Muscles advance the teeth in sand dollars and other sea urchins

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SUMMARY

We demonstrate the action of the dental promoter muscles in advancing the continuously growing teeth of sand dollars and sea urchins. Teeth wear at the occlusal end, while new calcite is added to the opposite end. Dental ligaments rigidly hold teeth during chewing, but soften and reform during advancement. The source of forces that advance the teeth was unknown until our discovery of the dental promoter muscles. The muscles, which underly the tooth, attach centrally to the stereom of the pyramid of the Aristotle's lantern (jaw) and peripherally to a membrane that covers the distal end of the tooth. The muscles shorten along an axis nearly parallel to the long axis of the tooth. We stimulated contraction by addition of acetylcholine, with increasing concentrations of acetylcholine generating higher forces. Forces exerted by this muscle are appropriate for its size and are 1000 times lower than forces exerted by interpyramidal muscles that generate chewing forces. In sand dollars, a single muscle contraction of the dental promoter muscle can account for half the mean daily advancement of the teeth.

1. INTRODUCTION

A mechanical understanding of morphogenesis requires identification of both changes in material properties and sources of forces. These two factors can also be studied during maintenance of continually growing structures such as hair, fingernails, or teeth. Here, we investigate these factors during the advancement of the continuously growing teeth of sand dollars and sea urchins.

The teeth are mounted radially on jaw ossicles (pyramids) in a five-sided jaw (Aristotle's lantern). Each tooth is mounted in a tooth slide along which it advances during growth. Advancement occurs at $140\text{--}210\ \mu\text{m d}^{-1}$ in sea urchins (Holland 1965; Märkel 1969); for sand dollars, a measurement of this rate is presented in the current paper. As a tooth advances, new calcite is added to the distal end of each tooth, thus replacing older calcite worn off at the occlusal end during chewing (Märkel *et al.* 1977). Teeth are held in place (figure 1) against chewing forces (Ellers & Telford 1991) by collagenous dental ligaments (Birenheide & Motokawa 1996; Lelievre *et al.* 1995).

Changes in the material properties of the dental ligaments may be involved in tooth advancement. One hypothesis is that the ligaments, which normally hold the teeth rigidly in place, may soften periodically to allow advancement of the teeth. In fact, the teeth in sand dollars are sometimes naturally loose (Ellers & Telford 1996), and the material properties of sea urchin

dental ligaments can be altered experimentally using neurotransmitters (Birenheide *et al.* 1996). Indeed, these ligaments are a special collagenous tissue typical of echinoderms, called mutable collagenous tissue. The viscous and elastic material properties of such tissue can change over several orders of magnitude under nervous control (Wilkie 1996). In addition to such changes, a source of force is required to advance the teeth.

We have discovered tiny muscles under each tooth (figure 1) that are oriented such that they could advance the teeth (Ellers & Telford 1996; Telford & Ellers 1997*a,b*). These muscles, which we named dental promoter muscles, consist of myoepithelial cells that are part of the membrane around the tooth. In a 10 mm sand dollar with teeth approximately 1 mm in length, there are 150–200 myoepithelial cells on each side of the tooth. At their 'origin' they are attached by fibrils of collagen to trabeculae of the pyramid at the beginning of the tooth-slide. Their 'insertion' is continuous with the tooth membrane, of which these cells are a part.

In this paper, we test whether contracting dental promoter muscles generate sufficient force to advance teeth. Because the motor nervous system associated with Aristotle's lantern in echinoids is cholinergic (Cobb 1984), we used acetylcholine to stimulate muscle contractions in extracted tooth and pyramid preparations. Both forces generated, and resulting movements of the teeth, were measured and compared with advancement rates during growth. We investigated dental promoter muscle contractions in one sand dollar and two sea urchin species.

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Figure 1. (a) A schematic diagram of a longitudinal section through a tooth and a pyramid in a sand dollar. The dental ligaments attach the tooth to the pyramid by wrapping around pillar bridges of the porous calcite or stereom of the jaw and of the tooth. The dental promoter muscle is attached proximally to the stereom of the pyramid, and distally to a membrane surrounding the plumular end of the tooth. (b) A photograph showing an aboral view of a pyramid after tooth removal. m, muscle; s, tooth slide.

