fischeri* Sharing a *lux*-Locus**

**Deletion.**

4-1 – The improved <i>hvnC</i> -PCR primer pair specifically amplifies <i>V. fischeri</i> ....	209
4-2 – Phylogenetic relationships among Plum Island Estuary <i>Vibrionaceae</i> .....	220-221
4-3 – Electrophoresis of PCR products...demonstrates variability in <i>lux</i> -region... 222-223	
4-4 – Molecular comparisons of the <i>lux</i> -operon among <i>V. fischeri</i> .....	228
4-5 – Full-genome comparisons among <i>V. fischeri</i> strains .....	229-230
4-6 – Agarose gel electrophoresis of products from <i>rscS</i> gene PCR.....	232
4-7 – Clonal frame reconstruction of <i>V. fischeri</i> .....	233
4-8 – Tree-based reconstruction of <i>V. fischeri</i> .....	234
4-9 – Growth on TCBS agar is not significantly different between <i>V. fischeri</i> ...	237
4-10 – Non-bioluminescent <i>V. fischeri</i> isolates are outcompeted by .....	239-240

4-11 – Single-strain <i>V. fischeri</i> colonizations of juvenile squid.....	241
--	-----

**Chapter 5. Experimental and Taxonomic Study of Bioluminescence Variation in Natural *Vibrionaceae* Isolates from New Zealand.**

5-1 – Bayesian analysis of 16S rRNA loci from selected NZ <i>Vibrionaceae</i> .....	273-274
5-2 – Rich-medium growth conditions promote bright-to-dark variation.....	275
5-3 – Dark variants out-compete their progenitors after several days' culture....	282

**Chapter 6. Synthesis and Future Directions**

6-1 – Initiating cell number is reduced in the E/V symbiosis.....	302
---	-----

**Appendix A. Population Structure of *V. fischeri* in Lab-Kept Adult *E. scolopes* Over a Month.**

A-1 – Population dynamics in a single <i>E. scolopes</i> adult over one month's time.	322
---	-----