lose, Sephadex G-200, and hydroxyapatite. The final product was then subjected to polyacrylamide gel electrophoresis, and the enzymatically active protein band was identified by the assay of gel slices. Gel slices that contained enzymatically active protein were collected, homogenized with 0.9 percent saline, mixed with Freund's complete adjuvant, and injected subcutaneously into rabbits. Four injections with a 2-week interval were required to produce high-titer antisera.
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Neuromuscular Patterns and the Origin of Trophic Specialization in Fishes

Abstract. *The pattern of muscle electrical activity in the pharyngeal muscles of the mollusc-eating sunfish* Lepomis microlophus *is highly specialized in comparison with the pattern displayed by most other members of the sunfish family and does not change when different prey are eaten. The closest genealogical relative of this species has the specialized muscle activity pattern for crushing prey when it feeds on snails but uses the primitive sequence of muscle activity during swallowing of other prey. The ability of species that crush snails to use molluscan prey effectively is due primarily to the evolutionary transformation of the neuromuscular program controlling the trophic apparatus.*

The process by which populations of organisms evolve to specialize on a particular resource in the environment is of considerable interest to evolutionary biologists. The phenomena of niche partitioning, character displacement, competitive exclusion of species, and evolutionary diversification within clades have all been linked to the ways in which organisms use environmental resources to obtain energy for growth and reproduction (*1*). Most analyses of trophic specialization have focused on morphological features of organisms as a reflection of their ability to collect and process food (*2*). I present experimental data from fishes on muscle activity patterns involved in the use of a specialized food resource. The results indicate that an evolutionary transformation in neuromuscular pattern resulted in a specialized method of acquiring energy.

The North American sunfish family Centrarchidae contains 32 species that display a wide range of food and habitat preferences (*3*). One species in the largest genus, *Lepomis microlophus* (redear sunfish), feeds primarily on freshwater snails (*4*). This food choice is specialized in that only one other species in the family, *Lepomis gibbosus*, feeds on snails to any significant degree; phylogenetically primitive species such as the bass *Micropterus* and the rock bass *Ambloplites*, as well as the other *Lepomis* species, are insectivorous and piscivorous; and, snails are not common elements of the diet in other morphologically generalized perciform fishes, which are predominately insectivores and piscivores (*5*).

Most teleost fishes capture prey by rapidly expanding the mouth and drawing water and the prey into the buccal cavity in a process known as suction feeding. Very little mastication occurs in the mouth, and prey are usually swallowed whole by movements of modified gill arch elements—the pharyngeal jaws (*6*). The pattern of pharyngeal muscle activity during intraoral prey manipulation and transport into the esophagus was studied by electromyographic recordings in six insectivorous and piscivorous sunfishes and in the perch *Perca* (*7*). All species exhibited a similar highly stereotyped pattern, with minor variations dependent on prey type and size (Fig. 1A). The key feature of the sequence of pharyngeal muscle electrical activity is the regular rhythmic pattern of activity (Fig. 1A) that may last a minute during prolonged swallowing sequences. For example, the muscle protracting the lower pharyngeal jaws alternates, occasionally in a double-burst pattern as in Fig. 1, with activity in the muscle retracting the lower pharyngeal jaws [pharyngohyoideus (PH) and pharyngocleithralis internus (PC_i), respectively, in Fig. 1A]. These features are consistently present in perch, as well as in all insectivorous and piscivorous sunfishes examined.

In the redear sunfish a radically different pattern of muscle activity occurs (Fig. 1B). All pharyngeal muscles are active together in a closely synchronized pattern to appose the pharyngeal jaws and crush the prey. When snails are being eaten, pieces of shell fall out of the mouth cavity as the shell is cracked, and sound recordings of snail crushing show that shell fracture occurs at the end of muscle bursts (Fig. 1B). Repeated crushing sequences occur until the snail shell has been completely fragmented and then the body and adhering shell pieces are swallowed. This same pattern of muscle activity occurs when the redear sunfish feeds on worms and fish. This species possesses only a single very stereotyped "crushing pattern" used without regard to prey consistency or size.