2. METHODS

Animals were collected as follows: sand dollars, *Dendraster excentricus*, near Los Angeles, California; sea urchins, *Eucidaris tribuloides* near Long Key, Florida, and *Strongylocentrotus purpuratus* near Bodega Head, California. All animals were kept in recirculating seawater aquaria in Davis, California at 13 °C for less than ten days prior to experimentation. Immediately before each experiment, Aristotle's lanterns were extracted, peripharyngeal membranes were removed, and pyramids were detached from each other by cutting the interpyramidal muscles. Each preparation therefore consisted of a pyramid with an attached tooth.

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To measure force exerted by dental promoter muscles when stimulated by acetylcholine, each isolated pyramid was held by a specially designed holder. For sand dollar pyramids, the distal end was supported by a U-shaped holder; for sea urchin pyramids, edges were gripped by an alligator clip. The mounted pyramids were attached, with tooth projecting upwards, to a motor-driven platform that could be driven at $80 \mu\text{m s}^{-1}$. Each pyramid was driven upwards until the occlusal surface of the tooth just began to push against a force beam situated above the tooth, i.e. until the force exerted on the beam was detectable but less than $2.5 \times 10^{-3} \text{ N}$. The signal from the force beam was amplified and digitized (14-bit) at 4 Hz, and displayed in real time using a signal analyser. Error in the force measurement was less than $\pm 2.5 \times 10^{-4} \text{ N}$. Within 30 s of halting the upward motion of the drive, each tooth and pyramid was gently flushed with 0.1 ml of acetylcholine at concentrations between 10^{-7} and 10^{-2} M in artificial sea-water (Dawson *et al.* 1959).

The force beam deforms slightly when detecting a force. Thus, the force recorded can be calibrated to reflect the distance that the tooth advanced while deforming the force beam. We calibrated distance (μm) as a function of force (N) by pushing a rod attached to a calibrated micromanipulator drive against the force beam.

Contraction of the dental promoter muscle in the Aristotle's lantern of *S. purpuratus* was also documented photographically. In *S. purpuratus*, the membrane to which the dental promoter muscle is attached has pigmented spots. We photographed these pigmented spots on an excised pyramid rigidly held under a dissecting microscope before and after application of 10^{-3} M acetylcholine. Distortion and movement of the dots reflects the contraction of the muscle investing the membrane.

Growth rate of the teeth of *D. excentricus* was measured using tetracycline marking. Individuals were kept in an aquarium for one month after collection, prior to being injected orally with 0.1 ml each of 40 mM of tetracycline-HCL (Ebert 1982). Two months after injection, individuals were sacrificed and their teeth were extracted. Addition of calcite to the plumular end of each tooth was measured relative to the tetracycline-marked growth line which was visualized using a multiband (254–366 nm) ultraviolet light.

3. RESULTS

Within 20 s following application of acetylcholine to the pyramid preparations from *D. excentricus* (figure 2a), *E. tribuloides* (figure 3a), and *S. purpuratus* (figure 3b), the dental promoter muscle contracted and force development began as the tooth pushed upwards against the force beam.

In 37 pyramids from eight *D. excentricus*, a cumulative dose response curve (figure 2b) shows that relative force was correlated with the logarithm of acetylcholine concentration, $\log[\text{Ach}]$ (non-parametric Spearman rank correlation coefficient = 0.80, $p = 0.0001$). Relative force was defined as the force developed by a dental promoter muscle stimulated by a given concentration of acetylcholine divided by the force developed by another dental promoter muscle on another pyramid from the same sand dollar stimulated with 10^{-3} M of acetylcholine. (Thus, the variance of the point shown for 10^{-3} M of acetylcholine is, of arithmetic necessity, equal to zero.) The significant dose response curve suggests that acetylcholine is stimulating the muscle, and provides a control for other imaginable sources of the measured force, such as physical stimulation of the

Figure 2. (a) Examples of force developed by the dental promoter muscle of an individual of *D. excentricus* when stimulated by the concentrations of acetylcholine shown. The muscle is stretched slightly to an initial force that stress-relaxes until acetylcholine is applied when indicated by the arrow. Lines drawn through sampled points are spline fits. (b) Dose response curve of maximum force developed by the dental promoter muscle when stimulated by concentrations of acetylcholine ranging over five orders of magnitude in several individuals of *D. excentricus*; this is a summary of many curves similar to those in (a). The numbers of pyramids tested are shown in brackets; error bars show 1 s.e.

preparation during addition of the test solution. (These other sources would be uncorrelated with acetylcholine concentration.)

