

Columellar Muscle of Neogastropods: Muscle Attachment and the Function of Columellar Folds

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Abstract. Malacologists often assume that ornamentation on snail shells is functional, and therefore adaptive. I conducted the first comprehensive test of the widely accepted hypothesis that columellar folds, a type of internal ornamentation, enhance the performance of the columellar muscle, which attaches the snail to its shell. Careful dissections of live, non-relaxed specimens reveal that the physical attachment between the columellar muscle and the columella is not restricted to a small, circular patch located deep within the shell. Instead, the attachment is long and narrow, extending approximately a full whorl along the length of the columella. I developed a novel technique for preparing three-dimensional reconstructions from photographs documenting the dissections. These reconstructions were then used to measure four parameters that describe the muscle: (1) the surface area of the physical attachment between the muscle and columella, (2) the total contact area between the muscle and the columella, (3) the depth of attachment, and (4) the length of attachment. None of these parameters differed significantly between species with and without folds. In light of the biomechanics of muscular hydrostats, values of the first parameter indicate that columellar folds probably do not guide the columellar muscle as the animal moves in and out of its shell. Values of the other parameters indicate that columellar folds neither increase an animal's ability to maneuver its shell nor facilitate deeper withdrawal. These results, and the fact that folds have evolved convergently several times, might indicate that folds are an easily evolvable solution to many functional problems, none of which are currently understood.

Introduction

Malacologists often assume that gastropod shell ornamentation is adaptive, but experiments that demonstrate the function of these presumed adaptations are rare (Morton, 1967; for notable tests of ornamentation function, see Palmer, 1977, 1979; Bertness and Cunningham, 1981; Appleton and Palmer, 1988; Marko and Palmer, 1991; West *et al.*, 1991; Carefoot and Donovan, 1995; Donovan *et al.*, 1999). Columellar folds, plications on the columella, or central column of a gastropod shell (see Fig. 1), are particularly intriguing ornaments because they evolved repeatedly in a number of clades, such as the Caenogastropoda, Opisthobranchia, and Pulmonata (Price, 2001). In the subclade Neogastropoda of the caenogastropods, the columellar folds may be an adaptation that is intimately related to the columellar muscle (Fig. 1). In fact, the muscle does attach the animal to the columella (Signor and Kat, 1984; Fretter and Graham, 1994) and has grooves that fit between each fold. In this paper, I test three hypotheses that purport to explain a functional relationship between the columellar folds and the columellar muscle.

Columellar folds are readily visible on the inner lip of many shells, and systematists have used the impressive diversity of fold shapes to distinguish species and higher taxa. I consider a fold to be any ridge on the inner lip of the aperture that extends along the columella for a number of whorls, usually extending all the way to the apex. Some folds occur at the bottom edge of the aperture, as in *Nassarius vibex*, whereas others, such as those in *Terebra dislocata*, are located in the center of the inner lip. Folds can be wide (*Triplofusus giganteus*) or narrow (*Nassarius vibex*), subtle (*Busycon contrarium*) or prominent (*Vasum muricatum*).

The columellar muscle conforms exactly to the shape of the folds where it lies over them, and this conformation has inspired the hypotheses that are most commonly cited to

explain the function of folds. These hypotheses must be considered in light of the fact that the columellar muscle functions as a muscular hydrostat (Thompson *et al.*, 1998), controlling protraction from, and retraction into, the shell. Like hydrostatic skeletons in general, the volume of a muscular hydrostat remains constant, so a contraction in one direction induces elongation in an opposing direction (Kier and Smith, 1985). The columellar muscle is composed of muscle fibers that are oriented longitudinally, transversely, and obliquely with respect to the long axis of the columellar muscle (Thompson *et al.*, 1998). Thus, the columellar muscle controls its own twisting, shortening, and elongating in addition to protraction and retraction.

Dall (1894; restated by Fretter and Graham, 1994) published the only nonfunctional explanation of folds. His explanation does not pertain to the columellar muscle, relying instead on the nature of the mantle, the tissue that secretes the shell. Dall surmised that folds, and all other internal ornamentation including the parietal ridge, lirae, and teeth on the outer lip, were deposited in the wrinkles that would form when an overly large mantle retracted into the shell. This idea predicts that ornamentation would be more pronounced at the aperture (Dall, 1894), but ornamentation around the aperture where the mantle is largest is exaggerated only in some species with determinate growth (Paul, 1991; Vermeij and Signor, 1992; Vermeij, 2001a); furthermore, contrary to Dall's prediction, not all of these animals have columellar folds. Indeed, the internal morphology of gastropods is highly stereotyped, not random, and it is unlikely that the mantle would always wrinkle uniformly (Signor and Kat, 1984). There are also no obvious differences in mantle size between species with and without internal ornamentation in general, or columellar folds in particular (pers. obs.).

Three interrelated hypotheses have been presented to explain how the folds affect the function of the columellar muscle, thereby explaining both why folds have evolved and why so many neogastropod lineages maintain them:

1. *Guidance.* Columellar folds guide the columellar muscle as the animal moves in and out of its shell (Signor and Kat, 1984; Ponder, 1972). The analogy here is that the folds act like a railroad track guiding a train; that is, they prevent the animal from slipping in its shell. One manner in which the folds may guide the muscle is by protruding far into the columellar muscle, restricting the muscle movement *along* the folds. In this event, the area of contact should be greater in species with folds than in those without. I test this prediction.

Signor and Kat (1984) limited their guidance hypothesis to high-spired, narrow (turritelliform) species, implying that the columellar muscle would slip within the shell unless folds guided its movement. Any attempt to extend this hypothesis to low-spired

shell shapes must account for the absence of folds in some large neogastropods, such as *Syrinx aruanus*, whose shell can reach almost a meter in length (Harasewych and Petit, 1989).

2. *Maneuverability.* Columellar folds enhance a snail's ability to maneuver its shell (Fretter and Graham, 1962; Vermeij, 1978). A number of authors have assumed that the shape of the muscle's physical attachment to the columella is small and circular, like the adductor muscle attachment in bivalves (*e.g.*, Linsley, 1978; Signor and Kat, 1984; Morita, 1993; Thompson *et al.*, 1998). Vermeij (1978) assumed that the attachment occurs immediately over the folds and predicted that animals with folds would consequently have a larger surface area of muscle attachment. An animal with greater attachment area might be better at maneuvering its shell, for example when it swings the shell back and forth to fend off a predator (Thompson *et al.*, 1998), or when it pries open the shells of prey (Taylor *et al.*, 1980). This hypothesis has remained untested because dissections usually begin by removing and discarding the shell, and because the muscle easily detaches from a cracked shell.
3. *Predator avoidance.* Snails with columellar folds can withdraw more deeply into their shells (Dall, 1894), increasing their ability to escape from predators. Dall (1894) commented that snails with columellar folds retract more deeply, but presented no data in support of this idea. Since then, others have documented that snails that retract more deeply are better at escaping predators: they are harder to reach, and they take so long to handle that the predator eventually abandons the task (Vermeij, 1978, 1987). Although it is not immediately obvious why these behaviors might be associated with columellar folds, the data required to confirm Dall's claim are easy to collect.

I have developed procedures for dissecting gastropods while keeping the columellar muscle intact; employed a novel mathematical algorithm that converts a photograph of a snail into a three-dimensional surface from which I can measure areas; and examined neogastropod species that represent a range of columellar fold morphologies, as well as species with smooth columellae. Contrary to what has been assumed, the muscle attachment to the columella is complex and leaves no scar. None of the three hypotheses outlined above adequately explain the functional relationship between the columellar folds and the columellar muscle.

Materials and Methods

Sample

Quantitative data on the columellar muscle were collected from seven species with folds (from five genera and

four families) and five species lacking them (from five genera and three families), affording phylogenetic breadth (Table 1). One of the species without folds, *Strombus alatus*, is not a neogastropod; it is a caenogastropod (a clade that contains the neogastropods) with shell shape similar to other species considered here. At least two and up to five specimens were studied for each species, for a total of 31 specimens for most measurements (36 specimens for attachment depth). These data were supplemented with qualitative observations from an additional nine species. All specimens were stored in 70% ethanol and deposited at the Field Museum of Natural History (FMNH), Chicago, Illinois. Except for *Oliva sayana*, all species have a functional operculum, eliminating any possible bias introduced by a relationship between folds and an operculum.

