Chapter 15
The Neogastropoda: Evolutionary Innovations of Predatory Marine Snails with Remarkable Pharmacological Potential

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Abstract The Neogastropoda include many familiar molluscs, such as cone snails (Conidae), purple dye snails (Muricidae), mud snails (Nassariidae), olive snails (Olividae), oyster drills (Muricidae), tulip shells (Fasciolariidae), and whelks (Buccinidae). Due to their amazing predatory specializations, neogastropods are often dominant members of the benthic community at the top of the food chain. In a dazzling display that ranges from boring holes to darting harpoons, neogastropods have developed several prey hunting innovations with specialized compounds pharmaceutical companies could only dream about. It has been hypothesized that evolutionary innovations related to feeding were the main drivers of the rapid neogastropod radiation in the late Cretaceous. The anatomical, behavioral, and biochemical specializations of neogastropod families that are promising targets in drug discovery and development are addressed within an evolutionary framework in this chapter.

15.1 Introduction

15.1.1 The Neogastropoda

Neogastropoda is an order of gastropod molluscs that are well characterized morphologically and are traditionally viewed as monophyletic (Ponder 1973; Taylor and Morris 1988; Ponder and Lindberg 1996, 1997; Kantor 1996; Strong 2003).

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This characterization of the Neogastropoda persists even after contrasting interpretations have been proposed (see e.g., Colgan et al. 2007; Kantor and Fedosov 2009). Strong (2003) has recently provided the most updated report of potential neogastropod synapomorphies. Anatomical characteristics of neogastropods include a very peculiar anterior foregut with a proboscis (pleurembolic or intraembolic), a valve of Leiblein, a gland of Leiblein (or a venom gland in Toxoglossa), paired primary and accessory salivary glands, an anal gland, and several radular peculiarities (Ponder 1973; Kantor 2002; Strong 2003). Figure 15.1 illustrates a generalized scheme of neogastropod anatomy.

The order Neogastropoda includes up to 25 families (Bouchet and Rocroi 2005) traditionally split into three superfamilies, Cancellarioidea, Conoidea, and Muricoidea, on the basis of anatomical features of the anterior foregut, including the radula. Cancellarioidea, also called Nematoglossa, comprised of the single family Cancellariidae, is perceived to be the basal offshoot of neogastropods (Kantor 1996; Strong 2003; Oliverio and Modica 2009; Modica et al. 2009). They are characterized by a nematoglossan radula with a complex mechanism of interlocking of the distal cusps (viewed as an adaptation to suctorial feeding: Petit and Harasewych 1986) and a mid-oesophageal gland that is generally not separated from the oesophagous (Fig. 15.2a). Conoidea, also referred to as Toxoglossa, include Conidae, Terebridae, and the "turrid" which are estimated to have more than 10,000 extant species, and whose taxonomy is under revision (Puillandre et al. 2008). In Conoidea, the radula is modified in various degrees until forming a harpoon (toxoglossan radula), and the dorsal mid-oesophageal gland is separated from the oesophagous and develops into a venom apparatus, with a muscular bulb and a secretory tubule producing neurotoxins (Fig. 15.2b). Muricoidea (also termed Rachiglossa) include the vast majority of neogastropod families, whose monophyly is currently debated (Kantor 1996, 2002; Oliverio and Modica 2009). The muricoidean radula is rachiglossate (Fig. 15.2c) and their anatomy is similar to the generalized model proposed in Fig. 15.1, but there are many modifications at different taxonomic levels. Variations include the presence/absence of radula, accessory salivary glands, valve and gland of Leiblein, anal gland and a number of other foregut, renal, and reproductive features.

According to the fossil record, the adaptive radiation of neogastropods has been particularly rapid (Taylor et al. 1980) and may be attributed to the evolution of a predatory lifestyle and diversification in a number of different trophic strategies. Such attributes allowed neogastropods to fully diversify their niches and to efficiently exploit their alimentary resources. In this scenario, the evolutionary role played by chemical innovations in feeding is unquestionable.

The Cancellarioidea, Conoidea, and Muricoidea possess a bountiful reservoir of bioactive compounds routinely used to sedate or capture prey. These compounds are the building blocks for future drug discovery targets. Outlined in this chapter are the anatomical features, specialty feeding strategies, and potential bioactive compounds found in the families of the Neogastropoda. Specific attention is given to the discovery and characterization of bioactive compounds from the Conoidea. Based on the successful characterization and implementation of cone snail toxins in

