

Sutural loosening and skeletal flexibility during growth: determination of drop-like shapes in sea urchins

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The shape of sea urchins may be determined mechanically by patterns of force analogous to those that determine the shape of a water droplet. This mechanical analogy implies skeletal flexibility at the time of growth. Although comprised of many rigid calcite plates, sutural collagenous ligaments could confer such flexibility if the sutures between plates loosened and acted as joints at the time of growth. We present experimental evidence of such flexibility associated with weight gain and growth. Over 13-, 4-, and 2-week periods, fed urchins (*Strongylocentrotus droebachiensis*) gained weight and developed looser sutures than unfed urchins that maintained or lost weight. Further, skeletons of fed urchins force-relaxed more than did those of unfed urchins and urchins with loose sutures force-relaxed more than those with tight sutures. Urchins (*Strongylocentrotus franciscanus*) fed for two and a half weeks, gained weight, also had looser skeletons and deposited calcite at sutural margins, whereas unfed ones did not. In field populations of *S. droebachiensis* the percentage having loose sutures varied with urchin diameter and reflected their size-specific growth rate. The association between feeding, weight gain, calcite deposition, force relaxation and sutural looseness supports the hypothesis that urchins deform flexibly while growing, thus determining their drop-like shapes.

Keywords: sea urchin; biomechanics; collagen; ligaments; growth; morphospace

1. INTRODUCTION

Thompson (1917) proposed that the shape of an urchin is determined by patterns of mechanical forces acting on its skeleton. His idea is based on an analogy with a liquid drop sitting on a solid surface. In a drop, forces due to pressure and surface tension determine the local radii of curvature of the surface. The analogous forces in urchins are due to coelomic pressure, podia and self-weight. Also essential to the analogy is that the skeletal surface of an urchin is flexible and thus able to conform freely to the pattern of forces imposed.

Thompson's original hypothesis has been examined in several papers. Some evidence supporting the theory has been presented (Dafni 1986); coelomic pressure has been measured (Ellers & Telford 1992); and explicit mechanical models have generated shapes that approximate reasonably to the shapes of urchins (Baron 1991; Ellers 1993). Most recently, the developmental morphospace generated by Ellers' model has been reviewed with other morphospaces (Foote 1997; Stone 1997; McGhee 1998;

Eble 2001). If they represent underlying causes of form, such morphospaces could be used to analyse macro-evolutionary trends. It is crucial to assess the validity of the underlying assumptions in morphospace models because the models will be interpreted to constrain or bias evolutionary trends. Thus, in this paper we are concerned with assessing the validity of one important assumption, namely the assumption of flexibility during growth.

Flexibility may seem unlikely since urchin skeletons are composed of rows of many calcium carbonate plates that are themselves quite rigid. The plates, however, meet at sutures, which are attached to each other in two ways. Firstly, calcite rods project from one plate into interlocking spaces on adjacent plates. Secondly, the plates are sewn together with collagenous sutural ligaments that pass through pores in the edges of the plates and wrap around struts called trabeculae located on adjacent plates. These sutural ligaments are sometimes responsible for all of the strength of the skeleton (Ellers *et al.* 1998). The sutures could act as flexible joints if the sutural gaps were sufficiently wide that the calcite projections did not interlock, thus leaving the ligaments as the principal determinants of sutural properties. In Thompson's hypothesis, that skeletal flexibility should be associated with growth.

Urchins increase in size by the addition of calcite to the surfaces and edges of plates and by the addition of new plates at the apex (Deutler 1926), but little evidence exists regarding changes at the sutures during growth. A narrowing of the sutural gaps was observed in *Heliocidaris erythrogramma*, which shrank under reduced rations

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(Constable 1993). Such shrinkage under reduced food availability has also been observed in *Strongylocentrotus purpuratus* (Ebert 1967) and in *Diadema antillarum* (Levitan 1991). Similarly, 23% of bleached skeletons of urchins (*S. purpuratus*) fed seaweed *ad libitum* for one year had visible sutural gaps, whereas none of an unfed group showed gaps (Ellers *et al.* 1998). However, that study was not designed to test for an association between growth and sutural gapping. No data were kept on weight or diameter changes and no tests were done to measure the mechanical impact of the gaps. Furthermore, the 1 year period of that experiment suggests that the unfed urchins may have been in an unusual physiological condition.

