

**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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