HOT TOPICS IN MARINE BIOLOGY 13.1

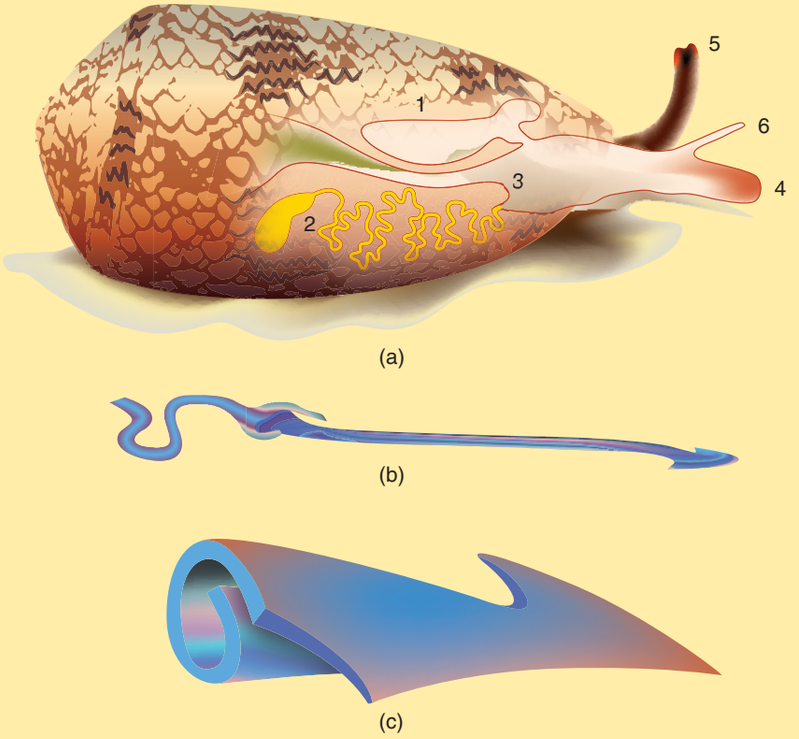
Diary of a Stinging Snail

Cone snails manage to excite shell collectors, scare divers, and inspire marine biologists, sometimes pretty much at once. The Academy of Natural Sciences of Philadelphia once displayed the shell of a deadly cone snail alongside a picture of its prey—a marine biologist it had killed in about a half hour! The geography cone, *Conus geographus*, has killed a large number of other humans. Cone snails belong to the genus *Conus*, whose species are united by a cone-shaped shell, a highly specialized radular tooth evolved for injecting venom, and venom that is remarkably toxic to many animals. Most species are found spread throughout the tropics, mainly in coral reef environments. In the past few years, a remarkable picture of the history and ecological diversification of cone snails has developed, along with an understanding of the molecular mechanisms that combine with morphology and behavior to establish cone snails among the world's most effective killing machines.

HOW THEY DO IT

No, they do not gallop like cheetahs; nor do they detect your blood a mile away and, like sharks, locate you and gobble you up. It's a bit more subtle than that, but no less deadly. Venom, consisting of a cocktail of toxins, is produced by epithelial cells in a venom duct (**Box Figure 13.1**) and injected into the proboscis by a squeeze of a specialized bulb. A highly modified radular tooth punctures the prey and delivers the venom. The harpoon-shaped radular teeth are useful in distinguishing among species. The shape of the proboscis varies greatly among species, and this variation is related to the method of capture and the type of prey. In some fish-hunting species, the snail has a part of the proboscis modified into a lure, which attracts fish. The attraction is fatal because the disposable tooth harpoons the fish, paralyzes it, and reels in the prey, which is swallowed whole.

The toxin is usually a mixture of highly specific peptides, which are short chains of amino acids that usually attack a group of cellular ion channels. The first venom to be studied was found to specifically attack the sodium channel, which paralyzed muscles but had no effect on nerves. After studies of a number of species, it became clear that the venom of any cone snail species was a group of peptides, which appeared in the venom duct as precursors that were cleaved by enzymes in order to obtain final toxicity as they were injected into the prey. The peptides are small, each only 10–30 amino acids long, and degrade rapidly after being produced in the venom duct. They are so small that there is not much three-dimensional structure, which is usually an important characteristic determining the function of most proteins. Instead, a large number of cysteine amino acid residues create disulfide bonds that result in a scaffold-like structure



BOX FIG. 13.1 (a) The stinging apparatus of a cone snail: (1) harpoon sac; (2) venom gland; (3) pharynx; (4) proboscis; (5) siphon; (6) eyestalk. (b) Harpoon-shaped specialized radular tooth. (c) Close-up of tip. (Courtesy of Dietrich Mebs)

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that can have highly specific toxic binding activity. The combination of different venoms overwhelms the prey and immobilizes it almost immediately after the harpoon-like tooth is injected.