The sample includes species with a variety of columellar fold shapes and numbers (Table 2), but all have fairly modest folds. Prominent folds, such as those in the Mitridae, Volutidae, and Cancellariidae could not be included, because they were too difficult to collect or purchase alive. *Oliva sayana* has plications on the inner lip of the aperture

that some authors describe as columellar folds (e.g., Abbott, 1974), but these do not continue inside the shell, and they therefore do not contact the columellar muscle, even when the animal is fully protracted. As such, these apertural plications cannot affect the function of the columellar muscle, so I consider *Oliva sayana* to be without folds.

Terminology describing orientation

A description of fold morphology requires terms that orient the reader to the snail shell (Fig. 1). In this paper, all terms refer to a snail shell in a standard orientation, with apex up and aperture visible (and on the right for dextral species). *Top* and *bottom* refer to positions along the coiling axis. The *bottom* of a whorl is farthest from the apex, whereas the *top* of the whorl is closest to the apex. The *width* of a feature is measured along a line parallel to the *top-bottom* axis, following the convention that the short axis of the columellar muscle represents *width*, whereas the long axis of the columellar muscle represents *length* (Fig. 1A, E, Fig. 2E). *Apical* and *apertural* refer to directions along the

Table 1

Species examined

Species	Collection site*	Family	Folds	Museum ID	Data type
<i>Busycon contrarium</i> (Conrad, 1840)	FS	Melongenidae	Yes	299449, 299459	Quantitative
<i>Busycon spiratum</i> (Lamarck, 1816)	FS	Melongenidae	Yes	299444, 299463, 299468, 299472	Quantitative
<i>Fasciolaria hunteria</i> (Perry, 1811)	FS, WFS, SM	Fascioliariidae	Yes	299450, 299492, 299493	Quantitative
<i>Fasciolaria tulipa</i> (Linnaeus, 1758)	HT	Fascioliariidae	Yes	299448, 299456	Quantitative
<i>Nassarius vibex</i> (Say, 1822)	FS, WFS	Nassariidae	Yes	299475, 299477	Quantitative
<i>Triplofusus giganteus</i> (Kiener, 1840)	HT	Fascioliariidae	Yes	299441, 299442	Quantitative
<i>Cantharus cancellarius</i> (Conrad, 1846)†	DIR	Muricidae	Yes	299486, 299487	Quantitative
<i>Chicoreus florifer distans</i> (Adams, 1855)	WFS	Muricidae	No	299451, 299458	Quantitative
<i>Melongena corona</i> (Gmelin, 1791)	FS, WFS	Melongenidae	No	299453, 299457	Quantitative
<i>Stramonita haemastoma</i> (Linnaeus, 1767)	WFS	Muricidae	No	299452, 299454, 301941	Quantitative
<i>Strombus alatus</i> (Gmelin, 1791)	P	Strombidae	No	301942, 301943	Quantitative
<i>Urosalpinx perrugata</i> (Conrad, 1846)	WFS, SM, ST	Muricidae	No	299481, 299482, 299483, 299484, 299485	Quantitative
<i>Columbella rusticoidea</i> (Heilprin, 1886)	DIR	Columbellidae	Yes	299473, 299474	Qualitative
<i>Leucozonia nassa</i> (Gmelin, 1791)	S	Fascioliariidae	Yes	299506, 299520, 299521, 299522, 299526, 299527, 299530, 299531	Qualitative
<i>Opeatostoma pseudodon</i> (Burrow, 1815)	IV	Fascioliariidae	Yes	299496	Qualitative
<i>Pleuroploca salmo</i> (Wood, 1928)	B	Fascioliariidae	Yes	299494	Qualitative
<i>Terebra dislocata</i> (Say, 1822)	HT	Terebridae	Yes	299488, 299489, 299490, 299491	Qualitative
<i>Vasum muricatum</i> (von Born, 1778)	G	Vasidae	Yes	299500	Qualitative
<i>Cypraea cervus</i> (Linnaeus, 1771)	G	Cypraeidae	No	299495	Qualitative
<i>Oliva sayana</i> (Ravenel, 1834)	SM, HT	Olividae	No	299460, 299461, 299466, 299469	Qualitative‡
<i>Polystira nobilis</i> (Hinds, 1843)	GC	Turridae	No	299497, 299499	Qualitative

* Collection sites: B, Bique, Panama (Pacific Ocean); DIR, Dog Island Reef, Florida (Gulf of Mexico); FS, Florida State University Marine Lab, Turkey Bayou, Florida (Gulf of Mexico); G, Galeta, Panama (Caribbean Sea); GC, Golfo de Chiriquí, Panama (Pacific Ocean); HT, Hammock Trail, near St. Joseph Bay, Gulf County, Florida (Gulf of Mexico); IV, Isla Venado near Playa Veracruz, Panama (Pacific Ocean); P, Purchased from Gulf Specimen Aquarium and Marine Biological Supply, Panama, Florida; S, Sebastian, Florida (Indian River County); SM, St. Mark's Wildlife Refuge, Florida (Gulf of Mexico); ST, Beach at St. Teresa, Florida (Gulf of Mexico); WFS, West side of Florida State University Marine Lab on oyster bar.

† Tentatively placed in *Soleneistra* by Vermeij (2001b).

‡ Only the attachment depth was measured.

Table 2

Fold morphology in species examined

Species	Fold morphology
<i>Busycon contrarium</i>	1 subtle fold at the bottom of the whorl, distinguished by a groove and angled obliquely; difficult to observe at aperture, but more prominent in older whorls
<i>Busycon spiratum</i>	As in <i>B. contrarium</i> , but slightly more defined
<i>Cantharus cancellarius</i>	1 fold at the bottom of the whorl, angled obliquely; broader than in other species
<i>Columbella rusticoidea</i>	1 fold immediately at the bottom of the whorl
<i>Fasciolaria hunteria</i>	2 folds at the bottom of the whorl, angled obliquely; the lower edge of the bottom fold coincides with the edge of the columella
<i>Fasciolaria tulipa</i>	As in <i>F. hunteria</i> , but with 3 folds
<i>Leucozonia nassa</i>	3 folds, angled obliquely and closely spaced
<i>Nassarius vibex</i>	1 well-defined fold at the bottom of the whorl, angled obliquely; square, rather than rounded, profile
<i>Opeatostoma pseudodon</i>	As in <i>L. nassa</i>
<i>Pleuroploca salmo</i>	As in <i>F. tulipa</i>
<i>Terebra dislocata</i>	2 large, broad folds spread throughout whorl; the bottom fold is more prominent than the top one
<i>Triplofusus giganteus</i>	As in <i>F. tulipa</i> , but top fold is more subtle than the others
<i>Vasum muricatum</i>	5 folds in "1 prominent—1 weak—1 prominent—1 weak—1 prominent" pattern; folds angled more perpendicular to coiling axis than in any of the other species and spread throughout the bottom two-thirds of the whorl

spiral of the shell towards the tip or opening respectively: the columellar muscle narrows apically, but is *wide* aperturally. A *junction* occurs where the bottom of one whorl meets the top of another. The *depth* of a feature indicates the number of revolutions between it and the aperture, where one revolution is 360°; for example, the columellar muscle attachment might begin at a depth of 300°.

Dissections

Dissections were performed on live, untreated animals. Surprisingly, any treatment of animals, such as freezing, storing in ethanol, or relaxing in magnesium sulfate (Epsom salts), caused the muscle to detach from the shell, even when tugged only slightly. Fresh material is therefore essential to study columellar muscle attachment.

To expose the soft tissues while keeping them and the columella intact, I used a Dremel rotary tool with a 1.6-mm-diameter carborundum abrasive wheel to cut away the exterior. The size of the blade limited the specimens that could be dissected with precision to those with whorls larger than half a centimeter. Thus, the

attachment morphology could be quantified in the largest specimens of *Nassarius vibex*, but the available specimens of *Columbella rusticoidea* were too small. The largest whorl of *Terebra dislocata*, a high-spired species, is only a few millimeters tall, so it could not be quantified with these methods. Although the most apertural point of the attachment was documented in *Oliva sayana*, the morphology of attachment throughout the rest of its length was inaccessible, because the delicate columella cannot withstand even slight pressure.