As force developed, a tooth advanced in its tooth slide. The distance advanced was calibrated (correlation = 0.99) against the force measured. In *D. excentricus*, 37 teeth advanced an average of $2.6 \mu\text{m}$ (s.e. ± 0.3), and the maximum advancement was $6.7 \mu\text{m}$. Those advancements were opposed by the force beam pushing against the teeth. When not opposed by a

Figure 3. Force development by a dental promoter muscle of (a) the cidaroid urchin, *E. tribuloides* and (b) the camarodont urchin, *S. purpuratus*. Stimulation was by 10^{-3} M acetylcholine.

force beam, in one *S. purpuratus* an advancement of $25\text{ }\mu\text{m}$ was observed (measured using an eyepiece micrometer).

In all tested pyramids from the sand dollar *D. excentricus*, teeth pushed against the force beam in response to acetylcholine. By contrast, in both sea urchin species, only some of the teeth pushed against the force beam (three out of 15 tested pyramids in three *E. tribuloides*, and two out of 15 from three *S. purpuratus*). The absence of detectable force exerted by the teeth was not due to non-responsiveness of the dental promoter muscles, but to the rigidity with which the teeth were held in the dental slide. That the dental promoter muscle contracted could be seen in the visible deformation of the membrane of which the dental promoter muscle is a part.

In *S. purpuratus*, we documented membrane deformation photographically. Addition of acetylcholine caused the pigment spots on the membrane to distort and to move in the direction that the tooth would have moved had it not been too rigidly held in the dental slide (figure 4).

In 40 teeth from eight individuals of *D. excentricus*, mean tooth length was 5.9 mm (s.e. ± 0.087). Over a

period of 43–54 days, the plumular ends of the teeth grew an average of $13 \pm 0.49\text{ }\mu\text{m d}^{-1}$ or $0.22 \pm 0.009\%$ d^{-1} .

4. DISCUSSION

Muscle contractions and tooth movements, elicited by acetylcholine, demonstrate that the dental promoter muscles can advance echinoid teeth. In sand dollars, the advancement during a single contraction of the muscles ranged up to $6.7\text{ }\mu\text{m}$, and could presumably have been larger without the added resistance of the force beam. By comparison, the advancement of teeth during seven weeks of growth averaged $13\text{ }\mu\text{m d}^{-1}$. Similarly, in sea urchins we observed an unopposed, muscle-driven advancement of $25\text{ }\mu\text{m}$, while natural tooth advancement in sea urchins of a similar size was $140\text{--}210\text{ }\mu\text{m d}^{-1}$ (Holland 1965; Märkel 1969). These comparisons imply that the muscles contract several times each day to advance the teeth.

Force generated by the dental promoter muscle corresponds to the muscle's size and function. Maximum muscle stress is peak force divided by cross-sectional area. In *D. excentricus*, peak force was 0.007 N (upper trace, figure 2a) and cross-sectional area is estimated as 10^{-7} m^2 , implying a stress of $7 \times 10^4\text{ N m}^{-2}$. That figure is slightly below the maximum muscle stresses (1.6×10^5 to $2.9 \times 10^5\text{ N m}^{-2}$) reported in vertebrates (McMahon 1984), and is thus within expected bounds. Although dental promoter muscles exert sufficient force (0.007 N) to advance teeth, they generate insufficient force to contribute to biting. Biting forces (7 N ; Ellers & Telford 1991, 1996) are generated by much larger interpyramidal muscles connecting the pyramids.

That the teeth are moved by small advancement forces, but are not dislodged by large biting forces presents a paradox. Time-dependent phenomena such as stress-relaxation and creep of the dental ligaments (Birenheide *et al.* 1996; Ellers & Telford 1996) might resolve this paradox. Perhaps, small advancement forces over long periods of time cause permanent deformation, whereas high biting forces over short periods of time cause temporary deformation (as has been suggested for rat teeth by Chiba & Komatsu (1993)). In sea urchins, this mechanism is unlikely, since biting duration is $10\text{--}160\text{ s}$ in sand dollars (Ellers & Telford 1991), while the duration of dental promoter muscle contraction was less than 200 s , and 50% stress relaxation occurred over 200 s at biting forces (Ellers & Telford 1996).

Another hypothesis is that teeth are periodically loosened. We have observed naturally loose teeth in *D. excentricus* (Ellers & Telford 1996) and creep rates of other sea urchin teeth were modified using acetylcholine (Birenheide *et al.* 1996). Thus, advancement could involve remodelling of dental ligaments, presumably by some combination of fibrocytes and material property changes typical of mutable collagenous tissue.