In this paper we specifically test for an association between feeding, weight change, calcite deposition and sutural looseness. We present short-term experiments with relevant weight and sutural looseness measurements taken at the beginning and end of the experiments. We test whether sutural loosening is reflected in the overall mechanical properties of the skeleton by performing force relaxation tests and testing for an association with sutural looseness. We test whether looseness is associated with calcite deposition and hence growth. Finally, we test whether there is an association between size-specific growth rates and sutural looseness in field populations.

2. MATERIAL AND METHODS

(a) *Growth and sutural changes in Strongylocentrotus droebachiensis*

For the 13-week growth experiment, 143 urchins (*S. droebachiensis*), between 25 mm and 82 mm in diameter, were collected subtidally in June from a kelp bed near East Bailey Island, Maine. For four- and two-week growth experiments, 91 urchins, between 43 mm and 71 mm in diameter, were collected subtidally in February from a coralline algae-encrusted ledge (i.e. an urchin barren) near Metinic Island, Maine.

At the start of the 13- and 4-week experiments urchins were assigned at random either to a pre-experimental (initial) group, an unfed group or a group fed mussel flesh *ad libitum*. Urchins were maintained in 380 l seawater aquaria at 9 °C. The two-week experiment was a continuation of the four-week experiment in which a random subset of the unfed group was fed mussel flesh *ad libitum* for an additional two weeks. Starting and ending wet weights (± 0.1 g) were obtained and at the end of the experiments, urchins were bleached and assayed to ascertain whether the bleached skeletons disarticulated into constituent plates under their own weight or whether they remained intact.

For the four- and two-week growth experiments, force relaxation tests were performed on urchin skeletons for all pre-experimental (initial) and experimental urchins. Prior to testing, spines were removed from the oral and aboral surfaces so that a force could be exerted directly onto the skeleton along that axis. Skeletons were compressed at 0.2 mm s^{-1} until a force of 15 N was reached and the subsequent decrease in force (force relaxation) was followed for 180 s. The force signal (error ± 0.01 N) was amplified and digitized (12 bit, 100 Hz). By definition, elastic solids (approximated by the calcite plates) do not exhibit force relaxation; by contrast, viscoelastic materials such as the ligaments do exhibit force relaxation (Wainwright *et al.* 1976). Thus the degree of relaxation should reflect changes in the ligamental contribution to the structural response. An experimental peak force of 15 N was chosen because it is much less than the break-

ing force of these urchins. (A force of 15 N is only 4% of the mean breaking force of intact *S. droebachiensis* of the same mean size ($n = 59$, mean weight = 69.4 ± 1.8 g, mean breaking force = 362 ± 13 N) that were collected at the same time from the same site as the urchins used in the 13-week experiment described above.)

To determine the relationship between size and sutural looseness, *S. droebachiensis* were collected during June from three intertidal sites (Giant Stairs, Ocean Point and Chamberlain, Maine), one subtidal kelp site (Jericho Island, Maine), and two subtidal urchin barren sites (Jericho Island and East Bailey Island, Maine). At each site, between 32 and 59 urchins were collected (total = 285 urchins), ranging in size from 23 mm to 84 mm in diameter. Test diameters were measured (± 0.7 mm) three times for each urchin. Urchins were then bleached, air-dried, and categorized as either disarticulated or intact.