To distinguish between muscle that was physically attached and muscle that was simply pressed against the columella, I used a blunt but flexible 34-gauge copper wire to probe between the tissue and columella. In all cases, only the top of the muscle (that closest to the junction between two whorls; see Figs. 1, 2) was physically attached. Since the bottom of the muscle was free, the attachment was not disturbed when probed.

Measurements

The measurements required to test the hypotheses (Table 3) were the standardized (see below) total area of muscle physically attached to the columella, the standardized total area of muscle in contact with the columella throughout the attachment, the depth of attachment, and the length of the attachment.

It was especially difficult to measure the total area of muscle physically attached to the columella (ATT in Figs. 1, 2). In these species, the columellar muscle attachment does not scar the shell, so the attachment area had to be observed directly. I tried removing strips of columellar muscle that were not attached to determine how much muscle was left on the columella, but this procedure inevitably destroyed the attachment. However, the attachment was so narrow relative to the total width of the columellar muscle that, when measured from digital photographs, it could be reasonably estimated as a thin but long box, only one pixel wide but many degrees long. The small bias introduced by this approximation should be the same in species with and without folds.

Another challenge was to measure the surface area of contact between the columellar muscle and the columella (CM in Figs. 1, 2G). The apertural end of the muscle grades into the foot (Voltzow, 1990; Thompson *et al.*, 1998), so the only point that could be consistently identified at the apertural end of the columellar muscle was the point at which the muscle *attachment* began (ATT in Fig. 2E). I measured the total area of muscle in contact with the columella: beginning with the attachment, extending down the width of the muscle to the bottom of the whorl, and extending across the columella to include the most apical part of the muscle (CM in Figs. 1, 2G). Because the muscle always spanned the columella between the attachment and the

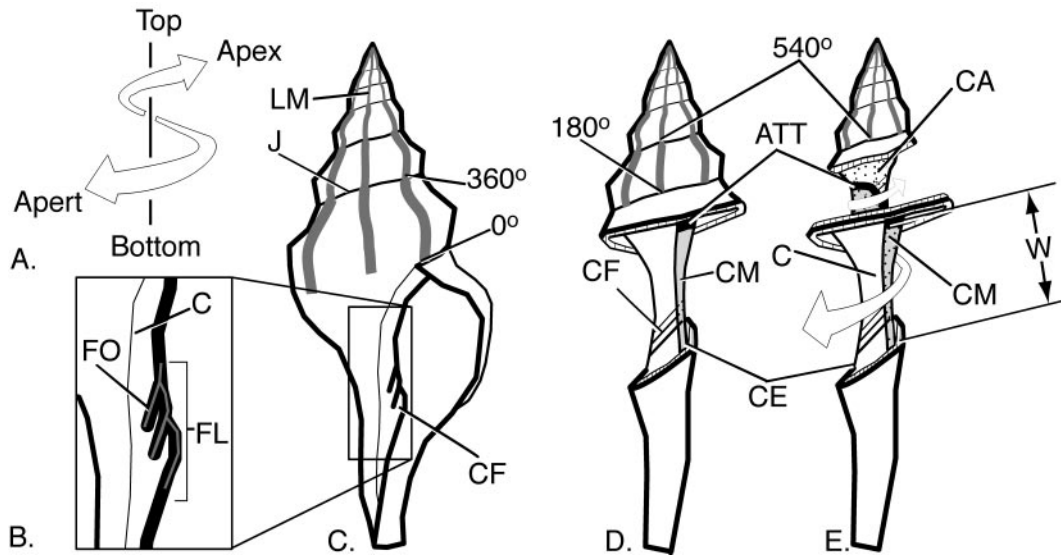


Figure 1. Definition of terms describing columellar morphology illustrated with *Triplofusus giganteus*. (A) *Top* refers to the part of a whorl relatively closer to the apex along a vertical axis (*adapical* in some literature, e.g., Vermeij, 1978); *bottom* refers to the lower part of that same axis (*abapical*). The open arrows wrap around the axis from the aperture to the apex. The *apertural* point of the muscle attachment is that closest to the aperture (also see D), and the *apical-most* point is that immediately after the attachment crosses the folds (also see E). *Width* (*W*) is the dimension from the *top* and to the *bottom* of a structure (e.g., the muscle is shown at its widest point in D and E; see also Fig. 2E). (B) Close-up of the inner lip of the aperture. The fold modifier is calculated as the ratio of FO:FL, where FL is the minimum length between folds, and FO is the outline of the folds (see Equation 1 in Materials and Methods). (C) Intact shell. (D) Same shell rotated 180° with sections of the shell exterior removed. (E) Same view as in D, but another 360° of shell exterior has been removed, and open arrows indicate the apertural-apical axis. Cut surfaces are indicated by fine, vertical hatching. Degrees indicate the *depth* of the landmark (LM): i.e., the number of degrees between the landmark and the aperture. The columellar muscle is shaded gray and passes over the columellar folds; the attachment area is a thick black line that is slightly exaggerated for illustrative purposes; stipples mark the area of the columella throughout the length of the attachment (CA). *Abbreviations*: Apert, aperture; ATT, columellar muscle attachment; C, columella; CA, columellar area; CE, edge of the columella; CF, columellar folds; CM, area of contact between columellar muscle and columella; FO, length of fold outline; FL, minimum length of folds; J, junction between two whorls; LM, landmark line; W, width.

bottom of the whorl, this measurement could be made in relaxed, contracted, and protracted animals. The position and length of the muscle attachment and the area under the attachment remain the same regardless of the animal's behavior. The exclusion of the area between the foot and the initial attachment was justifiable: it never included muscle that conformed to the morphology of the folds and was therefore not relevant to the present study; there was no reason to assume that it varied differently in species with and without folds.

Both the attachment area and the contact area were standardized to the columellar area (CA in Fig. 1; Table 3) to remove the effect of size. Columellar area is the area of the columella above, below, and including the length of attachment.

I used the depth of attachment as a proxy for depth of retraction, measured as the number of degrees behind the aperture. The two depths are intuitively related, because an animal cannot retract deeper than its attachment.

Depth of attachment was much easier to measure, and it is not subject to behavioral variation. This proxy assumes that the amount of space between the columellar muscle attachment of the contracted animal and its operculum varies equally among species with folds and those without.

The length of attachment was defined as the number of degrees between the depth of attachment and the apical edge of the attachment, which occurred in the crease between the columella and the rest of the shell (Fig. 2F, G). Some muscle fibers and connective tissue extended even farther apically beyond this point, but they were small and thus likely to function differently from the rest of the muscle. My methods were too coarse to dissect this tiny strand of tissue accurately.

Because the length of attachment and the depth of attachment were both measured as the number of revolutions behind the aperture (in degrees), they were independent of size and did not need to be standardized.