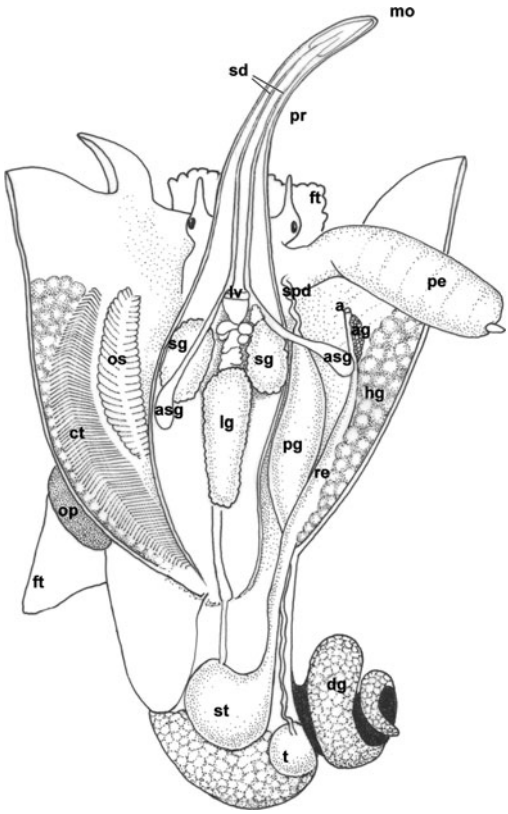

Fig. 15.1 Generalized scheme of neogastropod anatomy (male). Mantle longitudinally dissected, body wall not shown. Abbreviations are as follows: *a* anus; *ag* anal gland; *asg* accessory salivary gland; *ct* ctenidium; *dg* digestive gland; *ft* foot; *hg* hypobranchial gland; *lg* gland of Leiblein; *lv* valve of Leiblein; *mo* mouth; *op* operculum; *os* osphradium; *pe* penis; *pg* prostate gland; *pr* proboscis; *sd* salivary duct; *sg* salivary gland; *st* stomach; *t* testis. Modified after Ponder (1998a)

pharmacological approaches (Favreau and Stöcklin 2009; Twede 2009; Olivera and Teichert 2007; Fox and Serrano 2007), several groups within the Neogastropoda are highlighted as potential biodiversity targets for drug discovery.

15.1.2 Discovery and Characterization of Cone Snail Toxins

The gold standard for investigating toxins from marine snails is the discovery and characterization of neurotoxins from cone snails (*Conus*) (Fig. 15.2b). This extremely diversified group of marine snails comprises active predators that use biochemical substances to subdue their prey. Characterization of cone snail toxins begun almost a half century ago (Kohn 1956; Kohn et al. 1960; Endean et al. 1974), starting from empirical observations of envenomation episodes, and has blossomed into a successful research field (review; Norton and Olivera 2006). The characterization of conotoxins provides scientists with new, powerful tools to manipulate the function of ion channels and receptors governing the physiology of the nervous

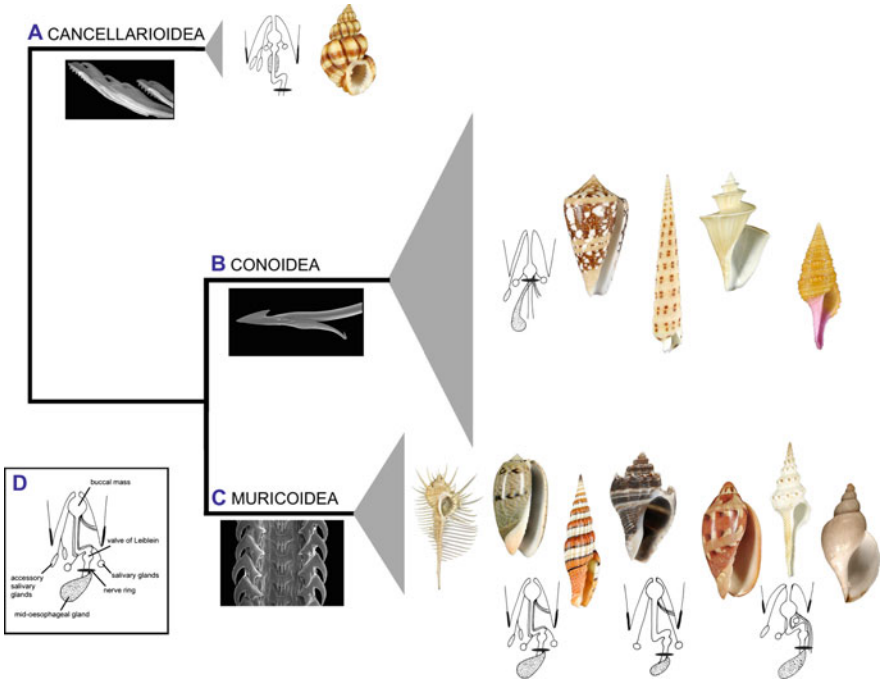

Fig. 15.2 The Neogastropoda radiation. Three major families of the Neogastropoda are shown: (**a**) Cancellarioidea, (**b**) Conoidea, and (**c**) Muricoidea. The *grey triangles* shown are proportional to the number of species included in each lineage. Shown for each superfamily are radula, scheme of the foregut, and some shell representatives. Shells shown, from left to right, by genus: (**a**) *Scalptia*. (**b**) *Conus*, *Terebra*, *Thatcheria*, *Gemmula*. (**c**) *Murex*, *Oliva*, *Vexillum*, *Melongena*, *Cymbiola*, *Fusinus*, *Volutopsius*. (**d**) Schematic arrangement of the foregut (modified after Kantor 1996). Shell images courtesy of Guido and Philippe Poppe. Radula pictures courtesy of Yuri Kantor (b) and Alisa Kosyan (c).

system. The pharmacological usage of ion channels and receptors as drug development targets for the treatment of neurological and cardiovascular diseases is rapidly gaining momentum. The discovery of Prialt (Ziconotide) (Miljanich 2004), the synthetic form of the *Conus magus* peptide ω-conotoxin MVIIA, an N-type calcium channel blocker, significantly highlight the potential of toxins from marine snails. Prialt was approved by the Food and Drug Administration of the United States in December 2004 for analgesic use in HIV and cancer patients.

Although Prialt is a significant breakthrough, *Conus* represents only a very small fraction of the diversity of Neogastropoda. *Conus* is one of the 20–30 recognized neogastropod families and includes ca 4–500 species out of 10–15,000 estimated in the Conoidea (Bouchet and Rocroi 2005). The pharmacological potential of neogastropods as a source for bioactive compounds is largely unrealized. Similar to cone snails, several other neogastropods have evolved specialized compounds as a result of their feeding ecology that may have potential in pharmacological applications.