To compare sutural looseness with growth, the size-specific growth rate was calculated using a Tanaka¹ function fitted by Russell *et al.* (1998). They present parameters of the Tanaka function for measurements of jaw height of *S. droebachiensis* urchins from their tide pools 2–7 in Maine; they also present a measured allometric power function relating jaw height to urchin diameter. As Russell *et al.* (1998) used those two relationships to determine a function that relates diameter to age, the authors also followed this method. The derivative of that function with respect to time was used to obtain a diametrical growth rate as a function of age. A parametric plot of these two functions of age produced a size distribution of growth rates to compare with the size distribution of looseness.

(b) *Sutural looseness and calcite accretion in Strongylocentrotus franciscanus*

To examine skeletal growth associated with feeding-induced weight gain, 25 *S. franciscanus*, collected subtidally near Bodega Head, California, were injected through the peristomial membrane with 0.2 ml each of 2 g per 100 ml (40 mM) tetracycline-HCL. The urchins were assigned at random to either an unfed group or a group fed mussel flesh *ad libitum*. Urchins were kept in a 300 l recirculating seawater aquarium at 10 °C under natural lighting conditions for two and a half weeks at the end of May. Individuals were weighed at the beginning and end of the period. Afterwards, all urchins were sacrificed, bleached and areas of calcite addition were visualized using a multiband (254–366 nm) UV light.

(c) *Calcium ions and ligament properties in Lytechinus pictus*

We tested whether sutural ligaments share, in common with mutable collagenous tissues (MCT), the property that they soften in solutions lacking calcium ions. To facilitate this, the breaking strength was determined of 54 *L. pictus* urchins that were assigned at random to one of three treatments: (i) soaked for 24 h in natural seawater, (ii) soaked for 24 h in divalent cation-free seawater (DCF) and (iii) bleached. The breaking strength was determined using the method described above for force relaxation except that skeletons were compressed until they broke.

3. RESULTS

(a) *Growth and sutural changes in S. droebachiensis*

The initial weights in the fed and the unfed *S. droebachiensis* were not significantly different for either the 13-week

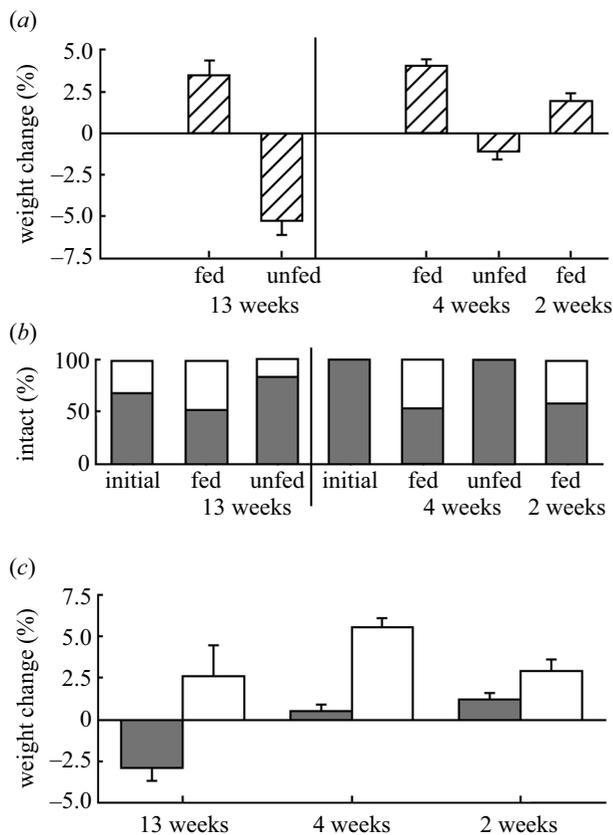


Figure 1. Growth and sutural loosening in *S. droebachiensis*. Fed urchins gained weight and unfed urchins lost weight (a). Feeding tended to loosen sutures (b) with the percentage remaining intact when bleached (grey bars) decreasing in all fed groups. Weight gains were highest in loose-sutured urchins (c). Error bars indicate one standard error.

experiment ($p = 0.52$, U -test; fed: $n = 25$, mean = 68.7 ± 2.4 g; unfed: $n = 28$, mean = 69.3 ± 3.1 g) or the four- and two-week experiment ($p = 0.57$, Kruskal–Wallis test; fed: $n = 38$, mean = 69.1 ± 2.9 g; unfed: $n = 22$, mean = 69.8 ± 3.6 g; unfed-then-fed: $n = 12$, mean = 74.7 ± 5.2 g).