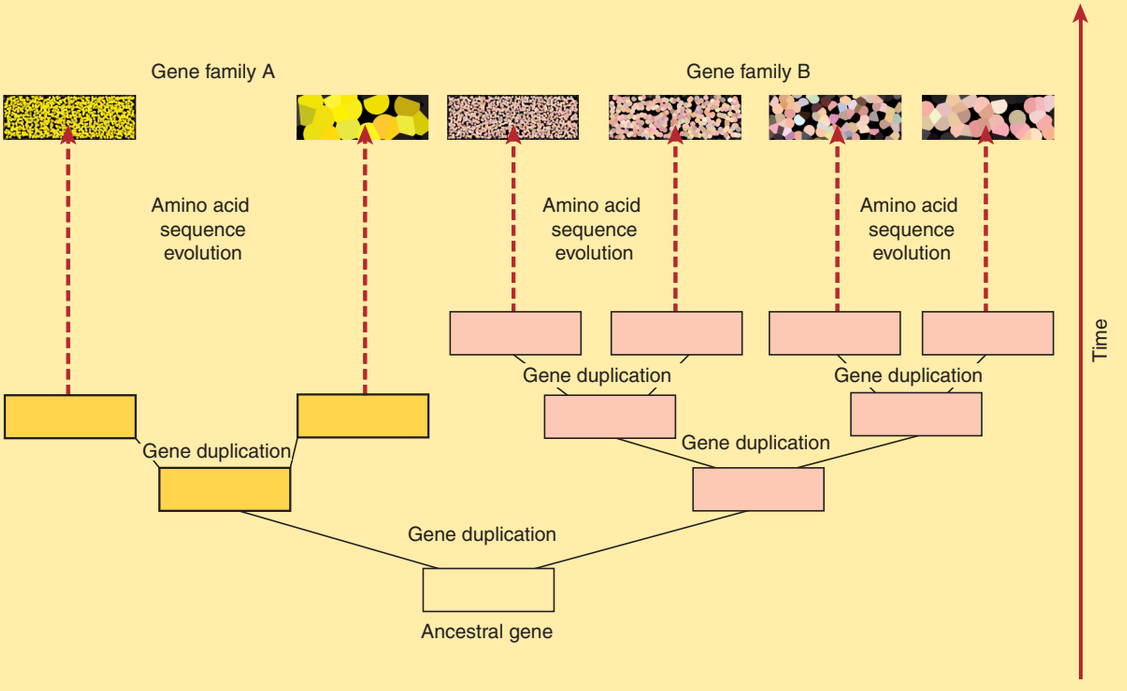
THE AMAZING MOLECULAR STORY OF A TOXIC BEAST

The peptides are produced with the aid of the genetic template of messenger RNA deriving from gene expression, which is followed by translation: the process where proteins are encoded with the aid of the ribosomes. But the final toxic peptide structure is determined by modification of the peptide after translation occurs. Such alteration involves addition of various components that determines final protein structure and is called posttranslational modification. They are further modified to become toxic within the venom duct of the snail.

What is truly spectacular is the diversity of venoms: there are at least 50,000 known conotoxins, each specified by a gene with a different DNA sequence. Any individual *Conus* species may have hundreds of such genes. The evolutionary history of these genes is also fascinating. One can see a hierarchy of evolutionary processes (**Box Figure 13.2**). At the largest and oldest evolutionary scale, the tens of thousands of conotoxins derived from mutations, which are variations on a functional theme spread over a few **gene families**. Each family has its own evolutionary history and produces toxins with high ion channel specificity. Within each family, new genes arise by **gene duplication**, which is a common error that occurs usually during meiosis and propagates a copy of the gene. In most cases, duplications do not function. But in conotoxins, some duplications are selected and evolve rapidly and diverge to perform a new toxic function, corresponding to a peptide with a new amino acid sequence.

One might expect intense natural selection for evolution of a more effective toxin. Duda and Palumbi (1999) were able to sequence DNA that codes for closely related conotoxins. The DNA sequences could be translated by means of the genetic code into amino acid sequences of the venomous peptides. They concluded that the rate of amino acid evolution in these peptides was far greater than for most other proteins. Evolution of changes in peptide length (number of amino acids) also was very rapid. Another study examined the rate of gene duplication of four closely related species of *Conus* and demonstrated that the rate of gene duplication was also very rapid—about twice as fast as in other gene family histories that have been studied (Chang and Duda, 2012). These phenomena are explained best by rapid adaptive evolution. As might be expected, even now *Conus* populations are experiencing strong positive natural selection for new toxins. Other evidence demonstrates that the part of the toxin gene that specifies the toxin evolves at the most rapid rate, whereas other parts of the gene evolve much more slowly and presumably have more general functions that are conserved in evolution.