15.2 Feeding Strategies in the Neogastropoda

From what is known about the diets of neogastropod families, the vast majority of neogastropods are carnivorous, with a degree of predatory activity that varies from actively seeking prey to grazing on sessile invertebrates, to scavenging. Some neogastropod families, such as Buccinidae and Muricidae, include many generalist species, which can feed on a variety of living and dead organisms. Most Muricidae feed on living bivalves, gastropods, polychaetes, bryozoans, sipunculids, barnacles, and other small crustaceans, but there are a few that also feed on carrions. A species of *Drupa* has been observed feeding also on holothurians (Wu 1965), while *Drupella* (Ergalataxinae) and all Coralliophilinae feed on corals (Taylor 1976; Ward 1965; Haynes 1990) (Fig. 15.4a). Some neogastropod families appear to be highly specialized, such as the Mitridae, which feed exclusively on sipunculids (Taylor et al 1980) and possess peculiar anatomical adaptations to this kind of prey (Harasewych 2009). An interesting feeding strategy is also displayed by the Volutidae, which has been reported for feeding on bivalves, gastropods, and in some deep-water species, on echinoderms (Darragh and Ponder 1998). Members of the Volutidae use their large foot to engulf the prey in a semiclosed environment, in which anesthetic substances are apparently released (Bigatti et al. 2009). Described in the following paragraphs are neogastropod feeding strategies that involve bioactive substances that may have pharmacological utility.

15.2.1 Harpooning

Cone snails, terebrids, and turrids make up the superfamily Conoidea (or Toxoglossa, "poisoned tongued"). Toxoglossans are a megadiverse group of hunting snails where the rapid evolution of venom peptide genes has led to an amazing molecular diversity. They feed on molluscs, polychaetes, acorn worms, and fish (Kohn 1959, 1968; Kohn and Nybakken 1975; Leviten 1980). The key evolutionary innovations enabling conoideans to hunt preys are a conspicuous venom apparatus made up of highly modified radular teeth (harpoon), a venom duct (a glandular duct connected to the oesophagous), and a muscular venom bulb (Fig. 15.2b). The radular tooth, held at the proboscis tip, is inserted into the prey and dispensed similar to a hypodermic needle (Olivera 2002). The mechanism of envenomation involves the contraction of the muscular venom bulb, which forces the secretion of the venom duct through the proboscis, until reaching the tooth. A single cone snail specimen may produce between 50 and 200 different peptides, which are known to target different ion channels (Terlau and Olivera 2004).

15.2.2 Shell Drilling

Shell drilling is the most common feeding technique in muricids, and it is achieved by the concerted action of the radula and a specialized glandular pad (the accessory boring organ) placed on the foot sole (Carriker 1961) (Fig. 15.3a). The drilling process may last up to 1 week (Palmer 1990; Dietl and Herbert 2005). Drilling is not restricted to muricids and has been observed in other rachiglossans, such as the marginellid genus *Austroginella* (Ponder and Taylor 1992), the buccinid *Cominella* (Peterson and Black 1995), and the nassariid *Nassarius festivus* (Morton and Chan 1997). Other feeding strategies developed by the muricids include the opening of the prey shell with the foot (Wells 1958), the cracking of the shells close to the apertural margin followed by proboscis insertion (Radwin and D'Attilio 1976) and the use of shell projections on outer lip (labial spines) to force the opening of the valves (Marko and Vermeij 1999).

15.2.3 Shell Wedging and Proboscis Insertion

As noted above, drilling has been reported for a few species of Buccinidae, but the majority of buccinids use the strengthened margin of their shells to wedge open bivalve shells (Nielsen 1975), in order to insert their proboscis (Fig. 15.3b). Buccinidae eat polychaetes, small crustaceans, and some species have been observed feeding on peculiar preys, e.g., *Neptunea antiqua* on priapulids, Taylor 1978). Buccinds can also insert their proboscis into the aperture of gastropod shells. Similar strategies of proboscis insertion with mild radular rasping or use of shell margins have been reported in families related to buccinids, such as: the Nassariidae, which feed on polychaetes, barnacles and carrion; the Fasciolariidae, which feed on bivalves, gastropods, sedentary polychaetes, and carrions; the Melongenidae, which feed on gastropods and bivalves; and the Columbellidae, which feed on ascidians, hydroids, small crustaceans, polychaetes, and algae (Taylor et al. 1980).

15.2.4 Suctorial Feeding

Suctorial feeding, or sucking the innards of prey organisms, is an evolutionary advanced feeding technique demonstrated by several neogastropod families. This form of feeding does not always result in the death of the prey, and several neogastropod species coexist with the prey. Two kinds of suctorial feedings are described: haematophagy and corallivory.