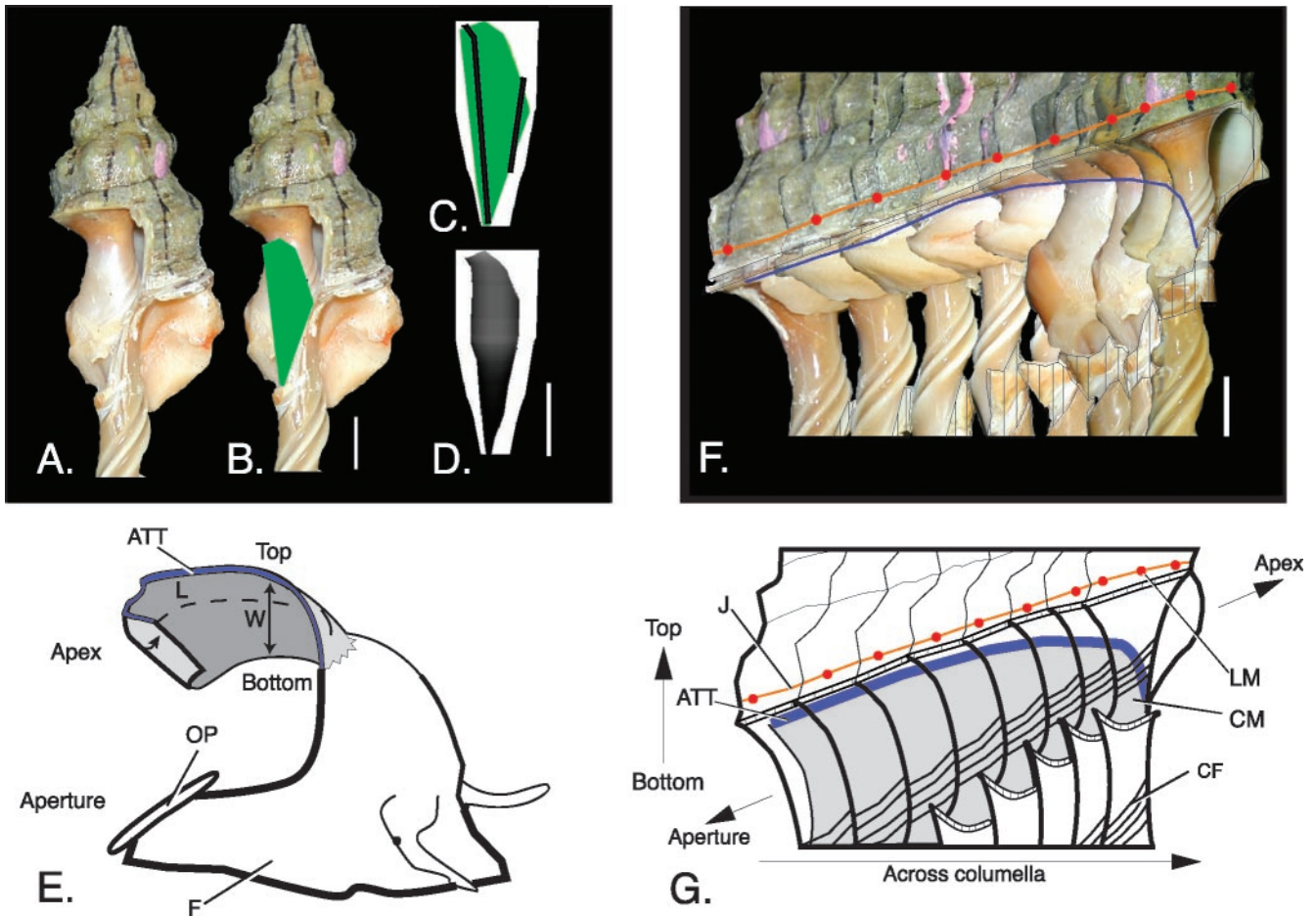


Figure 2. (A–D) An example illustrating the image processing technique. One photograph is shown from a series that documents the whole dissection. In this example, the contact area of *Triplofusus giganteus* (FMNH 299441) is reconstructed. The central landmark in this view has a depth of 540° (see Fig. 1D, E). (B) Contact area is highlighted (in green). (C) Comparison between contact area and a reference area (white). The reference area is a symmetrical stack of circles centered on the coiling axis (see Appendix). The black lines mark the middle 90° sector with the image (45° on either side of the landmark line), or “target area” as described in the Appendix. (D) Reconstructed contact area. Read the reconstruction as a topographic map: lighter points on the image are farther from the plane of the page. (E) Generic neogastropod with shell and viscera removed to illustrate the attachment and the manner in which the columellar muscle (in gray) coils throughout its length. The broken edge indicates that the columellar muscle grades into the musculature of the foot. Drawing based on figure 2 in Thompson, J. T., A. D. Lowe, and W. M. Kier. 1998. The columellar muscle of prosobranch gastropods: morphological zonation and its functional implications. *Invertebr. Biol.* **117**: 45–56. (F) Photographic montage and (G) graphic representation of the columellar muscle attachment in the same specimen illustrated in A and B. F and G are constructed from a series of photographs spliced together by aligning the attachment, and thus creating a single image that looks like an uncoiled, flattened shell. As indicated by the blue line, the attachment begins at the top of the whorl and gradually slides down the columella. Towards the apical end of the muscle, the angle of attachment changes dramatically, and the attachment crosses the folds. In F, much of the muscle has been removed to expose the columellar folds, but it remains largely intact in two of the views, demonstrating that the area of contact extends from the attachment to the bottom of the whorl. Hatching indicates shell broken to expose the columella and columellar muscle. All scale bars, 1 cm. *Abbreviations:* F, foot; L, length; OP, operculum; W, width; other abbreviations as in Figure 1.

Image processing

All measurements were taken from a series of digital photographs that documented each dissection. However, photographs distort three-dimensional data into two dimen-

sions, so the surface areas in the photographs under-represent true areas. I therefore developed a procedure to process the series of photographs for each dissection, as well as a geometric algorithm that calculates the three-dimensional

Table 3*Summary of measurements required to test hypotheses*

Measurement	Hypothesis	Use modifier to adjust for fold topography?
Standardized total area of muscle physically attached to the columella (ATT/CA in Fig. 1)	Maneuverability	Yes
Standardized surface area of contact between columella and columellar muscle throughout attachment when animal is relaxed (CM/CA in Fig. 1)	Guidance	Yes
Depth of attachment, in degrees	Retraction depth	No
Length of attachment, in degrees	Maneuverability	No

area projected into each photograph. The algorithm is presented in the Appendix.

Photographs were taken with a Nikon CoolPix 950 digital camera. The image plane of each photograph was parallel to the coiling axis, to which the optic axis was perpendicular. Each photograph also included a scale bar, was centered on a landmark, and showed the muscle attachment and the edge of the columella.

The outside of each shell was marked with radial lines running perpendicular to the junctions between whorls (LM in Fig. 1) every quarter of a whorl for small specimens and every eighth of a whorl for larger specimens (generally, those with shells longer than 2 cm). These landmark lines were used to locate the image within the series.

Each series consisted of between 4 and 12 photographs, with at least one photograph per quarter whorl. To create each series, all images were scaled and oriented identically. Only the middle 90° sector within each photograph was measured (45° on either side of the landmark line), thus eliminating overlap among the images (Fig. 2C).

The algorithm employed to estimate surface area converted tracings of photographs into three-dimensional surfaces (Fig. 2A–C). I used a mouse or mouse tablet (Wacom Graphire2) to trace the surface areas of the muscle attachment, the muscle in contact with the shell, and the columella within each image. Tracings were transformed according to the procedure described in the Appendix (implemented in MATLAB ver. 6.0; Fig. 2D), and then areas were summed from each image in the series. The procedure used to calculate the number of degrees from the aperture to the apertural and apical ends of the attachment is also described in the Appendix.

The 20 largest specimens were used to determine the precision of each measurement. I had two quarter-whorl series for these specimens, because they were photographed every eighth of a whorl; one series, for example, depicted the attachment centered on landmarks at 135°, 225°, 315°,

and 405°, and the other centered on landmarks at 180°, 270°, 360°, and 450°. I averaged the final measurements from the two series.

Fold modifier

The three-dimensional reconstruction technique employed here did not take into account the presence of folds. To account for folds, I employed a scale factor (“modifier”) defined as the length of the projected outline of the folds divided by the minimum distance between the two endpoints of the folds (Fig. 1B; Eq. 1):

$$\text{modifier} = \frac{\text{Length}_{\text{outline}}}{\text{Length}_{\text{minimum}}} \quad (1)$$

When there are no folds, the minimum length equals the length of the outline, so the lower limit of the modifier is 1.

I measured the fold modifier in each of the photographs of a series that clearly showed the folds’ silhouette. This measurement is highly sensitive to shell orientation and to the endpoints of the fold outline, for which I used inflection points. To compensate for this sensitivity, I always used the maximum fold modifier; this approach favored finding a statistical difference between groups with and without folds. Although there was considerable variability in fold modifier values within a species, the variance for the entire sample of species with folds was low (fold modifier variance, 0.003; range, 1.0 to 1.2; 114 measurements over 17 specimens).