One might ask why such spectacular complexity has evolved in this genus. One obvious guiding factor is the slow movement of the



BOX FIG. 13.2 The evolution of gene families. Gene families arise by gene duplications, and descendant genes can be traced from ancestral genes by means of similarities in DNA sequences. In this diagram, two generations of gene duplications result in two families, each with a different set of genes and related functions (sequence evolution also occurs in these stages, which enables difference of function, such as binding to different ion channels). The upper part of the diagram shows evolution within a gene of DNA sequences with no gene duplication, which allows a gene to improve function such as binding to an ion channel.

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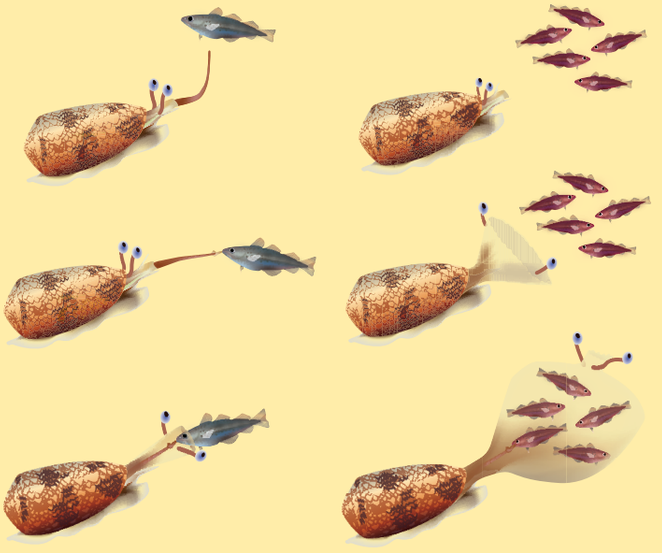
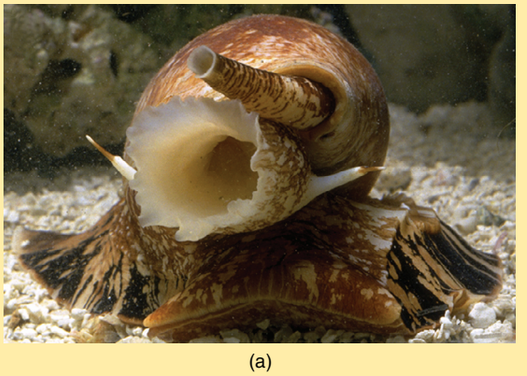


(a) (b)

BOX FIG. 13.3 Two different styles of prey capture. (a) *Conus striatus* has a thin proboscis, which is thrust at the prey; (b) *Conus geographus* has a broad, open proboscis that engulfs several fish at a time. Once within the cavity, the fish are stung. (After Olivera, 2002)



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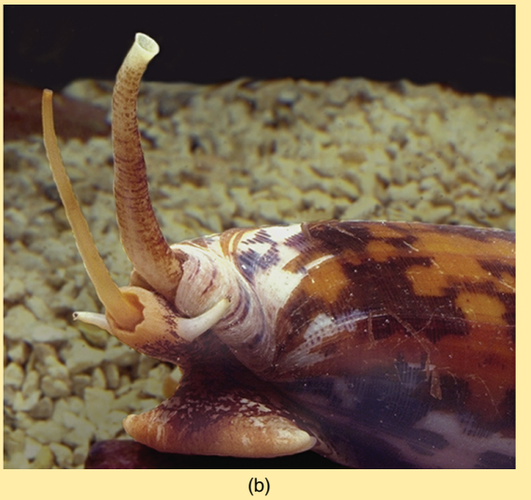

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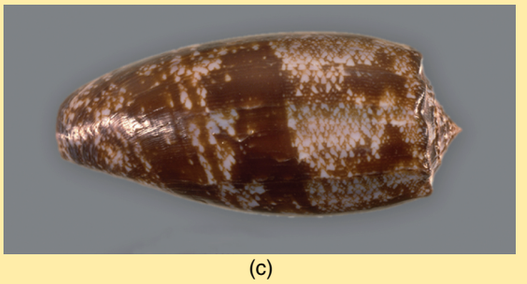

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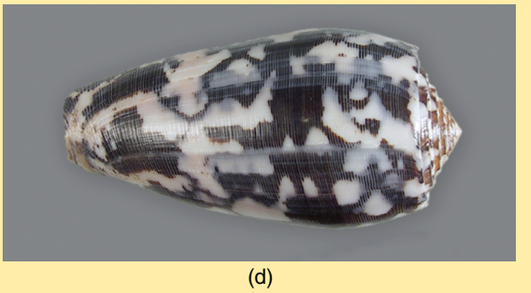

(d)

BOX FIG. 13.4 (a) *Conus geographus*; (b) *Conus striatus*; (c) shell of *C. geographus*; (d) shell of *C. striatus*. (Photographs courtesy of K. S. Matz)

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predator. If you are going to kill a mobile prey, even a worm, you must be able to rapidly subdue the prey. The specialized radular tooth and proboscis allowed the evolution of the rapid injection mechanism, and this was matched by the molecular toxin mechanism. It is not clear which adaptation came first, but the spectacular diversification of toxins would lead to more and better means of attracting and subduing prey.

