Is the *Vibrio fischeri*–*Euprymna scolopes* Symbiosis a Defensive Mutualism?

Eric V. Stabb and Deborah S. Millikan

6.1 Introduction

The light-organ symbiosis between the bioluminescent bacterium *Vibrio fischeri* and the Hawaiian bobtail squid *Euprymna scolopes* is a fascinating association rife with intriguing biology. This sepiolid squid is a small nocturnal inhabitant of shallow sandy reefs in the Hawaiian archipelago that allows *V. fischeri*, and only this bacterium, to colonize epithelium-lined crypts of a specialized light-emitting organ. This "light organ" is located just ventral to the squid's ink sac in the mantle cavity. Once they have colonized the light organ, the bacterial symbionts emit a bluish light, and adaptations by the host allow it to direct and modulate the emitted light. Below, we will discuss how the light organ's architecture suggests that it functions in the camouflaging behavior referred to as counterillumination (Figure 6.1), wherein marine animals emit light downward, roughly matching the downwelling light from above to obscure their silhouette from animals beneath them in the water column (Clarke, 1963; Dahlgren, 1916; Harper and Case, 1999; Latz, 1996; McFall-Ngai and Morin, 1991; Würmer et al., 1979).
To adjust to the host and other environments, *V. fischeri* possesses an impressive array of regulatory systems (Geszvain and Visick, 2006; Hastings and Greenberg, 1999). *V. fischeri* is well known for a group behavior known as "quorum sensing," wherein a diffusible pheromone mediates changes in gene expression when populations reach high cell density (Hastings and Greenberg, 1999). Quorum sensing and the redox-responsive ArcA/ArcB regulatory system tune the luminescence of *V. fischeri* to express maximally when cells are crowded, yet in relatively oxidative conditions (Bose et al., 2007). Because regulation of luminescence is governed in part by a diffusible pheromone, the potential exists for a sub-population of *V. fischeri* to incite a group decision to luminescence in the light organ. Understanding the basis for the behavior of *V. fischeri* in the light organ should help elucidate the role of luminescence for the bacterium (Stabb, 2005) and may reveal mechanisms by which the host manipulates the symbiont's luminescence.

6.2.3 Ecology and Behavior of *E. scolopes*

*E. scolopes* is a solitary nocturnal predator that feeds mainly on shrimps and polychaetes, and grows from hatchlings just a few millimeter in length to thumb-sized adults (Moylinah, 1983; Shears, 1988). The adults probably live less than a year (Hanlon et al., 1997; Singly, 1983). Females lay clutches of tens or hundreds of eggs, and as mentioned above, each new generation must acquire *V. fischeri* from the surrounding marine environment (Wei and Young, 1989), although infection is so rapid that no uninfected *E. scolopes* individuals have ever been found.

Most observations of *E. scolopes* are made in shallow sandy reef areas, but it is unclear how confined it is to such habitats. We, and others, typically use flashlights and nets to collect animals at night by wading in knee-deep water. It is not uncommon to find animals less than a meter from shore, sometimes in water no more than 10 cm deep. However, there are reports of *E. scolopes* being collected offshore near the surface (R. Young, personal communication) and even at depths around 200 m (Berry, 1912). So while most observations of *E. scolopes* are nearshore shallow water, it is not clear the extent to which this reflects the fact that this is where researchers tend to look for them. Similarly, we tend to find *E. scolopes* adults on the sandy bottom, not up in the water column; however, almost certainly the animals are aware of our presence before we spot them, so their behavior may be perturbed. In aquaria, *E. scolopes* spend most of their time on the bottom (Moylinah, 1983), and we tend to assume that this is their habit; however, it would be most accurate to say that little is known about their natural behavior.

Camouflaging seems to be a general strategy for *E. scolopes* (Anderson and Mather, 1996; Shears, 1988), which routinely buries or coats itself with sand and uses chromatophores to change colors from a palette that is similar to the background (Figure 6.2). The squid can even be observed swimming with a sand coat, which can apparently discard with remarkable speed and control (Shears, 1988). Even without the sand coat, their coloration blends in well with the sandy reef bottoms, as anyone who has collected these animals can attest. The animals also sometimes emit ink blobs and then jet away, threatened. However, in our observations and those of Anderson and Mather (1996), the animals rarely jet very far from the site of an encounter.