After measuring the surface area of the columellar folds, and transforming it according to the algorithm in the Appendix, I applied Equation 2:

$$\text{Area}_{\text{final}} = A_T - A_{fT} + (A_{fT} \times \text{modifier}) \quad (2)$$

where A_T is the area transformed by the algorithm into an estimate of the area of a three-dimensional surface (either attachment area or contact area), and A_{fT} is the similarly transformed area of the folds. In other words, I subtracted the transformed fold area from the attachment area, multiplied it by the fold modifier, added the product back to the attachment area, and then repeated the adjustment for the contact area.

The total surface area of the columella was not adjusted, because that measurement is used to standardize the other metrics. If fold surface area were added to columellar area, the difference between species with and without folds would be erased.

Statistics

Statistical comparisons between species with folds and those lacking them were performed with a Mann-Whitney U test using StatView 5.0 for Windows.

Results

Morphology of columellar muscle attachment

These dissections demonstrated that the columellar muscle attachment in neogastropods is far more complex than previously assumed (by authors such as Linsley, 1978; Signor and Kat, 1984; Morita, 1993; Thompson *et al.*, 1998). The muscle did not leave a scar, so there was no macroscopic feature on the columella that approximated attachment shape. The shape of the attachment was similar in all species, regardless of the presence of folds (Fig. 2E–G). In most of the species examined, the attachment began about three-quarters of a whorl ($\sim 275^\circ$) back from the aperture (Fig. 3C, raw data for the histogram is in Table 4). The depth of attachment might be much deeper in high-spined shells. In *Terebra dislocata*, attachments began about two whorls back from the aperture (pers. obs.). *T. maculata* and *T. subulata*, muscles were previously reported to attach more deeply at about 2.5 and 4.5 whorls back from the aperture, respectively (Signor and Kat, 1984). In general, the attachment was long and sometimes extended for more than one revolution; it was restricted to the upper edge of the muscle (blue line is ATT in Fig. 2E–G).

The relative position of the attachment changed as it

moved from the apertural edge of the muscle across to the apical edge. At its most apertural end, the attachment lay immediately under the junction of the two whorls. Tracing it apically along the rising junction, the attachment shifted in position progressively downward and across the columella toward the bottom of the whorl, with the rate of descent gradually increasing. Shortly before reaching the bottom of the whorl, the angle of the attachment changed dramatically, becoming more oblique to the junction and curving sharply down to the base of the whorl; this is where the attachment crossed the folds when they were present. Here the muscle was remarkably less robust than elsewhere along its length. Farther apically, the muscle was only a thin extension situated in the crease between the columella and the base of the whorl. Within the area where the muscle was attached, the muscle width was maximal at its apertural end and minimal towards the apex.

Functional hypotheses

Contrary to the prediction drawn from the functional hypotheses outlined in the Introduction, no significant differences were found between species with and without folds with respect to the surface area of muscle attachment ($P =$

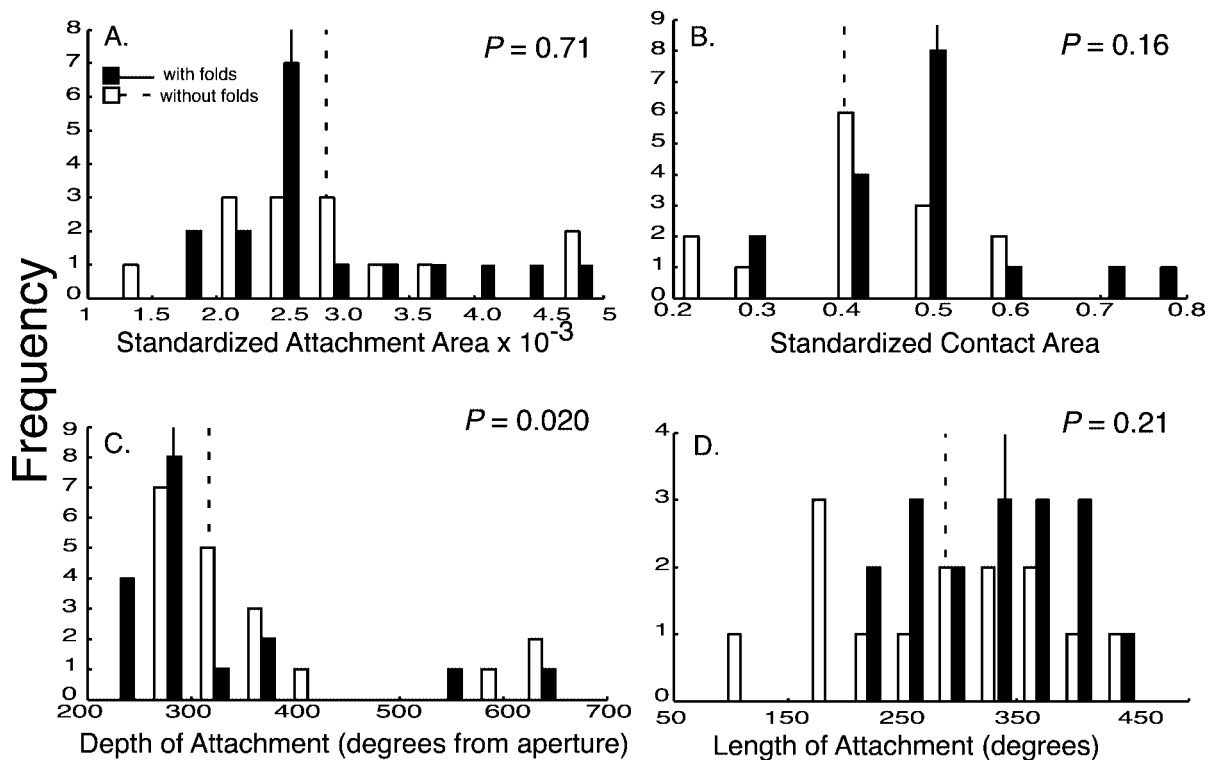


Figure 3. Frequency distribution of (A) attachment area, (B) contact area, (C) depth of attachment, and (D) length of attachment. Values for species with folds are indicated with black bars, and their median is a solid line; those without have white bars, median is dotted line. P values from Mann-Whitney U test. Raw data are provided in Table 4.

Table 4

Measurements for all specimens

Species	Museum ID	Folds	Standardized attachment area ($\times 10^{-3}$)	Standardized contact area	Depth of attachment (degrees)	Length of attachment (degrees)
<i>Busycon contrarium</i>	299449	Yes	3.0	0.5	210	330
<i>Busycon contrarium</i>	299459	Yes	2.7	0.5	240	330
<i>Busycon spiratum</i>	299444	Yes	2.2	0.4	280	230
<i>Busycon spiratum</i>	299463	Yes	1.8	0.3	270	290
<i>Busycon spiratum</i>	299468	Yes	2.3	0.4	270	450
<i>Busycon spiratum</i>	299472	Yes	2.4	0.4	250	270
<i>Cantharus cancellarius</i>	299486	Yes	3.6	0.5	350	330
<i>Cantharus cancellarius</i>	299487	Yes	3.4	0.3	360	230
<i>Fasciolaria hunteria</i>	299450	Yes	2.5	0.5	310	290
<i>Fasciolaria hunteria</i>	299492	Yes	2.4	0.5	280	370
<i>Fasciolaria hunteria</i>	299493	Yes	2.4	0.5	260	390
<i>Fasciolaria tulipa</i>	299448	Yes	2.5	0.6	260	390
<i>Fasciolaria tulipa</i>	299456	Yes	2.7	0.5	230	420
<i>Nassarius vibex</i>	299475	Yes	1.9	0.5	630	250
<i>Nassarius vibex</i>	299477	Yes	4.6	0.4	570	240
<i>Triplofusus giganteus</i>	299441	Yes	3.9	0.7	290	360
<i>Triplofusus giganteus</i>	299442	Yes	4.7	0.8	280	370
<i>Chicoreus florifer distans</i>	299451	No	3.1	0.4	270	190
<i>Chicoreus florifer distans</i>	299458	No	3.1	0.4	330	260
<i>Melongena corona</i>	299453	No	2.7	0.6	270	460
<i>Melongena corona</i>	299457	No	2.3	0.5	320	400
<i>Oliva sayana</i>	299460	No	ND	ND	280	ND
<i>Oliva sayana</i>	299461	No	ND	ND	260	ND
<i>Oliva sayana</i>	299465	No	ND	ND	370	ND
<i>Oliva sayana</i>	299466	No	ND	ND	300	ND
<i>Oliva sayana</i>	299469	No	ND	ND	290	ND
<i>Stramonita haemastoma</i>	299452	No	2.3	0.4	320	370
<i>Stramonita haemastoma</i>	299454	No	2.2	0.4	280	350
<i>Stramonita haemastoma</i>	301941	No	3.0	0.4	660	90
<i>Strombus alatus</i>	301942	No	2.4	0.2	590	210
<i>Strombus alatus</i>	301943	No	2.6	0.2	650	170
<i>Urosalpinx perrugata</i>	299481	No	1.2	0.4	310	300
<i>Urosalpinx perrugata</i>	299482	No	4.7	0.5	330	330
<i>Urosalpinx perrugata</i>	299483	No	3.4	0.3	360	320
<i>Urosalpinx perrugata</i>	299484	No	3.5	0.6	420	170
<i>Urosalpinx perrugata</i>	299485	No	5.0	0.5	360	310

ND—not determined. Measurements could not be taken because the columella is too delicate to survive dissection.