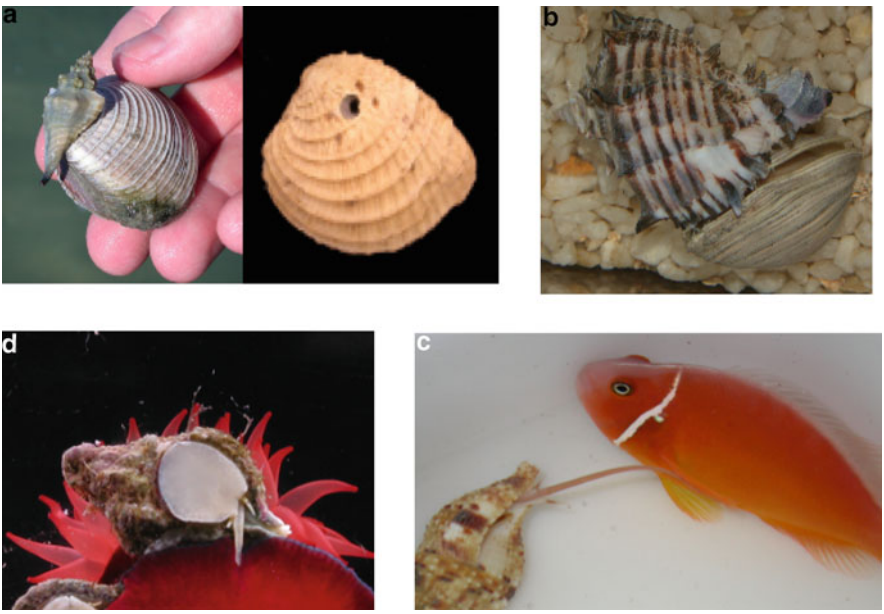

Fig. 15.3 Examples of neogastropod feeding strategies. (**a**) An ocinebrine Muricidae drilling the shell of a venerid bivalve (photo G. Herbert). (**b**) A *Muricanthus* sp. (Muricidae) using the shell margin to wedge open a bivalve shell (photo G. Herbert). (**c**) *Colubraria muricata* (Colubrariidae) feeding on a clownfish in aquarium; the proboscis is inserted under the pectoral fins (photo M. Oliverio). (**d**) *Coralliophila meyendorffi* (Coralliophilinae) feeding on *Actinia equina* (photo P. Mariottini)

15.2.4.1 Haematophagy

Three different neogastropod families, Cancellariidae, Marginellidae, and Colubrariidae, have independently evolved haematophagous feeding on fish (Fig. 15.3c). The buccinoidean family Colubrariidae includes at least six species involved in a parasitic association with different species of fish, mainly belonging to the family Scaridae (Johnson et al. 1995; Bouchet and Perrine 1996). *Colubraria* specimens can extend their proboscis to a length exceeding three times the shell length. When the extended *Colubraria* proboscis is in contact with the skin of the prey, a scraping action with its minute radula allows access to the blood vessels of the fish. The snail then apparently takes advantage of the blood pressure of the fish to ingest its meal (Oliverio and Modica 2009). Experimental observations on different *Colubraria* species (Modica and Oliverio, unpublished) suggest that adaptation to haematophagy involves the use of anesthetic and anticoagulant compounds. In fact, the fish appears to be anesthetized when the snail is feeding. Anesthetization is reversible, and the fish usually recovers its full mobility in a few minutes after the interruption of the contact with the snail. The anesthetic compounds used are not lethal as the prey recovers, in agreement with field observations

that *Colubraria* usually feed on fish sleeping in crevices of the reef (M. Oliverio pers. comm.; Bouchet and Perrine 1996; Johnson et al. 1995).

A similar strategy has been reported for the cancellariid *Cancellaria cooperi* (Cancellarioidea), which has been observed using its proboscis to ingest blood from open injuries on the body of the electric ray *Torpedo californica* (O'Sullivan et al. 1987). Cancellariidae are likely to include exclusively suctorial feeders, as inferred from foregut and radular characteristics. Dissection of *Cancellaria cooperi* evidenced a peculiar oesophageal structure (M.V. Modica, J. Biggs, and M. Holford, unpublished observations). In fact, the mid oesophagous is extremely long (up to 5 times the shell length) and glandular, similar to what is found in *Colubraria*, suggesting a convergent adaptation to haematophagy. Other examples of haematophagous feeding are the very minute species of Marginellidae, *Kogomea ovata*, *Hydroginella caledonica*, and *Tateshia yadai*, that live attached to the pectoral fins of their host (Kosuge 1986; Bouchet 1989).

15.2.4.2 Corallivory

Feeding on the living tissues of corals and other Anthozoans is reported in Muricidae for *Drupella* (Ergalataxinae) and for the subfamily Coralliophilinae (Taylor 1976; Ward 1965; Haynes 1990). Coralliophilinae includes over 200 marine tropical to temperate species, from shallow to deep waters. The few species for which alimentary preferences are known (about 10% of the shallow water species, Oliverio et al. 2008) feed exclusively on anthozoans (Fig. 15.3d). A variety of feeding strategies and preferences are displayed for this group. Some species are stenophagous, with very strict host specificity; they are mostly sessile on corals, and many groups have developed interesting eco–morphological adaptations. In fact, while *Quoyula* has a limpet-like shell suitable for external life on stony corals, *Rhizochilus* lives and feeds on anthipatharians with the shell deformed to adhere to the black coral branch. A second group lives embedded in the host skeleton: *Rapa* lives inside alcyonarian octocorals, *Magilopsis* and *Leptoconchus* have ovoid shells and bore holes into corals, while *Magilus* is sessile inside corals and possesses an uncoiled adult shell (Robertson 1970). Some others are mobile as *Latiaxis*, which is probably associated with deep-water gorgonians, or *Babelomurex* that mostly feeds on shallow water hexacorals. In a few cases mobile euryphagous species can feed on anthozoans belonging to different orders, such as some species of *Coralliophila* associated with sea anemones, scleractinians, and zoanthids (M. Oliverio, unpublished observations). Among coralliophilines some anatomical modifications related to parasitism on corals are widespread, such as the loss of the radula and jaws, viewed as an adaptation to suctorial feeding, and brooding of embryos in capsules kept in the pallial cavity (Richter and Luque 2002).