The closest genealogical relative (sister species) of *L. microlophus* (*8*) possesses a versatile activity pattern that can be modulated for different prey types. *Lepomis gibbosus* uses the primitive muscle activity pattern when eating fish and worms (Fig. 1A) but employs a stereotyped crushing pattern when eating snails (Fig. 1C). Pharyngeal muscle activity during snail crushing is similar in all respects to that of *L. microlophus* except that the duration of the burst in all muscles is significantly shorter [$t(70) = 5.46$, $P < .001$].

These results allow reconstruction of the sequence of evolutionary modification in muscle activity patterns involved in utilizing a specialized food type. A rhythmic pattern of coordinated pharyngeal muscle activity (Fig. 1A) transported prey into the esophagus in early sunfishes. Use of a new prey type, snails, in addition to fishes and invertebrates, was associated with the addition of a distinctive crushing pattern of muscle activity in which all the pharyngeal muscles are nearly synchronously active (Fig. 1C). The primitive pattern of rhythmic activity is retained as a component of the behavioral repertoire and is elicited by other types of prey. Finally, the snail-crushing pattern became the only component of the neuromuscular output involved in feeding to the extent that all prey are treated as though they were snails.

Morphological modifications associated with snail crushing are found in *L. microlophus* and include an increase in the proportion of molariform teeth on the pharyngeal jaws and an increase in physiological cross section of several of the pharyngeal muscles (*9*). However, no major structural reorganization has oc-

curred in the musculoskeletal system of the redear sunfish, and the lines of action of the pharyngeal muscles and their fiber lengths are similar to those of primitive piscivorous and insectivorous species.

The key modifications involved in trophic specialization in sunfishes thus occurred by a major reorganization of the neuromuscular pattern regulating movement of the trophic apparatus. Sunfishes that do not exhibit the crushing pattern of muscle activity (for example, *L. macrochirus*) cannot crush mollusc shells. The evolution of trophic specialization (indicated by restricted dietary diversity) has involved a phylogenetic stage in which flexibility of the neuromuscular pattern mediating motor output to pharyngeal muscles has increased (illustrated by *L. gibbosus*). The specialized taxon *L. microlophus*, however, has a neuromuscular repertoire restricted to only one component of the ancestral pattern. An initial behavioral shift in food preference may have provided the stimulus for increased breadth in the repertoire of neuromuscular responses to different prey in populations ancestral to *L. gibbosus*.

These data provide an interesting contrast to recent work on trophic differentiation in cichlid fishes in which both trophic polymorphism within a species and a wide diversity of food-processing morphologies are well documented (*10*). Neuromuscular patterns associated with intraoral prey manipulation are similar even in taxa with distinct pharyngeal morphologies and vary little with prey type (*11*). This similarity in neuromuscular output may be a phylogenetic specialization that reflects structural constraints on the intraoral trophic apparatus that are not present in sunfishes, and may provide a basis for understanding the radically different patterns of trophic diversification in centrarchid and cichlid fishes.