Different degrees of loosening are required for advancement in sand dollars and sea urchins. In sand dollars, the dental promoter muscles always advanced the teeth, whereas in sea urchins, they rarely did so. This difference is in part due to the tightness of fit

Figure 4. Contraction of the dental promoter muscle in *S. purpuratus* can be detected as movement and distortion of pigmented epithelial spots. The spots change location (e.g. small arrow) and elongate as a result of muscle contraction that occurs after addition of acetylcholine. Compare left image (before) and right image (after) application of acetylcholine. Legends: p, pyramid; st, styloid process to which dental promoter muscle attaches; t, tooth; the large arrow indicates the direction in which the tooth would have advanced had it been loose. For scale, the tooth is 1 mm wide.

between each tooth and its pyramid. The teeth of sand dollars fall out when their dental ligaments are dissolved in bleach. Rearrangement of the collagenous fibres alone would be sufficient after or during dental promoter muscle contraction to allow tooth advancement. In sea urchins, however, teeth often remain held in place by the pyramids after bleaching. A pyramid consists of two hemipyramids that meet at a suture immediately underneath the midline of the tooth. The hemipyramids of sea urchins may exert lateral pressure that serves to stabilize a tooth even in the absence of dental ligaments. Thus, changes in both the dental ligaments and in the lateral pressure exerted by the hemipyramids must accompany tooth advancement in sea urchins.

Furthermore, the mounting and shape of the teeth differ between these two taxa. The teeth of sand dollars are straight and mounted nearly parallel to the flat oral surface; those of sea urchins are curved and angle upwards. Therefore, in sand dollars, biting forces are aligned with the long axis of the teeth; but in sea urchins, biting forces generate moments tending to rotate teeth. Correspondingly, ligaments of sand dollars are angled backwards (figure 1), nearly aligned with biting forces; but sea urchin ligaments are arranged in a crossed fashion (Birenheide & Motokawa 1996) that resists rotation. Thus, when dental promoter muscles contract, the ligaments presumably merely shift in angle in sand dollars, so allowing advancement; but in sea urchins, some of the crossed fibres directly oppose the contraction and thus tend to counteract advancement.

In both taxa, a combination of material property changes, remodelling of dental ligaments and a source

of force, e.g. dental muscle contraction, is required to advance teeth. There could be other forces advancing teeth (such as plumular pressures or fibrocytes among the dental ligaments), but the advancement forces and material properties that we measured suggest that other sources of force are unnecessary to explain tooth advancement in echinoids.

There are general similarities and specific differences between vertebrate and echinoid systems of tooth advancement. Material property changes, rearrangements of collagenous ligaments, and a source of force are required in both systems. For vertebrates, however, the source of force has not been definitively identified for continuously growing teeth (e.g. rats) or for teeth of limited eruption (e.g. dogs, humans). Although proximal addition of calcite could cause pressures that drive eruption, this mechanism is unlikely because removal of the calcifying proximal ends (roots) of the teeth in both rats and dogs fails to halt eruption (Moxham & Berkovitz 1982; Marks & Cahill 1984). Alternatively, ground substance of the periodontal ligament may generate eruption forces in rat teeth (Kirkham *et al.* 1993). And, although periodontal ligament cells from dogs can generate forces sufficient for eruption *in vitro* (Kasugai *et al.* 1990), that ligament may not be attached to adjacent bone during eruption (Marks & Cahill 1984). The dental follicle, however, is necessary for eruption and may mediate calcifying bone pushing up under the teeth (Marks *et al.* 1995).

In addition to eruption forces, changes in the extracellular matrix are necessary during morphogenesis in vertebrate teeth and other tissues. In mouse teeth, for example, receptors for interstitial matrix proteoglycans became strongly reactive during periods of extensive

morphogenetic movements, suggesting that the matrix is either being rearranged or stretched during these periods (Thesleff *et al.* 1988). In rats, the glycosylaminoglycan content of the periodontal ligament increased during accelerated eruption, but decreased in the absence of eruption relative to controls (Kirkham *et al.* 1993). Changes in extracellular matrix properties are known also in other systems. For instance, in gilts, softening of the cervix in late pregnancy is due to a reduction and reorganization of collagen bundles (Winn *et al.* 1993). Thus, collagenous tissues are constantly being rearranged, most especially during morphogenetic changes.

Hence, morphogenesis and maintenance of tissues involves a combination of material property changes, rearrangement of collagenous tissues, and forces generated by cells. For the specific case of advancement of continuously growing echinoid teeth, we have shown that material property changes and rearrangement of the dental ligaments act in concert with forces generated by dental promoter muscles.

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