The amazing display of feeding strategies developed by neogastropods is possible due to the diversity of innovative anatomical features and chemical compounds that can be readily employed to overcome their prey.

15.3 Neogastropod Specialized Anatomy and Predatory Chemical Substances

Most neogastropod snails have developed specialized glands or other anatomical features that enable them to produce and use chemical substances to subdue their prey. It can be argued that the development of specialized foregut glands, such as the venom gland in Conoidea, or salivary and accessory salivary glands in other neogastropod groups, has lead to the successful radiation of neogastropods. The biochemical weaponry developed in the foregut and other glands is an evolutionary advantage that has enabled neogastropods to thrive.

15.3.1 Foregut Glands

The foregut glands described here include the venom gland, primary, and accessory salivary glands (Figs. 15.1 and 15.2). Toxins may be produced in a specific venom gland, as is the case with most Conoideans, or in primary and/or accessory salivary glands (Andrews 1991) for species that do not have a venom gland. In some cases, the production of toxins might involve other foregut organs/tissues, such as the glandular mid-oesophagous of the haematophagous *Colubraria* and *Cancellaria*.

15.3.1.1 Venom Gland

The presence of a venom apparatus is characteristic of the Conoidea (Fig. 15.2b). Generally it is a conspicuous organ, constituted by a proximal muscular bulb and a very long, convolute duct (the gland itself). The tubular gland always passes through the nerve ring and opens into the buccal cavity, posterior to the radular sac opening. The active exocrine secretion of the venom is due to a single cell type: cuboidal ciliated cells, accumulating venom granules at their apex, until they are discharged into the lumen (Smith 1967). The venom gland may be lined with such secretory cells for its whole length or, as happens in some species, the secretory tissue may be confined to the region posterior to the nerve ring, while the anterior-most region is a simple ciliated duct (Taylor et al. 1993). The terminal muscular bulb is usually constituted by two muscular layers, internal and external, separated by connective tissue; the relative thickness and development of these layers is variable between species. According to Ponder (1973) the tubular venom gland originated from the dorsal glandular folds of the oesophagous while the gland of Leiblein gave rise to the muscular bulb. Some conoideans, mostly radula-less species, do not possess a venom apparatus.

All cone snails (*Conus*) have a venom apparatus and the toxins found in their venom glands have led the field in characterizing peptide toxins from marine snails. When venom is injected into a prey, the conotoxins work in a concerted manner to

shut down the prey's nervous system. Conotoxins are potent neurotoxins that target ion channels and receptors. The complement of peptides found in any one *Conus* venom is strikingly different from that found in the venom of any other *Conus* specimens (Romeo et al. 2008). Thus, in the whole genus, many tens of thousands of distinct active peptides have evolved. A question that immediately arises is why individual cone snails should need so many different peptides. It has been speculated that the complement of peptides in a venom may be used for at least three general purposes: An individual peptide may play a role in (1) prey capture, directly or indirectly; (2) defense and escape from predators; or (3) other biological processes, such as interaction with potential competitors. Not all terebrids and turrids have a venom apparatus, but those that do also produce toxins to subdue their prey. Unlike conotoxins, less is known about terebrid and turrid toxins, teretoxins and turritoxins, respectively. Preliminary characterization of terebrid and turrid toxins (Imperial et al. 2003, 2007; Watkins et al. 2006; Heralde et al. 2008) indicate a similar three-domain conotoxin structure consisting of a highly conserved signal sequence, a more variable pro-region, and a hypervariable mature toxin sequence. While conotoxins have been identified as potent neuropeptides, no known molecular target has been identified for teretoxins or turritoxins. However, given their similarities to conotoxins it is expected they will also be effective modifiers for ion channels and receptors in the nervous system.

15.3.1.2 Primary Salivary Glands

Primary salivary glands are usually acinous, with a very small lumen and a system of narrow branched ducts (Fig. 15.1). In some species, the paired glands may be fused together in a single glandular mass, but two salivary ducts are always present and run along the oesophagous (or, in some groups, embedded in the oesophageal walls) until opening into the roof of the buccal cavity. Two cell types have been identified in the secretory epithelium, mixed with one another: (1) basal cells with apocrine secretion and (2) superficial ciliated cells secreting mucus (Andrews 1991). Ciliary movement is responsible for delivering the secretion, as the outer layer of muscle fibers is poorly developed (Andrews 1991). Acinous salivary glands are present in all neogastropod, although their role in toxin production may be variable, depending on whether other secreting structures, such as venom gland or accessory salivary glands, are present.

Only acinous salivary glands are present in Buccinidae and related families, such as Nassariidae, Melongenidae, Fasciolariidae, and Columbellidae (accessory salivary glands are missing). Species of the buccinid genus *Neptunea* (as e.g., *N. antiqua*) have very large salivary glands containing high quantity of tetramine (Fänge 1960; Asano and Itoh 1959, 1960; Saitoh et al. 1983; Fujii et al. 1992; Shiomi et al. 1994; Watson-Wright et al. 1992; Power et al. 2002), which blocks nicotinic acetylcholine receptors (Emmelin and Fänge 1958). A number of human intoxication has been reported so far, caused by consumption of snails of these species (Fleming 1971; Millar and Dey 1987; Reid et al. 1988). Further studies have

shown the presence of three additional unidentified toxins in the salivary glands of *N. antiqua* that appear to inhibit neuronal Ca^{2+} channels (Power et al. 2002). Other whelks are known to produce histamine, choline, and choline esters (Endean 1972). Nassariidae possess three types of secreting cells in their salivary glands, one of which secretes a glycoprotein rich in disulphide groups like the accessory salivary glands of the muricid *Nucella lapillus* (Fretter and Graham 1994; Minniti 1986; Martoja 1964).