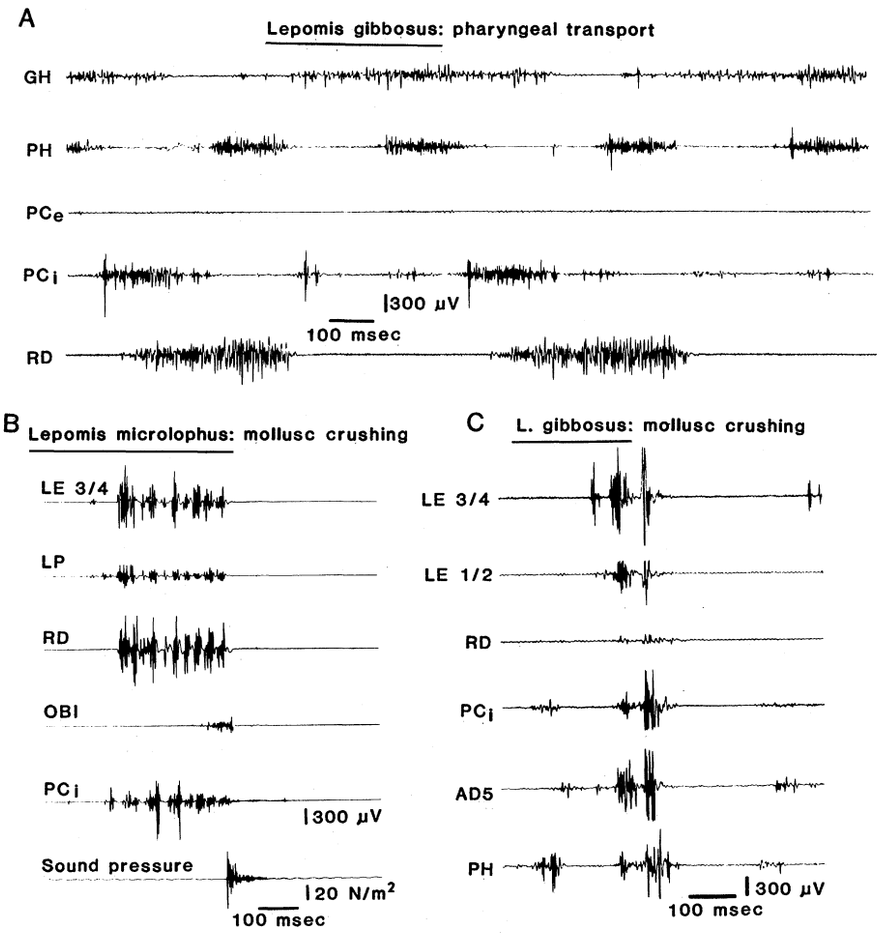

Fig. 1. Electrical activity patterns of muscles in the pharyngeal apparatus. All muscles in each panel were recorded simultaneously. (A) The rhythmic pattern in *Lepomis gibbosus* with consistent phase relationships between pharyngeal muscles controlling the upper and lower jaws during feeding on a worm (*Lumbricus*). This pattern is present in all primitive sunfishes and in *L. gibbosus* except when this species eats snails. (B) The very different pattern occurring during snail crushing by *L. microlophus*. Nearly all pharyngeal muscles are synchronously active. The pattern of activity in one hypobranchial muscle (*OBI*) together with a recording of high-frequency shell-cracking sounds is also illustrated. (C) Crushing pattern displayed by *L. gibbosus* when feeding on snails. Note contrast to muscle activity shown by this same species when feeding on a worm in (A). Muscle abbreviations: *AD5*, fifth adductor arcus branchialum; *GH*, geniohyoideus; *LE* 1/2 and *LE* 3/4, levatores externi muscles 1 to 4; *LP*, levator posterior; *OBI*, obliquus inferioris; PC_e and PC_i, pharyngocleithralis externus and internus; *PH*, pharyngohyoideus; and *RD*, retractor dorsalis.

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Dorid Nudibranch Elaborates Its Own Chemical Defense

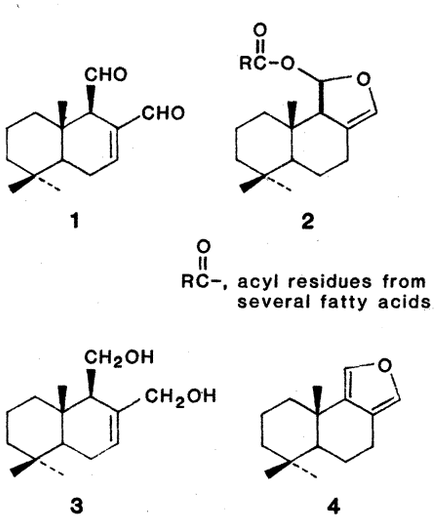
Abstract. *A biosynthetic experiment with mevalonic acid labeled with carbon-14 showed that the nudibranch* Dendrodoris limbata *elaborates polygodial, a sesquiterpenoid dialdehyde stored in the mantle, which constitutes its chemical defense against predators. Previously described nudibranchs drew defensive chemicals from their preys.*

Among the different defensive mechanisms (*1*) employed by nudibranchs to escape predators, chemical defense has received attention only recently. Several of these shell-less mollusks secrete a mucus that decreases the animal's likelihood of being eaten (*2*), since the mucus may contain toxic (*3*) or unpleasant compounds (*4, 5*). Most of the nudibranchs studied from a chemical point of view belong to the suborder Doridacea; these invertebrates feed mainly on sponges (*6*), which are said to be repellent to most other animals.