0.71), the amount of contact between muscle and columella ($P = 0.16$), or the length of muscle attachment ($P = 0.21$) (Fig. 3; Table 4). The depth of attachment in species with folds was significantly closer to the aperture than in species without folds ($P = 0.020$), which is opposite to what was predicted.

Three specimens (*Busycon contrarium* FMNH299449, *B. contrarium* FMNH299459, and *Cantharus cancellarius* FMNH299487) had fold modifier values close to unity (1.02, 1.00, and 1.04, respectively), meaning that the folds were almost flush with the columella and thus did not protrude enough to increase the topographic relief above that of specimens lacking folds. The data were consequently re-analyzed categorizing these three specimens as lacking

folds. Results were identical for the contact area, attachment area, and length of attachment. However, the depth of attachment was no longer significantly different between species with folds and those lacking them ($P = 0.19$).

Discussion

Using novel methods to examine the columellar muscle *in situ*, I have described, for the first time, the surprisingly complex attachment of the columellar muscle in neogastropods. This attachment is remarkable for its delicate structure, lack of discernible muscle scars, and considerable length. The data presented here do not support any of the previously published hypotheses thought to explain the

function of columellar folds, although the species included do not exhibit the most exuberantly developed folds. These data cast doubt on the hypothesis that folds act as struts, guiding the columellar muscle as the animal protracts from, and retracts into, its shell. Furthermore, the muscle probably does not need to be guided, because it is a muscular hydrostat. The muscle probably mirrors the morphology of the folds simply because it is physically adjacent to them. I reject the hypotheses that animals with folds maneuver better because their attachment area is larger, and that these animals avoid predators by retracting deeper. While I reject these hypotheses as general explanations for columellar fold function in neogastropods as a whole, future work may demonstrate that some of them adequately explain function within groups of species with similarly shaped folds.

Methodology

I developed two novel methods in this research: one is a practical dissection technique, and the other is an analytical approach for measuring distances and areas from photographs of specimens. With the dissection technique, I described the columellar muscle attachment in 20 neogastropods and one caenogastropod (Table 1). The soft tissues of other gastropods can be dissected without damaging the columella, and future applications of this approach will provide a more general understanding of how soft tissues are situated within the shell. With the analytical approach, I determined the relative position of soft tissues and shell features. I quantitatively characterized the columellar muscle in 11 species of neogastropods and one caenogastropod (Tables 1, 3, 4). This algorithm will work for any organism arranged as a stack of circles—echinoderms, cnidarians, foraminifera, diatoms, and mushrooms, for example.

The measurements and their analysis rest on three assumptions. First, to measure the area of contact between the columellar muscle and the columella, I assumed that the amount of muscle apertural to the attachment varies equally in species with and without folds. Second, I also assumed that the attachment area was only one pixel wide, which seems justified by the observation that the attachment was quite thin relative to the total width of the muscle. Third, areas were inflated by the algorithm that transformed the two-dimensional data, because the columella was assumed to be perfectly symmetrical (see Appendix). All of these assumptions were justified, especially in this first attempt to quantify differences in the columellar muscle. Furthermore, there is no *a priori* reason to believe that they would affect species with folds differently than species lacking them.

Note that the measurements are coarse (Table 4), and that subtle differences in contact area, attachment area, depth of attachment, and length of attachment cannot be resolved with these methods.

Columellar muscle attachment

The complexity and extent of the columellar muscle attachment has gone unrecognized because the attachment itself is remarkably delicate. A slight tug in the wrong direction (away from the aperture, toward the apex) tore the muscle from the columella, and the strength of the attachment deteriorated rapidly in preserved or relaxed animals. Its tenuous nature probably reflects the interplay between the physical mechanism of adhesion and the shape of the adhesive surface. Although the muscle was easily detached from the columella, the animal never naturally experiences a force pulling on the attachment from the angle or location I used during dissection; if the shell is opened as I have opened it, the animal is already too exposed to save itself from predators. An adhesive joint that can withstand large shear stresses will frequently fail when peeled (Portelli, 1986). The muscle attachment is analogous to a long piece of tape on a tabletop. When peeled backward, perpendicular to the table, the tape detaches easily, but when pulled along its length, it has great strength.

Surprisingly, none of the species studied here have muscle scars, even though scars are found in all major groups of shelled molluscs throughout their history (*e.g.*, Abbott, 1974; Lindberg, 1985; Pojeta, 1985; Doguzhaeva and Mutvei, 1996; Isaji *et al.*, 2002). In vertebrates, however, muscles frequently do not leave attachment scars (McGowan, 1982; Bryant and Seymour, 1990), especially when they insert directly into bone instead of attaching to a tendon which inserts into bone. In snails, the columellar muscle inserts into an epithelium that in turn inserts into the shell (Tompa and Watabe, 1976). Additional work is required to determine whether neogastropods and vertebrates lack muscle scars for similar reasons.

The fact that the muscle attachment is so much longer than previously thought (by authors such as Linsley, 1978; Morita, 1993; Thompson *et al.*, 1998) has interesting implications for how the columellar muscle functions. Thompson *et al.* (1998) calculated, on theoretical considerations, the force required to buckle the columellar muscle and the amount of torsion the muscle could exert. The muscle, which has a crescentic cross section, buckled more easily and could not exert as much torsional force as a cylindrical muscle with a circular cross section. However, these authors assumed that the muscle was attached at only one end rather than throughout its length. The true, side-long attachment should help the muscle resist compressive forces (J. T. Thompson, St. Joseph's University, pers. comm.), so that the mechanical disadvantage due to buckling may not be so severe. Similarly, the net torsion exerted must be reconsidered in light of the new attachment data.

Guidance

I hypothesized that folds guide the columellar muscle during protraction and retraction by protruding far into the columellar muscle, compelling the muscle to move along the folds. I reasoned further that, if folds act as struts, then they should protrude far enough into the muscle to significantly increase the amount of contact between the muscle and columella. For the species analyzed here, however, there was no significant difference in the contact area or length of attachment between species with folds and those lacking them. Therefore, the folds-as-struts hypothesis, as explained here, was not supported.

From another perspective, the methods were relatively coarse, and the data were poorly resolved. If these methods are applied to species with a subtle fold topology, then no significant increase in the contact area may be detectable. In species with folds, I compared the unadjusted contact area to the contact area after it had been adjusted with the fold modifier. The two metrics differed by more than 2% in only one specimen, and the average difference was only 0.2% ($n = 17$). This difference is less than the precision of the measurement of contact area, which is only to one decimal place. Therefore, although the topography of folds does not increase the contact area in the specimens considered here, the contact area may be greater in species with more prominent folds, such as those in the Volutidae, Mitridae, and Cancellariidae. Thus, additional work with these species and with methods providing higher resolution may lend support to the guidance hypothesis and its corollary that folds act as struts.

A consideration of the association between the area of contact and the presence of folds suggests other functional relationships. For example, if the advantage offered by strut-like folds were offset by the benefit of a large contact area, species with folds should have a significantly smaller area of contact than those without. Similarly, species with folds could have the same area of contact as species without folds, provided that the increase in contact due to the protruding folds was offset by a shorter attachment length. However, no such significant differences were observed.