Little is known about which predators *E. scolopes* may be hiding from. Hawaiian monk seals occasionally feed on *E. scolopes* (Goodman-Lowe, 1998), and we have observed lizard fish struggling with a catch of *E. scolopes*; however, we have a poor understanding of the threats it faces. Also lacking are observations of luminescence by these squid in their natural habitat. Every wild-caught *E. scolopes* tested has been found to emit bioluminescence; however, this was determined upon placing the animals in a sensitive luminometer and not by observations in the wild. There are reports that the animals "flash" their luminescence when disturbed (Moylinah, 1983; A. Wier and M.J. McFall-Ngai, personal communication; R. Young, personal communication), but this has not been documented in a natural setting.

6.2.4 Evidence of Mutualism in this Symbiosis

The selective advantage of the symbiosis for *V. fischeri* appears clear. *V. fischeri* is provided a privileged niche in the *E. scolopes* light organ, where it grows rapidly (Ruby and Asato, 1993). Not only are they provided nutrients to support this growth, but because the squid can prevent infection by other microorganisms, *V. fischeri* cells would appear to have the host immune system effectively protecting them from predatory grazing or competitive antibiosis by other microbes. Moreover, colonization of the *E. scolopes* light organ is not a dead end. Each morning these squid settle to the bottom, cover themselves with sand, and the light of dawn triggers the expulsion of most *V. fischeri* cells into the environment (Boetecher et al., 1996a). The *V. fischeri* left behind grow to repopulate the light organ by dusk. Presumably, because of this diurnal "venting" process, *V. fischeri* populations are relatively high in habitats occupied by *E. scolopes* (Lee and Ruby, 1994b). Further ecological studies (Lee and Ruby, 1994a) support the theory that in the Hawaiian reefs occupied by *E. scolopes*, the ability to colonize this host is a strong selective force on *V. fischeri*. So, while *V. fischeri* can be found in many marine environments, its populations appear to be enhanced by *E. scolopes*.

Presumably, the host also benefits from the association, and the *V. fischeri*—*E. scolopes* symbiosis is a mutualism. The *E. scolopes* light organ grows and maintains a monospecific culture of *V. fischeri* and has the means to control the release of its bioluminescence. Moreover, we (and others) have observed that stressed animals appear to clear their light organs of *V. fischeri* symbionts altogether, suggesting that the host has ultimate control and is not suffering an unavoidable infection. The symbiosis probably provides a benefit to *E. scolopes* by giving it a controllable source of bioluminescence for some behavior, and this is usually attributed to a camouflaging "counterillumination" behavior mentioned above and discussed at greater lengths below. Although an additional nutritional or other benefit of the symbionts cannot be ruled out, *E. scolopes* raised through a complete life cycle in the absence of *V. fischeri* did not appear to suffer due to lack of symbionts (Claes and Dunlap, 2000).

6.3 Counterillumination Model

Strategies to avoid predation are numerous and varied in the marine environment and can involve highly specialized adaptations. Familiar examples include animals with the ability to camouflage themselves through pigmentation or shape that mimics the surrounding substrate, such as rock or kelp beds, or the
ability to bury into sand or hide among rocks. However, in the open ocean, the ability to "hide" from predators is difficult, given the lack of substrate. In spite of this difficulty, many mesopelagic organisms including luminous fishes, crustaceans, and cephalopods have found a way to camouflage themselves by producing light in discrete regions of their body (Clarke, 1963). This use of light is called counterillumination, which is most often used to describe the ability to match downwelling light using ventrally directed luminescence (Dahlgren, 1916; Harper and Case, 1999; Latz, 1996; McFall-Ngai and Morin, 1991; Warner et al., 1979; Young, 1977). In this way, light production along the ventral surface of an organism can be used to hide the animal's silhouette if viewed from below in the water column (e.g., Figure 6.1).