The metabolites found in dorid nudibranchs have been traced in some cases to a particular sponge (*3, 4, 7–10*), suggesting that these mollusks concentrate metabolites from their sponge diet in skin glands and employ these metabolites as a defensive secretion (*10*).

When such a predator-prey relation was not determined, it was suggested that the metabolites found in the nudibranchs arose by predation on unidentified sponge species (*7, 11*).

We reported earlier (*12*) the presence of polygodial (structure **1**) in the skin extracts of *Dendrodoris limbata* (phylum Mollusca, class Gastropoda, subclass Opisthobranchia, order Nudibranchia, suborder Doridacea). This compound showed antifeedant activity against marine and freshwater fish. A mixture of sesquiterpenoid esters (structure **2**), which were inactive in the feeding inhibition bioassay (*12*), was isolated (*13*) from the digestive gland of the same nudibranch.



We were unable to find either compound **1** or **2** in several sponges collected in the area in which *D. limbata* was located, and we therefore investigated the possibility that these metabolites are synthesized in the nudibranch. Since both compounds **1** and **2** have a sesquiterpenoid skeleton, mevalonic acid was selected as the most suitable precursor for the biosynthetic experiments in vivo. Seven specimens of *D. limbata* were placed in aerated seawater (3 liters), and 2 μCi of [2-^{14}C]mevalonic acid–dibenzylethylenediamine salt (Amersham; 31 mCi/mmole) in distilled water solution (0.01 ml) was injected into the hepatopancreas of each animal by means of a syringe.

After 24 hours, the animals were killed by freezing and were carefully dissected. The hepatopancreas and the mantles were separately extracted with acetone. The solvent was removed at reduced pressure from the acetone extracts, and the residues were partitioned between diethyl ether and water. The resulting ether layers were evaporated and then subjected to chromatography on two distinct silica gel columns with petrol and increasing amounts of diethyl ether (*12, 13*).

Polygodial (compound **1**; 15 mg) was recovered from the mantle extract. This compound was pure, as judged by thin-layer chromatography; to ascertain whether the radioactivity found (Table 1) was due to polygodial itself or to accompanying impurities, compound **1** was further purified by preparative silica gel thin-layer chromatography (Merck; C_6H_6–diethyl ether, 8:2) to yield 9 mg of material that was subsequently reduced with $NaBH_4$ in methanol to the dialcohol (structure **3**) and purified again by preparative thin-layer chromatography (petrol–diethyl ether, 3:7), with 5 mg recovered.

The radioactive counts in the samples were determined (*14*) after each purification step (Table 1); the specific radioactivity in disintegrations per minute per milligram remained practically constant. The results indicate good incorporation (1.6 percent) of mevalonic acid into polygodial. The esters (**2**) obtained from the column chromatography were contaminated by colored material; they were therefore purified by preparative thin-layer chromatography (petrol–diethyl ether, 95:5), and the radioactive counts were determined.

To establish whether the radioactivity found was associated, as expected, with the terpenoid part of the molecule, we converted mixture **2**, by heating in the presence of a catalytic amount of silica gel (*13*), into the furan (structure **4**) and into the corresponding mixture of free fatty acids. The furan was purified by preparative thin-layer chromatography (petrol). The mixture of the fatty acids was purified with the same system, but with a different eluent (petrol–diethyl ether, 1:1). The esters, **2**, were also biosynthesized de novo by the nudibranch. The labeled mevalonic acid was specifically incorporated into the terpenoid part of the molecule, the radioactivity, after conversion, being almost completely associated with the furan (*15*).

These results show that the nudi-

Table 1. Radioactivity found in the metabolites of *Dendrodoris limbata* and in their derivatives after each purification step. A total of 14 μCi (30.8 × 10^6 dpm) of [2-^{14}C] mevalonic acid–dibenzylethylenediamine salt was injected into seven specimens.

Compound	Amount (mg)	Radioactivity (dpm/mg)
1 (first purification)	15	32,400
1 (second purification)	9	28,000
3 (from reduction of **1**)	5	32,500
2 (second purification)	53	6,500
4 (from thermolysis of **2**)	10	13,100
Fatty acids (from thermolysis of **2**)	15	330