During the 13-week period, weight change differed between fed and unfed *S. droebachiensis* ($p = 0.0001$, U -test), with fed urchins gaining 2.2 ± 0.7 g ($p = 0.005$, paired t -test) and unfed urchins losing 3.7 ± 0.4 g ($p < 0.0001$, paired t -test; figure 1a). Fed urchins were more likely to disarticulate under their own weight after bleaching than were unfed urchins ($p = 0.02$, χ^2 -test; figure 1b). The weight gain (figure 1c) of urchins that disarticulated was higher than the weight gain of those that remained intact ($p = 0.008$, U -test; 17 disarticulated with a mean weight change of 1.9 ± 1.1 g and 36 remained intact with a mean weight change of -2.2 ± 0.5 g). Both disarticulation and the feeding regime were predictive of weight change (multiple regression of weight change versus disarticulation and feeding regime: $r^2 = 0.57$; effect tests: disarticulation, $p < 0.01$; feeding regime $p < 0.0001$).

During the four-week period, weight change differed between fed and unfed urchins ($p = 0.0001$, U -test) with fed urchins gaining 2.5 ± 0.3 g ($p = 0.0001$, paired t -test) and unfed urchins losing 0.7 ± 0.1 g ($p = 0.0001$, paired

t -test; figure 1a). Fed urchins were more likely to disarticulate under their own weight after bleaching than were either unfed urchins ($p < 0.0001$, χ^2 -test, figure 1b) or the initial controls (19 initial urchins, $p < 0.0001$, χ^2 -test). No unfed urchins and no initial controls disarticulated.

For both the four- and the two-week experiments, the weight gain (figure 1c) of urchins that disarticulated was higher than the weight gain of those that remained intact (four weeks: $p = 0.0001$, U -test; 20 disarticulated with a mean weight change of 3.2 ± 0.3 g and 40 remained intact with a mean weight change of 0.4 ± 0.3 g; two weeks: $p = 0.06$, U -test; five disarticulated with a mean weight change of 1.9 ± 0.5 g) and seven remained intact with a mean weight change of 0.9 ± 0.3 g. At four weeks, both disarticulation and the feeding regime were predictive of weight change (multiple regression of weight versus disarticulation and feeding regime: $r^2 = 0.64$; effects tests: disarticulation, $p < 0.001$; feeding regime $p < 0.0001$).

There were differences in force relaxation after 180 s among the four treatments (figure 2b; $p = 0.03$, ANOVA). The only significant, pairwise comparison was that, at four weeks, fed urchins force-relaxed more after 180 s (mean fraction of peak force = 0.72 ± 0.018) than did unfed urchins (mean fraction of peak force = 0.81 ± 0.016 ; *a posteriori* $p = 0.003$, Fisher's protected least-significant difference (PLSD)). All other pairwise comparisons of means were not significantly different (all other *a posteriori* $p > 0.19$, Fisher's PLSD). Skeletons that disarticulated came from urchins that underwent greater force relaxation (figure 2c, mean fraction of peak force = 0.71 ± 0.017 , $n = 25$) than those that remained intact (mean fraction of peak force = 0.78 ± 0.013 , $n = 54$; $p = 0.0005$, U -test).

The size distribution of looseness in field populations (figure 3) resembled that of the size distribution of growth rates reported by Russell *et al.* (1998). Spearman's rank correlation statistics, calculated only for size categories collected from all six locations, indicated that smaller urchins were more likely to disarticulate ($p = 0.002$; $\rho = -0.5$).