Downwelling light in the mesopelagic zone is unidirectional and predictable in its wavelength and low intensity, but in shallow water light is more variable both in quantity and quality. Moreover, light in shallow water is multidirectional owing to reflection, shadows, and surface effects. This potentially renders counterillumination a more difficult strategy for shallow-water animals to use effectively. However, McFall-Ngai and Morin provided convincing evidence that counterillumination behavior does occur in shallow-water leegnathid fishes, although they suggest that "disruptive illumination" might be a better description of the mottled appearance that the ventral bioluminescence imparts on the animals (McFall-Ngai and Morin, 1991).

The concept of "disruptive illumination" is an important distinction from most discussions of counterillumination, and it may be especially relevant to the role of the E. scolopes light organ. However, for most part we will use "counterillumination" to encompass partial or complete illumination, disruptive illumination, or intermittent countershading, with the commonality to all of these being the ability of the animal to produce light that mimics its surroundings to whatever extent, in order to camouflage themselves. One exception is that of the cookie cutter shark discussed next, where camouflage is not the apparent role of counterillumination. In general though we will use "counterillumination" in reference to hiding behaviors, and we will sometimes use "camouflaging" in conjunction with "counterillumination" to reinforce this distinction.

Several factors may contribute to the effectiveness of counterillumination. It has been argued that animals should have many evenly distributed light sources, because those with fewer or less-distributed sources of bioluminescence would be less able to produce an even field of light, minimizing or negating any camouflaging effect of counterillumination (Johnsen et al., 2004). Consistent with this argument, counterillumination is usually attributed to an array of ventrally directed photophores. In Histioceutis squids, for example, many small light organs are scattered over the ventral surface of the body, head, and arms. However, the majority of shallow-water luminous animals, including E. scolopes, have a single light-emitting organ. It has also been pointed out that effective counterillumination should mimic both the quantity and the quality (e.g., wavelength) of downwelling light (Johnsen et al., 2004; McFall-Ngai and Morin, 1991). Otherwise, luminescence could have the opposite effect and become an attractant for predators. Finally, the habits and visual acuity of the predator being hidden from are important.

6.4 Weighing the Evidence for Counterillumination and Other Models

6.4.1 Does Light-Organ Anatomy Suggest Counterillumination?

The idea that the E. scolopes light-organ functions in counterillumination gathered momentum from close examination of its architecture (McFall-Ngai and Montgomery, 1990). The light organ is situated in the mantle cavity just ventral to the bulk of the ink sac, and it is oriented such that light is emitted ventrally. The light-organ crypts occupied by bioluminescent symbionts are situated between a reflective layer of reflectin protein (Crookes et al., 2004) and a muscle-derived lens (Montgomery and McFall-Ngai, 1992), which together appear to direct and control ventral light emission. In the adult animals, the ink sac apparently can be drawn around the light organ, perhaps in the manner of a shutter, and anatomical observations of several fixed specimens suggest that controlled movement of reflective tissue and ink sac diverticula could modulate the emission of light (McFall-Ngai and Montgomery, 1990).

The animals may also be able to control luminescence by modulating the oxygenation of the light organ (Boettcher et al., 1996b). This could effectively control luminescence because oxygen is one of the reactants of the V. fischeri luciferase reaction. Thus, the E. scolopes light organ has elements consistent with counterillumination, specifically a controllable ventrally emitted light.

In at least one respect, the architecture of the light organ seems imperfectly adapted for camouflaging by counterillumination. The E. scolopes light organ does not cover the entire underside of the animal the way multiple photophores of some mesopelagic squid do. This would arguably represent a poor or incomplete counterillumination apparatus. On the other hand, symbiotic luminescence would provide counterillumining cover for a large opaque structure in the squid, the ink sac integral to the light organ (this is illustrated with a juvenile in Figure 6.3), which might provide a selective advantage.

6.4.2 Does Host Behavior Fit the Counterillumination Hypothesis?

It is difficult to say whether E. scolopes behavior supports the counterillumination hypothesis simply because very little is known about this animal's behavior. As discussed in the final section, answering many questions about the behavior and ecology of E. scolopes would help put light-organ function in a natural context and inform our discussion of its potential role(s). At least it seems clear that E. scolopes is nocturnal and spends at least some time in the water column, which is consistent with counterillumination.