The finding that conopeptides are expressed in the salivary gland of *Conus pulicarius* (Biggs et al. 2008) suggests that salivary glands may play a role in the envenomation process. Crude extracts of salivary glands of the haematophagous *Colubraria reticulata* have been observed to increase coagulation time of human blood (S. Rufini, M.V. Modica, and M. Oliverio, unpublished). Current research by Modica and colleagues is underway to identify the anticoagulant transcript using cDNA analysis.

15.3.1.3 Accessory Salivary Glands

Accessory salivary glands are considered to be an informative synapomorphy of Neogastropoda, although they are missing in several families. Accessory salivary glands are present in the basal family Cancellariidae (Fig. 15.2a) and in several Toxoglossa, where in some vermivorous cones they coexist with the venom gland (Marsh 1971). Two pairs of accessory salivary glands are also found in Muricidae, Mitridae, Costellariidae, Volutidae, and Olividae, while in Volutomitridae only one gland is found. In Marginellidae, Harpidae, and in the buccinoideans, accessory salivary glands are generally missing, but are present in *Busycon* (Andrews 1991). A common anatomical organization of the glands is shared by all neogastropods. The paired glands are tubular in shape, with a lumen lined by a columnar secretory epithelium surrounded by a subepithelial muscular coat richly innervated. External to the muscle layer there is an outer layer of gland cells, with long necks opening in the central lumen of the gland (Ponder 1973; Andrews 1991) producing a peculiar granular secretion (Andrews 1991). Exceptions to this model include olives, volutids, and some mitriform species (Marcus and Marcus 1959; Ponder 1970, 1972). The structure is very similar to the venom gland of Conoidea (West et al. 1996). The glandular accessory salivary glands open at the tip of the buccal cavity with nonciliated ducts.

In Muricidae, accessory salivary glands are usually large and well developed. In *Nucella lapillus* and *Stramonita haemastoma*, the only muricids studied so far at the biochemical level, accessory salivary glands produce a glycoprotein rich in cysteines (Martoja 1971; McGraw and Gunter 1972), similar to conotoxins. Extracts of the glands are able to elicit flaccid paralysis in *Mytilus edulis* which can be drilled or not, and, in the case of *S. haemastoma*, in barnacles, which are never drilled (Carriker 1981; Huang and Mir 1972; Andrews 1991; West et al. 1996; Andrews et al. 1991). *S. haemastoma* also produces a toxic secretion in the primary salivary glands that decreases cardiac activity in mammals and induces vasodilatation,

hypotension, and smooth muscle contraction (Huang and Mir 1972). A similar response was demonstrated in a combined primary/accessory salivary glands extract of another muricid, *Acanthina spirata* (Hemingway 1978). *N. lapillus* extracts also disrupt neuromuscular transmission in rat phrenic nerve–hemidiaphragm preparations (West et al. 1996). In some Volutidae, the accessory salivary glands have been reported to produce a narcotizing compound, with a very low pH, inducing muscular relaxation in the preys (Bigatti et al 2009).

15.3.2 Hypobranchial Gland

The hypobranchial gland is constituted by a thickening of the epithelium in the roof of the pallial cavity and produces large amounts of mucus. Its primary function is currently viewed to be the cleaning of the mantle cavity; the mucous secretion binds together the particulate matter, which is then eliminated from the mantle cavity. However, the hypobranchial gland comprises at least three different cell types that may correspond to distinct chemical activities, which have only been partially identified (Naegel and Aguilar-Cruz 2006). In many muricid species, the hypobranchial gland produces chromogens, which, exposed to light and oxygen, develop into a purple pigment that has been used for centuries as a dye (Tyrian purple). Similarly, in the Mitridae, the hypobranchial secretion once exposed to air becomes yellowish, then purple, and finally dark brown (Harasewych 2009), while in Costellariidae it remains predominantly yellow-green (Ponder 1998b). The production of small compounds, mainly choline esters, but also biogenic amines, has been detected in the hypobranchial gland of several species of muricids and buccinids. These substances elicit neuromuscular blocking, with paralyzing effects both in invertebrates and vertebrates (Roseghini et al. 1996). Due to the low concentrations in which these toxic compounds are found in the snails, it is not sure how effective they are in prey hunting (West et al. 1996). The functions of the hypobranchial gland and the role it played in the evolution and diversification of the Neogastropoda are still to be clarified; nevertheless, hypobranchial secretions may have useful pharmacological properties.

15.4 Neurotoxins, Anesthetics, and Anticoagulants: Prominent Bioactive Compounds from Neogastropod Snails

As stated in the introduction of this chapter, conotoxins, with the approval of the analgesic drug Prialt, have demonstrated the utility of translating basic research of marine snail compounds into drug development targets. The identification of novel neurotoxins, anesthetics, and anticoagulants are three areas in which harvesting the bioactive compounds of the Neogastropoda could prove very fruitful. The following

section highlights the success of conotoxins as neurotoxins and outlines the potential of identifying anesthetic and anticoagulant compounds from neogastropod snails.