ECOLOGICAL DIVERSIFICATION

The cocktail of venoms of a given species is specialized with regard to prey. Cone snails that hunt fish have far more paralyzing venoms than species that hunt for worms. Venoms also have a variety of effects. Many have a powerful and rapid ability to simply stop neuromuscular function. In one case, a venom has an analgesic effect, slowing the prey down. Different venoms appear to be specialized to disable specific groups of organisms.

Even the morphology of the proboscis varies with regard to the mode of capture (**Box Figures 13.3 and 13.4**). *Conus striatus* has a long, thin proboscis that is thrust toward the fish prey. The radular tooth at the end harpoons the fish, venom is injected, and the proboscis is then retracted and the paralyzed prey is eaten. *Conus geographus*, probably the most toxic species, has a broad, funnel-shaped proboscis, which engulfs several fish at a time and then a radular tooth within the funnel injects the venom. The work of biochemist Baldomero M. Olivera (2002), a great pioneer in this field, demonstrated that in *C. striatus*, the cocktail of venoms causes an instantaneous massive tetanic paralysis, completely immobilizing any neuromuscular activity in the vicinity of the puncture wound. But in the case of *Conus geographus*, the venom has a much more subtle effect, suppressing neuromuscular function more slowly. In this species, the funnel-shaped proboscis has already engulfed the prey before injection, so the venom need not act so rapidly. In other words, the venom function matches the morphology of the proboscis and the behavioral strategy for seizing prey. Overall, it is likely that the tremendous diversity of toxic peptides is associated with morphological and behavioral diversity. ■

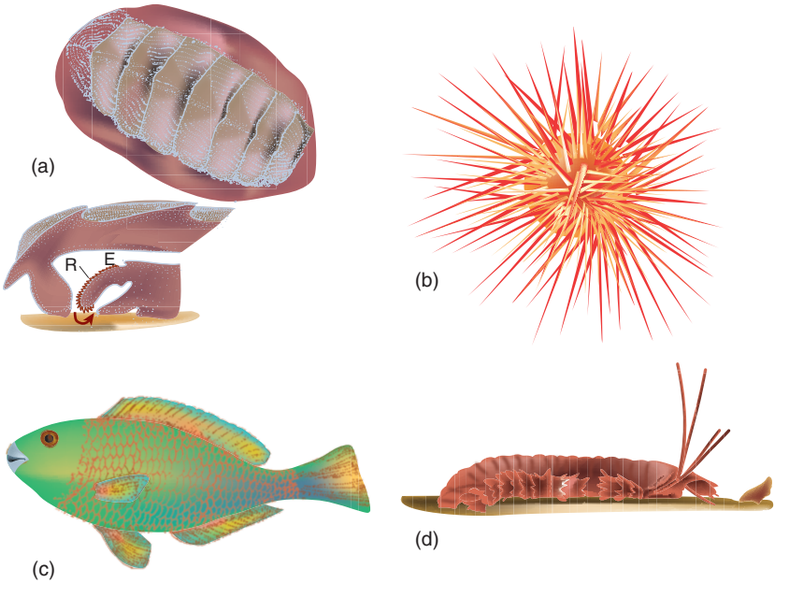

FIG. 13.22 Some benthic herbivores. (a) The chiton *Tonicella*, a scraper of microalgae; inset shows anterior sagittal cross section, indicating the action of the radular tooth belt in scraping algae from the substratum (R, radula; E, esophagus). (b) The sea urchin *Arbacia*, which uses a toothed Aristotle's lantern to scrape microalgae or to tear apart seaweeds. (c) A parrot fish, which uses specialized fused teeth to scrape algae from coral surfaces. (d) The nereid polychaete *Nereis vexillosa*, which tears apart sea lettuce with buccal hooks. (Copied from an original by K. Fauchald)

microalgae include a variety of groups, such as diatoms, cyanobacteria, and microscopic stages of seaweeds. These organisms may form a thin layer on a rock surface or on the surface of sediment. **Microphages** have a range of morphological features that allow them to graze efficiently on this layer. Chitons, limpets, and other grazing mollusks employ a radula, a belt of teeth that scrape along the surface. The movement of the subradular membrane over a cartilaginous portion of the buccal mass erects the teeth and scrapes them over the surface. The radula and buccal mass are retracted, and food trapped on the teeth is delivered to the buccal cavity. This feature can be used on rocks, and limpet