One mysterious behavior of E. scolopes deserves special mention. Multiple investigators have seen E. scolopes "flash" bioluminescence (Moynihan, 1983; A. Wier and M.J. McFall-Ngai personal communication; R. Young, personal communication). Typically, in situations when these animals were

FIGURE 6.3 Backlit juvenile E. scolopes. This animal has retracted its chromatophores to small points rendering its mantle largely translucent. A white arrow points to the light organ and ink sac. The other prominent dark (opaque) shaded structures are the eyes. The mantle length of this typical hatching juvenile is ~1-1.5 mm. (Photo credit of Dawn M. Adin.)
severely disturbed or roughly handled, they often released ink. This suggests that flashes might be an attempt to distract or confuse predators that get too close, perhaps as part of an escape response. Obviously, visible flashes are inconsistent with camouflage, and it may be that flashes reflect the real role of the light organ or that the light organ has two very distinct roles.

6.4.3 How Conclusive Are Attempts to Measure Counterillumination in E. scolopes
Jones and Nishiguchi (2004) have provided the most important and convincing data for evaluating counterillumination in the V. fischeri–E. scolopes symbiosis. They placed adult E. scolopes in small downwelling light. To differentiate the room lights from animal bioluminescence, the overhead lights were turned off and animal luminescence was measured immediately thereafter. Controls that included correlate with overhead light intensity, was not mistaken for bioluminescence. As predicted by the counterillumination hypothesis, bioluminescence released from animals was more intense as the intensity observed at the highest light intensities, when animals began to dim the luminescence they emitted. Although not predicted by the counterillumination hypothesis, it seems plausible that at uncommonly high light intensities the animals would not be able to match the downwelling light the experimental setup, because it is unclear how any nonbiological explanation for the data could yield this result at high light intensity.

Two results of the Jones and Nishiguchi study hint at either the imperfection of counterillumination or the technical difficulty of such experiments. First, although luminence emitted by the animals correlated with the intensity of downwelling light, the animal luminescence appeared dimmer than that of the light. In perfect counterillumination might have a selective advantage over no counterillumination turned off, but presumably before the squid could react and alter the luminescence. Therefore, an unexpected quick reaction by the squid could explain this aspect of the data. This brings up a second result, sometimes animals were remarkably slow in dimming their luminescence after the lights were turned out. In such animals, luminescence continued for trying to be stealthy, but then again the animals may never (or rarely) encounter such a rapid and absolute change in light intensity, in nature.

Overall, the work of Jones and Nishiguchi strongly supports the counterillumination hypothesis; however, skeptics can still point to an obvious gap in our observations. Specifically, to our knowledge At least in the laboratory, this might seem easy, by simply putting E. scolopes in a clear-bottom container put in this situation are prone to simply sitting on the bottom, with their arms curled under them and animals are up in the water column, and as discussed below future work aimed at viewing animals in this way could provide important information.

It is also worth noting that to our knowledge the wavelength of light emitted from E. scolopes has not been compared to the background light in its habitat(s). This is important, because for counterillumination and Nishiguchi measured) and in quality. Moyuhi (1983) reported that flashes seen from disturbed E. scolopes juveniles (Stahl, 2005). It seems unlikely that a green light would effectively match the emitted light and environmental light are needed to resolve the issue.

6.4.4 Counterillumination Is Not Necessarily a "Defensive" Camouflage
If counterillumination is used as camouflage by E. scolopes, this might reflect an offensive, rather than defensive, strategy. E. scolopes might use counterillumination to hide from potential prey. However, at least in aquaria we have not observed E. scolopes approach the prey from above. Rather they seem to attack horizontally with their tentacles to capture shrimps. It is also uncertain whether the prey that E. scolopes feeds on would be easier to catch if the squid possesses a ventral counterillumination mechanism; however, with so much unknown about E. scolopes behavior (e.g., its feeding habits in deeper water), it is impossible to rule out counterillumination as an offensive strategy.