The guidance hypothesis assumes that, without the resistance offered by folds, the muscle would slip along the columella during both retraction and protraction, presumably causing the animal to expend more energy. However, the arrangement of fibers within the muscle, including some fibers that are wound obliquely around the robust, apertural end of the muscle, suggests that the fibers, and not the columellar folds, control the path of the muscle when it contracts (Thompson *et al.*, 1998). Because the muscle is attached along its top edge (Fig. 2), it will shift across the columella when the oblique fibers contract. In light of this newer evidence about the columellar muscle in particular and the muscular hydrostats of molluscs in general (Kier

and Smith, 1985, 1990; Hodgson and Trueman, 1987; Kier, 1988; Marshall *et al.*, 1989), the muscle seems to function in the way that was previously thought inefficient (Signor and Kat, 1984).

Signor and Kat (1984) formulated the guidance hypothesis in part because Signor (1982) correlated burrowing behavior in high-spired gastropods with columellar folds: 55 of 59 burrowing species had folds, but only 1 in 46 non-burrowing species did. He concluded that columellar folds guide the columellar muscle in burrowing animals (Signor and Kat, 1984). However, 40 of his 59 burrowers were in the Terebridae, and only 4 of his 11 families included non-burrowing species. Thus, his results may be explained by phylogenetic bias and should be reanalyzed with comparative methods based on phylogenetic contrasts (as in Harvey and Pagel, 1991) once the appropriate estimates of relationship are available. The advantage of folds to burrowing animals is not obvious, especially if the guidance hypothesis is not true. Furthermore, many species with folds do not burrow (G. J. Vermeij, University of California, Davis, pers. comm.). The species considered here cannot be used to explore the relationship between folds and burrowing behavior, because species were not sampled randomly across burrowing and non-burrowing habitats.

In conclusion, since the inner surface of the columellar muscle is an exact impression of the columellar folds, it is only reasonable to assume that the muscle moves along the folds. This assumption does not require, however, that the folds dictate the muscle's motion. I suspect that the similarity in the shape of the muscle and folds is due simply to their proximity, and that the direction the muscle moves is governed instead by attachment morphology and muscle fiber orientation.

Maneuverability

As with contact area, the folds added insignificantly to the attachment area. The attachment was so long, and so much of it was distant from the folds, that the folds would need to protrude into the muscle six times more than they do (*i.e.*, multiply the fold modifier by 6) to significantly increase attachment area in species with folds at the $\alpha < 0.05$ level. If animals with folds are better able to maneuver their shells, it is not because they have a greater surface area of muscle attachment.

Signor and Kat (1984) suggested that the columellar muscle is divided by the folds into functionally discrete units joined only by connective tissue. However, judging by the observations presented here, the divisions they describe are probably part of a gradation between the robust, most apertural, part of the muscle and the weak, more apical part (left and right sides of Fig. 2E–G). Since Signor and Kat (1984) did not mention the frequency or placement of their histological sections, nor illustrate their results, it is difficult

to reevaluate their conclusions in the light of the newly recognized attachment morphology. The muscle histology of high-spired species with and without folds should be compared to determine whether the muscle is subdivided, and if so, whether those subdivisions are constrained by, or at least correspond to, columellar folds.

Maneuverability in a number of terrestrial pulmonates does appear to be enhanced by physically distinct subdivisions of the columellar muscle (Suvorov, 1993, 1999a, b, c). In these taxa, the columellar muscle originates from the most apical point (this is the only part of the muscle that is attached) and is divided into left and right pedal retractors, and left and right buccal mass retractors. These four branches continue to subdivide closer to the aperture. The four functional groups of the muscle are separated by a septum of connective tissue, but apertural teeth and columellar folds may play a secondary role in keeping subsets of branches separated. Thus, columellar ornamentation in pulmonates apparently evolved for a different reason than it did in neogastropods.

Predator avoidance

I was unable to substantiate Dall's (1894) claim that animals with folds retract deeper into their shells. Instead, animals lacking folds retract more deeply, because they have a significantly deeper attachment site. A larger sample of species is required to confirm this conclusion. Because all of the specimens from which quantitative data were obtained were collected in spring, seasonal variability in growth rate and attachment site were not considered. Variability in growth rate may be especially important to consider in genera such as *Busycon*, which exhibit highly episodic growth.

The depth of attachment was surprisingly constant among most species considered here (median depth = 295°; $n = 36$). Interestingly, the distribution of attachment depth was bimodal, with medians at 280° ($n = 31$) and 630° ($n = 5$) (Fig. 3), although, admittedly, there were few specimens in the higher mode. One species, *Stramonita haemastoma*, had specimens in both modes. This bimodality may reflect episodicity in growth.

Despite these overall similarities, the shallower attachment depth of species with folds implies that columellar folds do not help gastropods to escape from their predators by retracting into their shell. Moreover, my qualitative laboratory and field observations suggest that neogastropods are not subject to particularly intense predation. For example, a number of species (*Leucozonia nassa*, *Stramonita haemastoma*, *S. rustica*, *Pisania tinctoria*, and *Melongena corona*) neither retract quickly nor re-orient themselves quickly when their shells are overturned (pers. obs.). I have found wild specimens of *Latirus mediamericus* (fascio-

lariid, with folds) lying on coral, each with the aperture pointed upward and the foot hanging outside the aperture.

Do folds have a single function?

Although the function of columellar folds remains unidentified, there are potentially fruitful paths for future research. As discussed above, the correlation between the presence of folds and burrowing habit in high-spired gastropods (Signor, 1982) must be studied in more detail. Also, columellar folds might strengthen the shell, thereby protecting the animal from predators. External features of the shell, such as thickness and the presence of spines, have been shown to increase resistance to predators, making it more difficult for a durophagous predator to break a snail's shell (reviewed in Vermeij, 1993; Kohn, 1999). In fact, some species have a corrugated shell, which presumably increases strength while minimizing the costs associated with building and moving a heavy shell (Vermeij, 1993). Perhaps, in a similar manner, columellar folds protect against predators by increasing the strength of the inner lip while minimizing the cost of thickening the entire columella. Supporting this idea, Hughes and Elner (1979) report that crabs open *Nuccella lapillus* shells that have thin columellae by snapping the inner lip, breaking the shell in half. In contrast, *N. lapillus* individuals with thickened columellae are attacked at the apex of the shell. Both *N. lapillus* phenotypes lack folds, but these observations imply that strengthening the inner lip makes the columella harder to break more apically. However, predators of gastropods rarely break the inner lip. Most predators either crush the shell at its apex or peel back the shell at the outer lip (Vermeij, 1982; Johannesson, 1986); folds are simply ill-placed to affect either of these actions directly. There may be no evidence of damage on columella, as there are with failed attempts at predation in other parts of the shell, because when these attacks succeed, the shell fails catastrophically (G. J. Vermeij, pers. comm.).

Vermeij (1978) has observed that, within at least the families Vasidae and Mitridae, columellar folds are more common in tropical species, suggesting that the presence of folds might be correlated with latitude and increased predation intensity. This observation should certainly be tested quantitatively, although it is difficult to interpret the meaning of latitudinal diversity trends (Roy *et al.*, 1998).

Columellar folds may not have a function. Still, convergence is considered to be some of the best evidence for adaptation (Raup and Gould, 1974; Harvey and Pagel, 1991; Larson and Losos, 1996), and columellar folds have evolved at least six times within different families of neogastropods (Price, 2001). Direct observations on how fold shape has evolved over time may reveal patterns of evolution that are consistent with adaptation. This approach would require a phylogenetic context (Harvey and Pagel, 1991; Larson and Losos, 1996), although there are currently no well-resolved

phylogenies that include a sufficiently large number of species with and without folds to give power to such an analysis. With the phylogenetic comparative method, it would be possible to determine whether folds evolved by “hitchhiking” along with demonstrably adaptive characters (Maynard Smith and Haigh, 1974).