15.4.1 Neurotoxins

In the Conoidea, the best-characterized venom components are small, highly structured disulfide peptides, individually encoded by a separate gene. Every *Conus* species has its own distinct repertoire of 50–200 venom peptides, with each peptide presumably having a physiologically relevant target in prey or potential predators/competitors (Olivera 2002). Most conotoxins are small peptides (6–40 amino acids in length), with the majority being in the size range of 12–30 amino acids (Olivera et al. 1990; Terlau and Olivera 2004). Conotoxins are comprised of a highly conserved precursor structure including a signal sequence, followed by a propeptide region and then a mature toxin that is cleaved from the prepro-structure. The mature toxins are highly disulfide rich and are classified according to their cysteine framework. Cone snails practice combinatorial drug therapy in that it is not one conotoxin that attacks the prey, but instead a cocktail of the 50–200 venom peptides working together to shut down the prey's nervous system. The conotoxin cocktail contains ion channel and receptor modifiers that can affect neuronal signaling. For example, conotoxins that inhibit Na^+ channel function prevent the formation of action potential, while conotoxins that target Ca^{2+} prevent vesicle fusion, which impedes the release of neurotransmitters. There are presently more than 3,000 different *Conus* venom proteins reported in the literature (Conoserver: http://research1t.imb.uq.edu.au/conoserver/). Less than 10% of the described conotoxins have been functionally characterized. Of those characterized, at least 25 different functions have been described (Olivera 2006; Conoserver). Several conotoxins are at various stages of drug development with the more promising examples being: MrIA (active on norepinephrine transporters), Vc1.1 (active on nicotinic receptors), and Conantokin-G (active on NMDA receptors) (Olivera 2006). While the majority of conotoxins in therapeutic development are analgesic compounds, conotoxins are also being considered as viable targets for epilepsy or myocardial infarction, as well as disorders concerning neuroprotective/cardioprotective properties (Twede et al. 2009).

Another promising group to investigate in order to discover new neurotoxins and/or substances capable of inactivating toxins is the corallivorous subfamily Coralliophilinae (Muricidae). The Anthozoa, such as sea anemones, and stony and soft corals, which are included in the Cnidaria along with the jellyfishes (Scyphozoa), sea-wasps (Cubozoa), hydrocorals, and hydromedusae (Hydrozoa), are known to produce a neurotoxin-rich venom as well as other toxic defensive compounds, from which the Coralliophilinae appear to be immune. Envenomation by cnidarians represents a remarkable sanitary problem for humans. An estimated 40,000–50,000 marine envenomations occur annually due to several species of Cnidaria. Cubozoan alone have been responsible for over 5,000 human deaths in

the last 130 years (Brinkman and Burnell 2009). Antivenom is available only for a very limited number of species. If, as is suggested by reported observations, coralliophilines have antivenom-type compounds, they may potentially be useful in cases of cnidarian envenomations. The immunity of Coralliophilinae raises a number of interesting evolutionary questions, such as: What are the physiological adaptations related to corallivory? Do corallivorous species secrete bioactive compounds interacting with and inactivating anthozoans' toxin? Are there specialized organs involved in the production of the antivenom (e.g., salivary glands)? Is host switching in euryphagous and host specificity in stenophagous correlated with biochemical variations in the secretion? The answers to these questions may translate into a modern physiological and biochemical understanding of gastropod innovations related to feeding.

15.4.2 Anesthetic and Anticoagulant Compounds

As pointed out in Sect. 15.3, three different neogastropod families have haematophagous species, which produce anesthetic and anticoagulant compounds that may be useful in elucidating cellular communication in the nervous system and as antithrombotic agents.

In Colubrariidae, anticoagulants are produced in the salivary glands, but the anatomical structures responsible for anesthetic secretion are not yet known. In addition to the salivary glands, it might be worthy to investigate the glandular mid-posterior oesophagous, a peculiar derived structure that may be related to the haematophagous lifestyle (Oliverio and Modica 2009). Furthermore, the peculiar mid-oesophagous of *Cancellaria cooperi* is a very advantageous tissue to test for bioactive compounds production, as cancellariid mid-oesophagous may be homologous to toxoglossan venom glands (Ponder 1973; Kantor 1996, 2002). Another issue of interest is the presence in Cancellariidae of both primary and accessory salivary glands. The roles these anatomical structures play in prey subduction and in the production of bioactive substances, as well as their interactions, are still to be investigated. Are the bioactive substances the same in the different haematophagous lineages? Intriguing evolutionary questions may be addressed studying and comparing anticoagulant and anesthetic molecules in Colubrariidae and Cancellariidae.

15.5 Investigating Genetic Evolution and Expression of Neogastropod Toxins

The early evolution, and the first diversification of venom toxins, has been interpreted as the result of a process of neofunctionalization in which strong positive selection acts on redundant genes produced in duplication events, originating new

functions (Ohno 1970). This evolutionary mechanism was reported also for conotoxins (Duda and Palumbi 1999). The evolutionary pressure promoting the variability of these "specialty genes" (also called exogenes, as their products act outside the organism; Olivera 2006) is related with a predator–prey arms race process in which the availability of a particular kind of prey may produce an evolutionary force acting on ecologically important genetic loci. Conotoxins are particularly prone to rapid genetic variations, due to their extremely reduced size. It is still unclear at which level the results reported for *Conus* might be generalized in the neogastropods, but it is plausible at least to hypothesize that the same organs produce the same type of bioactive substances across the entire order Neogastropoda. According to the amount of variation that will be detected at the different taxonomic levels in neogastropods, it will be possible to clarify the evolutionary patterns acting at each level. In snakes, where the same neofunctionalization mechanism is responsible for the evolution of the toxin gene families, the genes that have been recruited to constitute the venom proteome have been partly identified (Fry 2005). In neogastropods, including cone snails, the origin of the toxin sequences has yet to be investigated.