6.4.5 Alternatives (or Amendments) to the Counterillumination Camouflage Hypothesis
If E. scolopes does not use bioluminescence in a camouflage counterillumination behavior, or if this is not the primary selective advantage of symbiotic bioluminescence, then what might be the main role of the light organ? Proposing alternate answers to this question may prove to be a useful exercise, both by preventing tunnel-visioned interpretation of existing data and by pointing future research in new directions. Below, we discuss a few possible uses for the light organ other than as a silhouette-obscuring camouflage.

E. scolopes might use counterillumination as a prey attractant. Widder presented evidence that the cookie cutter shark, Isistius brasiliensis, uses counterillumination not to obscure its entire ventral face, but rather to obscure all but an image shaped like a smaller fish (Widder, 1998). He convincingly argues that this might be used as a lure to attract predators of small fish, including the swordfish, tunas, and porpoises that I. brasiliensis attacks. It is not clear to us whether a distinct luring image would be produced by the shape of the E. scoloea light organ, but we cannot rule this out. Nonetheless, this model predicts the same sort of correlation between ambient light and emitted luminescence that was observed by Jones and Nishiguchi (2004).

Another possibility is that luminescence per se, and not the counterillumination of a particular alluring shape, might be used to attract prey. This would seem to be a risky proposition for E. scoloea hunting in the water column. E. scolopes may have defensive strategies other than avoiding detection, but these are not immediately apparent (e.g., it lacks spines or known predator-deterring toxins), making stealth an important asset for E. scolopes. On the other hand, it seems plausible that the shrimp or polychaetes that E. scolopes feeds on might display photosis, and with so little knowledge about the behavior and ecology of E. scolopes we cannot rule out the possibility that E. scolopes exploits such a behavior of its prey.

Perhaps, the most plausible alternative model for the role of the light organ center is its potential to flash brightly. As mentioned above, such flashes have been observed when animals were collected or held in captivity (Moyuhi, 1983; A. Wier and M.J. McFall-Ngai personal communication; R. Young personal communication). The ability to flash visible luminescence could suggest a role in intraspecies communication; however, the fact that it has been observed when the animals are disturbed (e.g., physically grabbed) suggests a role as a startling tactic (Herring, 1977). Another sepiolid squid in Hawaii, Heteroteuthis hawaiiensis, found in open ocean waters at depths of up to 600 m, can use its light organ to create a glowing cloud of bacteria mixed with mucus (Dilly and Herring, 1978; Young, 1995; Young and Roper, 1976). If E. scolopes can emit a brief flash of light with or without expelling bacterial symbionts, this might be used to startle or confuse the predators. Like the expulsion of ink blooms by E. scolopes (Anderson and Mather, 1996), this behavior could be used once camouflage has failed.
6.5 Future Directions

Many big-picture questions regarding the V. fischeri-E. scolopes symbiosis remain unanswered. Notably, as discussed in this chapter, the functional significance of the light organ for the host is uncertain, although evidence points to a role in camouflage by counterillumination. Future behavioral and ecological research on E. scolopes would be welcome and could shed light on one of the most central issues of the symbiosis.

6.5.1 The Life and Times of E. scolopes

Many fundamental questions about the ecology and behavior of E. scolopes remain opaque. How much of their life is spent in shallow water? Do they often venture deeper? Can reports of E. scolopes in the mesopelagic zone be substantiated? What are they feeding on in such habitats? How much time do they spend in the water column or on the bottom? What is the quality of the light in their environment? Does this match the light they emit? Do they hunt exclusively in the water column? What are their main predators? What are the hunting techniques and visual acuity of these predators? Answering these questions may not be easy. Observing the natural habits of these nocturnal well-camouflaged animals has obvious barriers. For example, it is difficult for divers to follow the nighttime behavior of these animals without disrupting their routine. Remote tagging and tracking devices are probably not feasible, although the advent of nanotechnologies and miniaturization could facilitate such approaches.