On the other hand, folds may have evolved as a common solution to a number of problems, especially because the function of folds in pulmonates obviously differs from that in neogastropods. Functional experiments on species from a number of smaller clades that contain closely related species with and without folds should be conducted to identify functions unique to those smaller clades. If columellar folds serve different functions in different clades, then they might be an easy-to-evolve, flexible solution to a number of problems.

Conclusions

Columellar folds probably do not guide the columellar muscle. Rather, the motion of the muscle is likely determined by muscle fiber orientation and by the attachment to the shell, which is along the upper edge of the muscle. The geometry of the attachment is probably responsible for its adhesive strength, and the weak physical connection between muscle and shell is not. The methods employed here show no association between the presence of folds and the length of columellar muscle attachment, surface area of attachment, or depth of attachment. The widely observed similarity in morphology between the folds and the columellar muscle may be due simply to their physical juxtaposition rather than to any functional relationship, with folds having some other and presently unknown functions. Because folds have evolved multiple times, the most plausible explanation for their existence might be that they are an easy-to-evolve solution to a number of functional demands.

Acknowledgments

I thank Dan Reuman, Moon Duchin, and Chris Degni for generously devoting their time to help develop the algorithm to reconstruct the 3-D surface of the shell. Jason Goodman and Gidon Eshel helped debug the programs that implemented the algorithm. Deirdre Gonsalves-Jackson, Greg Farley, Cheryl Swanson, Jessica Cande, the Smithsonian Marine Station, and the Florida State University Marine Lab helped with field collections. Jochen Gerber, Janeen Jones, and Martin Prydzia at the Field Museum of Natural History helped deposit specimens. Additional thanks to Michael LaBarbera, David Jablonski, Rüdiger Bieler, Susan Kidwell, Peter Wagner, and colleagues at the University of Chicago for support throughout this research and helpful comments on the manuscript. Geerat J. Vermeij, two anonymous reviewers, and especially Michael J. Green-

berg provided helpful suggestions that greatly improved this paper. This material is based upon work supported by the National Science Foundation under Grant No. 0073248. NSF grant 9903030 to David Jablonski, the Conchologists of America, the Link Foundation and Smithsonian Marine Station, and the University of Chicago Hinds and Gurley Funds provided additional support.

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Appendix

Measuring True Surface Area and Location on a Shell from a Series of Photographs

Measuring surface area

A photograph defines an *XY*-plane that contains an object (Appendix Fig. 1A), and the pixels within the object are “on,” whereas those outside the object are “off.” That object is a projection of a surface, *Z*, onto the *XY*-plane. The projected surface area, *SA_Z*, is defined as

$$SA_Z = \int \int_x \left(1 + \left(\frac{\partial z}{\partial x} \right)^2 + \left(\frac{\partial z}{\partial y} \right)^2 \right) dx dy \quad (A1)$$

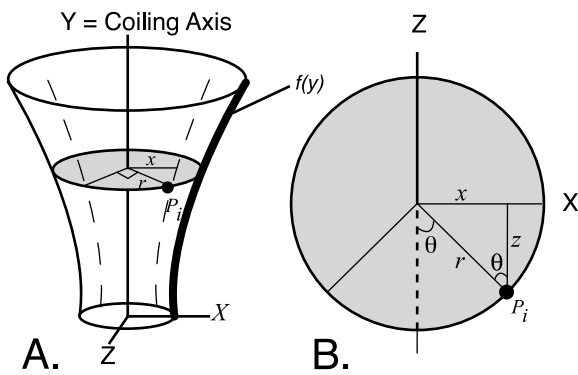
(equation 6a in Thomas and Finney, 1984).

Because a digital photograph is pixelated, I approximated *SA_Z* by calculating the surface area of each pixel, *i*, then summing those values over all *n* pixels:

$$SA_Z = \sum_{i=1}^n \sqrt{1 + \left(\frac{\Delta z}{\Delta x_i} \right)^2 + \left(\frac{\Delta z}{\Delta y_i} \right)^2} A_i dx dy, \quad (A2)$$

where *A_i* is the area of the pixel. Since the area of a pixel is 1, Equation A2 reduces to

$$SA_Z = \sum_{i=1}^n \sqrt{1 + \left(\frac{\Delta z}{\Delta x_i} \right)^2 + \left(\frac{\Delta z}{\Delta y_i} \right)^2} dx dy. \quad (A3)$$



Appendix Figure 1. (A) A shell in a photograph can be thought of as a stack of circles centered on a coiling axis, *Y*. *r* is the radius of a circle, measured as one-half the number of “on” pixels in a row. Dashed lines define the target region, 45° on either side of the coiling axis; *x* is the distance from the coiling axis to the edge of the target region. The curve in bold is *f*(*y*) = *z*. (B) The projection of a circle onto the *XZ*-plane. The *Y*-axis is perpendicular to the page. *θ* is the angle from the coiling axis, and the coiling axis always corresponds to a landmark line in these photographs. Since *r* is known, and *x* is the projected distance from the coiling axis to *P_i*, both *z* (the surface function) and *θ* (the angle between *P_i* and the landmark) can be calculated.

I found the partial derivative of *Z* with respect to *x* and the partial derivative of *Z* with respect to *y* by assuming that the object was a series of circles stacked on top of each other. Each row in the object corresponded to one circle, and all circles were centered about the *Y*-axis, which is also the coiling axis. Anyone familiar with the shape of neogastropods will recognize that the columella is somewhat asymmetrical. Imposing symmetry on the columella inflated true surface area, but this deviation was uniform across randomly sampled specimens with and without folds (28% inflation, st. dev. = 15%, *n* = 10).

Consider one row of pixels, *i*, of the photograph. The number of “on” pixels in that row equals the diameter of the circle, because the circle was projected straight onto that row. Thus, the circle’s radius and origin were both known, the surface function, *z_i*, for a given row was

$$x_i^2 + z_i^2 = r_i^2 \quad (A4)$$

(Appendix Fig. 1B), and the partial derivative was

$$\frac{\partial z_p}{\partial x_p} = \frac{-x_p}{\sqrt{r_i^2 + x_p^2}} \quad (A5)$$

where *p* was any pixel in row *i* of the object.

The partial derivative of *Z* in the *x*-direction represented the distortion of the projection due to the curvature of the circle about the coiling axis. Therefore, a pixel located immediately on or above the coiling axis did not have any horizontal distortion, and the two pixels 90° from the coiling axis were the most severely distorted.

To calculate the partial derivative of *Z* with respect to *y*, I expressed the edge of the image as *f*(*y*) = *r*. Rewriting Equation A4 gives:

$$x_i^2 + y_i^2 = f(y_i)^2. \quad (A6)$$

where *f*(*y*) was found empirically by fitting a second-degree polynomial to the edge. The partial derivative *∂z/∂y* was evaluated from this polynomial.

The resulting area was measured in pixels.

Identifying a target region

A curved surface projects a 180° sector onto a row, so when a series contains photographs taken every 90° about a coiling axis, the photographs overlap. I eliminated overlap by using only the middle of each image (dashed lines in Appendix Fig. 1). This approach has the added benefit of using the region of the photograph with the best focus and least distortion.

One row of “on” pixels represented a circle projected onto its diameter. The coiling axis marked the midpoint of the diameter, and the end points of the row were 45° and -45° from the coiling axis. If we draw the circle, we can see that its radius is the hypotenuse of an isosceles right triangle (Appendix Fig. 1B). The sides of this triangle equal the x -coordinate for the boundary of the target region.

Measuring degrees from aperture

I used the same circle to locate point P_i relative to the aperture (Appendix Fig. 1), to calculate the edges of the attachment. In this case, I determined the X -coordinate of the point from the photograph, which is simply the distance

between the coiling axis and P_i . Here, the X -coordinate was known, but the triangle was not necessarily isosceles, so the angle, θ , was not known. The angle that defined the arc between the coiling axis and P_i was calculated:

$$\theta = \text{asin}\left(\frac{x}{r}\right) \quad (\text{A7})$$

If P_i was apertural to the nearest landmark, I subtracted θ from the landmark’s depth. Suppose, for example, that P_i was depth of attachment, the curve $f(y)$ in Appendix Figure 1 was coincident with the landmark at 270° , and θ equals 20° . The value of attachment depth would be 290° . If P_i were apical to the nearest landmark, then I would have added θ to the landmark’s depth.