(b) Sutural loosening and calcite accretion in *S. franciscanus*

Growth statistics for the tetracycline-marked *S. franciscanus* were as follows. The initial weights in the fed and the unfed groups were not significantly different ($p = 0.3$, U -test; fed: $n = 15$, mean = 299 ± 27 g; unfed: $n = 10$, mean = 370 ± 5 g). During the two-and-a-half-week period, the fed urchins gained an average of 6.5 ± 0.9 g (paired t -test, $p < 0.0001$). In contrast, the unfed urchins lost an average of 2.7 ± 1.3 g, which was a nearly significant loss (paired t -test, $p = 0.07$). These weight changes in the fed and unfed groups were significantly different from each other (U -test: $p < 0.0001$). The weight changes represented a gain of $2.5 \pm 0.4\%$ and a loss of $0.6 \pm 0.2\%$, respectively. When bleached, all urchin skeletons in the fed group disarticulated at plate edges under their own weight, whereas all skeletons from the unfed group remained intact. All skeletons in the fed group glowed at the plate margins when viewed under UV light, whereas those in the unfed group did not (figure 4). Thus, fed urchins were incorporating calcite whereas unfed urchins were not.

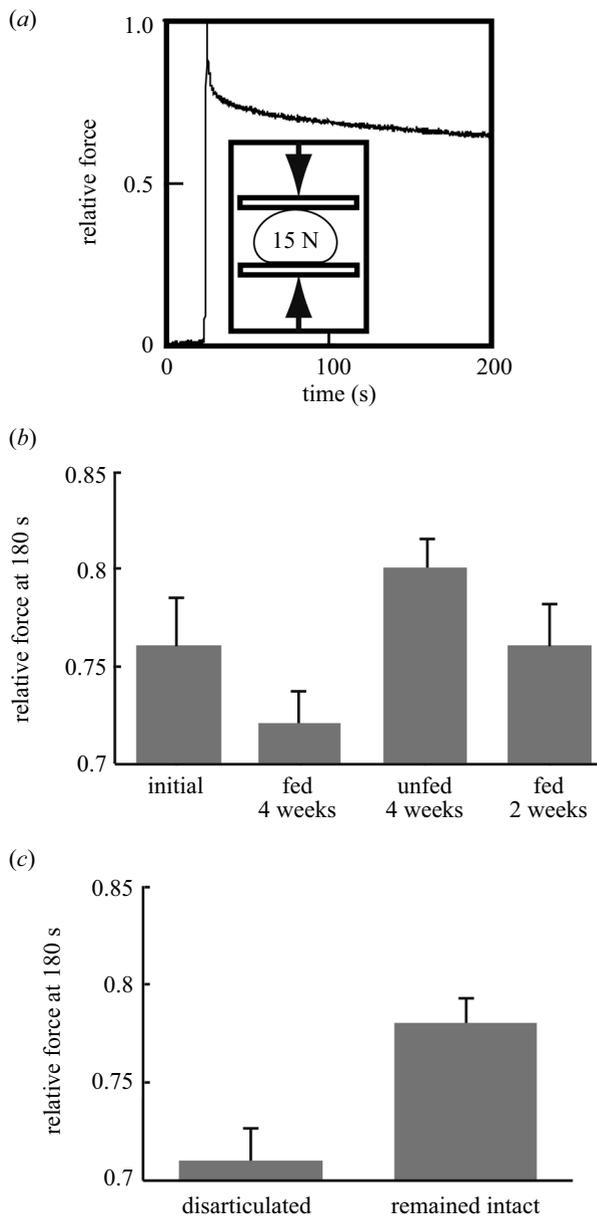


Figure 2. Force relaxation and sutural looseness in *S. droebachiensis*. Force relaxation was measured on whole urchins (a). The more an urchin force-relaxes the lower the relative force at 180 s. Fed urchin skeletons force-relaxed more than unfed urchins (b) and loose-sutured urchins force-relaxed more than did tight-sutured urchins (c). Thus, the material properties of an urchin's skeleton are changed by feeding and are associated with weight gain. Error bars indicate one standard error.