The role of differential gene expression and posttranscriptional modifications in modulating toxin diversity is also an intriguing area requiring further investigation. This line of research could be addressed at different taxonomic levels: (1) Between different species – a particular focus should be dedicated to host specificity, to verify if the inverse correlation between the degree of specialization and the diversity of the venom in *Conus leopardus* (Remigio and Duda 2008) can be generalized to other neogastropod groups. (2) In individuals of the same species – the high levels of intraspecific variability observed in *Conus ventricosus* (Romeo et al. 2008) raise the possibility that fine-scale modulatory mechanisms may act in response to environmental and ecological variations. And (3) at different ontogenetic stages – juvenile neogastropods have often a largely different diet from the adults, implying a different suite of toxins. How and under which mechanisms does venom composition change during ontogenesis? To address these and other toxin evolution and expression topics, a robust phylogenetic hypothesis and an integrated strategy for the characterization of bioactive compounds are required.

15.6 Conclusion: Integrated Strategies for Building a More Robust Evolutionary Framework and Effective Drug Development Methods

The major challenges in characterizing bioactive compounds in snails are the complexity of sampling, the scarcity of the biological material, and the absence of databases for determination of peptide and protein sequences. Venom profiling may thus prove an elusive target, unless molecular biology techniques are coupled

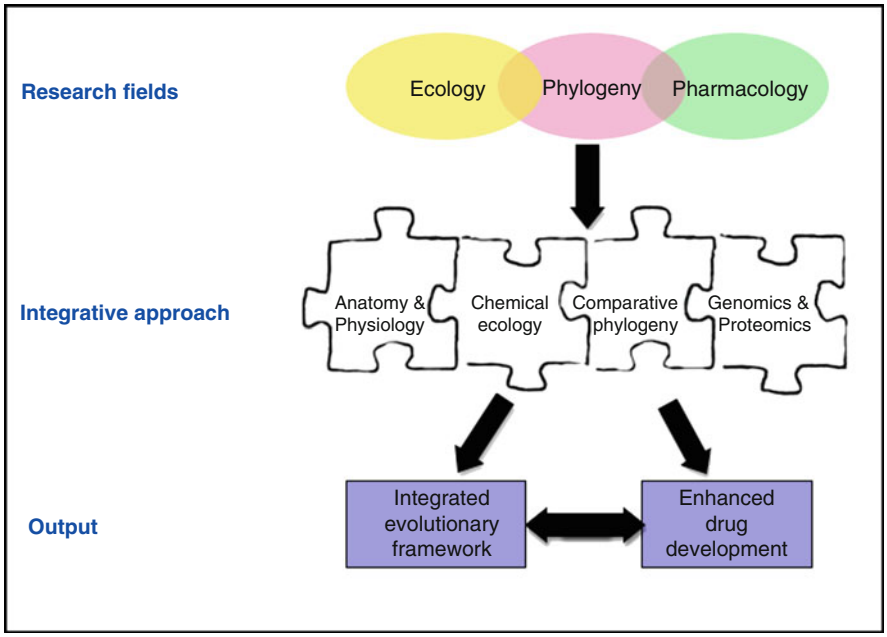

Fig. 15.4 Integrated research strategies for investigating biodiversity. The integration of different approaches to diversity may lead to a more complete evolutionary framework and enhance the rate of drug discovery and development

with biochemical analysis of polypeptide composition. A multidisciplinary platform, combining modern genomic and proteomic techniques, as well as phylogeny and descriptive approaches to ecology and anatomy, is necessary to increase the rate of pharmacological characterization of new bioactive compounds. Genomic libraries can be obtained from tissues of interest and their analysis can be integrated with proteomic techniques, such as venom fractionation, peptide purification, mass spectrometry, and sequence analysis using automated Edman degradation. Spider venoms have recently been analyzed by a three-dimensional approach, combining calculated, predicted, and measured data obtained with different techniques such as cDNA sequences and LC-MALDI analysis (Escoubas et al. 2006). The use of such "venom landscapes" may constitute a significant improvement in venom profiling and can also be effective as molecular markers in taxonomic and phylogenetic studies. A similar strategy has been applied to snake venoms (Nascimento et al. 2006). Molecular phylogeny, combined with anatomical and ecological data, can guide us through the maze of snail biodiversity, toward the species or group of species which are likely to possess bioactive compounds worthy to investigation to find new therapeutics (Fig. 15.4). This strategy was successfully applied to the Terebridae, outlining particular genera/species important for teretoxin discovery (Holford et al. 2009a, b).

Interestingly, the relationship between drug discovery and phylogeny is a two-way street. In fact, exogenes mostly belong to gene superfamilies with highly conserved sequence elements, enabling the use of standard molecular techniques. In what has been called a "concerted discovery strategy" venom toxins are revealed to be useful characters for the taxonomy and phylogenetic relationships of their producers (Olivera 2006; Olivera and Teichert 2007; Bulaj 2008). This integrated approach has been used in non-molluscan toxin-producing groups such as snakes to garner insight into the molecular evolution of snake venoms and to correlate the appearance of other morphological evolutionary novelties (Fry and Wüster 2004). For the Neogastropoda, whose phylogeny cannot be readily elucidated using standard taxonomic approaches, an integrated approach has several possibilities. Proteomics of the venom as well as the characterization of its biochemical and functional properties successfully separated two closely related, morphological indistinguishable pit-viper species (Angulo et al. 2007).

The use of genomic analysis and venom profiling techniques, along with more traditional approaches such as anatomical and physiological studies, will allow a better understanding of the correlation between venom composition, trophic preferences, and adaptive radiation of the Neogastropoda, creating the basis for a modern integrated evolutionary framework and an effective drug discovery strategy (Fig. 15.4).

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