One technology that could provide a powerful tool for studying E. scolopes in the environment is the use of autonomous underwater vehicles (AUVs). Fidopiastis and Clark (personal communication) have proposed that a robot with the capacity to swim and crawl might be trained to follow E. scolopes individuals, monitoring and recording their behavior. This promises to be much less disturbing to the animals than observations by humans, and could yield unparalleled insights into the behavior of E. scolopes over a range of environments. Remotely operated vehicles have been used to monitor marine animals such as jellyfish, and AUV technology has been applied to tracking fish (Zhou et al., 2007). Moreover, an AUV was used to measure patterns of marine bioluminescence (Blackwell et al., 2002). If an AUV can be trained to track E. scolopes individuals and potentially to monitor their bioluminescence, this has great potential for answering many unresolved questions about their behavior.

6.5.2 What about the Flashes?

The fact that E. scolopes occasionally emits bright flashes may belie the true function of the light organ; however, observations of this behavior are rare, and to our knowledge it has only been observed in disturbed captive animals. A squid-tracking AUV might be able to document flashes in the wild, and to determine the behavior of the animal before and after the flash. Is it used when the animals are threatened by a predator? Is it used in conjunction with the release of ink? Do the animals change their swimming direction in an evasive manner following a flash? Do the flashes have a temporary blinding or distracting effect on a would-be predator? Or are the flashes used as intraspecies signals perhaps eliciting some altogether unknown behavior? Any observation of light-organ flashing in the wild would be tremendously important.

Observations of flashing in the laboratory may also provide insight into the function of the light organ for E. scolopes. Because the flashes seem to be rare, it may be useful to set up a digital image recording system that monitors the animals constantly, detects flashes, and then saves the data before and after a flash event. A clear-bottom tank could be used with a camera (or array of cameras) so that observations could be made of the animal's ventral surface, although flashes should be detectable regardless of how the animals direct them. To test whether flashes are used in defensive responses or communication, it will probably be necessary to place multiple squid or predators in the same tank, which could require a relatively large setup. Although such an experiment is not without obstacles, it could yield important data and might be easier than observations, in nature.

6.5.3 More Tests of Counterillumination

One conspicuously missing piece of evidence for counterillumination by E. scolopes is the lack of an image documenting this effect. A squid-tracking AUV might capture such an image in the environment, which would provide strong support for this theory. However, carefully crafted laboratory studies might provide similar and compelling evidence. As noted above, animals observed in clear-bottomed containers tend to simply sit on the bottom. A clear-bottomed raceway with water flowing through it (e.g., a clear pipe) might force the animals off the bottom, allowing visualization from underneath the swimming E. scolopes. Alternatively, a useful setup might include a camera underneath a clear-bottomed container set to capture several hours worth of images, or to be triggered by the motion of the animals. In the absence of a human investigator, the animals may eventually leave the motion (e.g., to capture prey or mate) and could then be visualized. Sensitivity digital cameras capable of capturing low-light images could document the squid's silhouette and whether or not it is obscured by counterillumination.

A more advanced apparatus for measuring counterillumination would also be useful, if counterillumination can be elicited by animals going about relatively normal behaviors in the laboratory. One such methodology that has been employed productively is to use an overhead light that provides illumination that is seen as constant by the animals, by that is actually "chopped" into short bursts of light, such that a photomultiplier detector set "out of phase" with the incident light will measure only the counterillumination (Latz and Case, 1992). In this way, quantitative measurements of counterillumination can be made on animals that are not forced into cramped confines as they were in the apparatus used by Jones and Nishiguchi (2004), and it may provide a telling picture of natural counterillumination behavior. For example, the apparent under-illumination by animals observed by Jones and Nishiguchi may simply reflect a perturbation of the animals associated with putting them in the experimental apparatus.

The ultimate proof that camouflaging provides an antipredatory selective advantage would be an ecological prey study in which E. scolopes with luminescent symbiotic bacteria and animals raised without luminescent symbionts or no symbionts are presented to an appropriate predator and in an environment that mimics their natural one. Such experiments are obviously difficult to set up; however, such an approach was used to demonstrate an antipredatory effect of counterillumination in the fish Porichthys notatus (Harper and Case, 1999). A potential predator in these experiments could be the lizardfish of the Synodontidae family, which we suspect may be an important natural predator of E. scolopes, although as noted above more research needs to be done in this regard. Such experiments would need to be done with pools of animals isolated from one another, so that cross contamination of luminescent bacteria to nonluminescent animals cannot occur. The animals would also have to be monitored closely for potential flashing behavior, to distinguish their possible antipredatory value from that of counterillumination. If done convincingly, such experiments could settle the issue of the selective advantage of symbiotic bioluminescence in the V. fischeri-E. scolopes mutualism.

ACKNOWLEDGMENTS

The authors are especially indebted to Margaret McFall-Ngai and Richard Young for reading drafts of this chapter and providing helpful suggestions. We also thank Dawn Adin and Jeffrey Bose for the photographs of E. scolopes, Bryan W. Jones, Michele Nishiguchi, and Pat Fidopiastis for helpful conversations, and numerous colleagues who have provided healthy skepticism of counterillumination in the V. fischeri-E. scolopes symbiosis. EVS was supported in this work by a grant from the National Science Foundation (CAREER MCB-0347317).
REFERENCES


Defensive Mutualism in Microbial Symbiosis


# Entomopathogenic Nematode and Bacteria Mutualism

## CONTENTS

7.1 Introduction ................................................................................................................. 99
7.2 Life Cycle .................................................................................................................... 101
7.3 Offensive Mutualism: Tag-Team Killing of an Insect ................................................... 104
7.4 Resource Harvest Mutualism: What Is for Dinner?...................................................... 105
7.5 Defensive Mutualism: Protection of the Insect Cadaver ................................................. 107
7.6 Applications .................................................................................................................. 108
7.7 Conclusion .................................................................................................................... 109
Acknowledgment ............................................................................................................. 110
References ....................................................................................................................... 110

---

**7.1 Introduction**

Entomopathogenic nematodes of the families Heterorhabditidae and Steinernematidae are lethal insect endoparasites characterized by their association with bacteria in the genera *Photorhabdus* and *Xenorhabdus*, respectively. This association is obligate mutualism in nature, with each partner requiring the other to complete its life cycle. Nematode growth and reproduction depend upon conditions established in the insect host by the bacterium. On the one hand, the bacteria contribute anti-immune proteins to assist the nematode in overcoming host defenses and antimicrobials that suppress competitors (Forst and Clarke, 2002). On the other, the bacteria lack invasive powers and are dependent upon the nematode to locate and penetrate suitable hosts. The nematode–bacterium complex is an important natural enemy of soil insects and plays a significant role in the regulation of soil food webs. Interest in their potential for use as biological insecticides has fueled decades of research.

Entomopathogenic nematodes show a broad, worldwide geographical range and are common in the soil environment. Seventy-five (61 steinernematid and 14 heterorhabditid) nematode species have been described. The two families share the same general life history but belong to different clades within the order Rhabditida. Heterorhabditids were derived from free-living bacteriovorous ancestors, whereas steinernematid origins are ambiguous. Twenty-three bacterial species (20 *Xenorhabdus*, 3 *Photorhabdus*) have been described, but most bacterial associates are yet to be examined. The bacteria are Gram-negative, nonfermentative rods, belonging to the family Enterobacteriaceae (Thomas and Poinar, 1979). *Photorhabdus* and *Xenorhabdus* are a unique group phenotypically and genotypically distinct from other genera within the family. They are insect pathogens although one species, *Photorhabdus asymbiotica*, has been occasionally reported as an opportunistic human pathogen (Pecl et al., 1999). Although similar, *Photorhabdus* are bioluminescent and secrete antiaquaginone pigments and catalase, which are traits absent in *Xenorhabdus*. Similarities in the mutualism and parasitism of the bacteria–nematode complexes resulted from convergent evolution (Poinar, 1993). That is, each complex independently evolved its pathogenic and symbiotic relationships.

Other bacteria may show conditional associations with an entomopathogenic nematode, but the natural symbiont is the most efficient partner for pathogenicity, reproduction, and development of the nematode.