(c) Calcium ions and ligament properties in *L. pictus*

The breaking strength of *L. pictus* differed significantly between all treatments (overall ANOVA, $n = 18$ urchins per treatment, d.f. = 2,52, $p < 0.0001$ that mean breaking strength was the same for all treatments; *a posteriori* Fisher PLSD, each pairwise $p < 0.05$). The breaking strength was greatest for urchins soaked for 24 h in natural sea-water (mean = 45.4 ± 3.4 N), was reduced by soaking for 24 h in DCF (mean = 26.3 ± 1.5 N) and was least for the bleached urchin skeletons (mean = 6.2 ± 0.25 N). Since the bleached skeletons broke at the lowest force, sutural

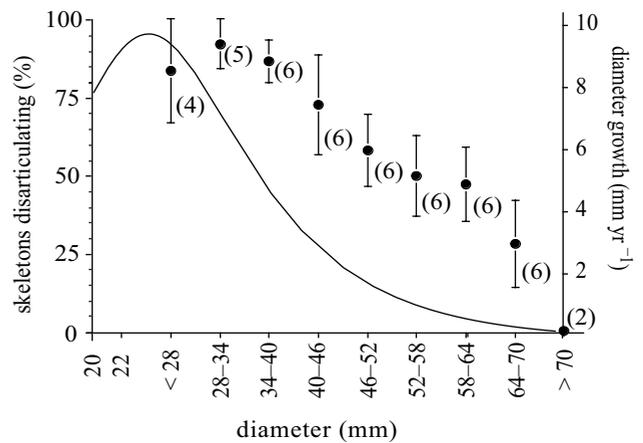


Figure 3. Diametrical growth rate (solid line, right axis) and percentage of skeletons disarticulating (points with s.e. bars, left axis) plotted as functions of diameter in *S. droebachiensis*. The diametrical growth rate shows a similar pattern of change with size as does the sutural looseness. Size-specific looseness was quantified by the percentage of bleached urchin skeletons in each size class that disarticulated. The disarticulation assay was done on 285 urchins sampled from six field sites in Maine. Numbers of locations represented in each size class are shown in brackets beside s.e. bars. Size-specific growth rates were estimated from Tanaka functions for the same species using data from Russell *et al.* (1998).

ligaments were responsible for the higher breaking forces in the other treatments.

4. DISCUSSION

The growth we quantified is associated with longer-term size increase, as can be seen in the associated deposition of calcite (figure 4). The growth rates observed in the current experiment can be compared with field growth rates. The fastest growing urchins, those with loose sutures, had growth rates of 5.5% and 2.9% by weight over four and two weeks, respectively (figure 1c). If sustained, these rates would translate into increases of 26% and 28% by diameter per year.² But field populations in the same size range (43–71 mm) have lower yearly diametrical growth rates of between 10% and 1%, respectively (figure 3). These discrepancies between field and laboratory growth rates suggest that these high rates are unsustainable for urchins of this size. Indeed, the growth rate in the fed group in the 13-week experiment was 3.5% by weight per 13 weeks (or 5% by diameter per year), which is more consistent with rates seen in the field. Thus, urchins show an initially rapid weight increase in response to the availability of food, but sustained growth appears more limited.

Field growth rates are highly variable and, in particular, field populations of smaller urchins (10–20 mm diameter) can have higher, diametrical, growth rates of 40–100% (Russell *et al.* 1998). The rapid response to food seen in the experimental groups in this study is one mechanism by which such variability may be generated. Growth rates observed in the four- and two-week experiments indicate that even larger urchins can double or triple growth rates at least temporarily.

During the periods of growth observed in our experimental urchins, sutures loosened (figure 1). Such loosening

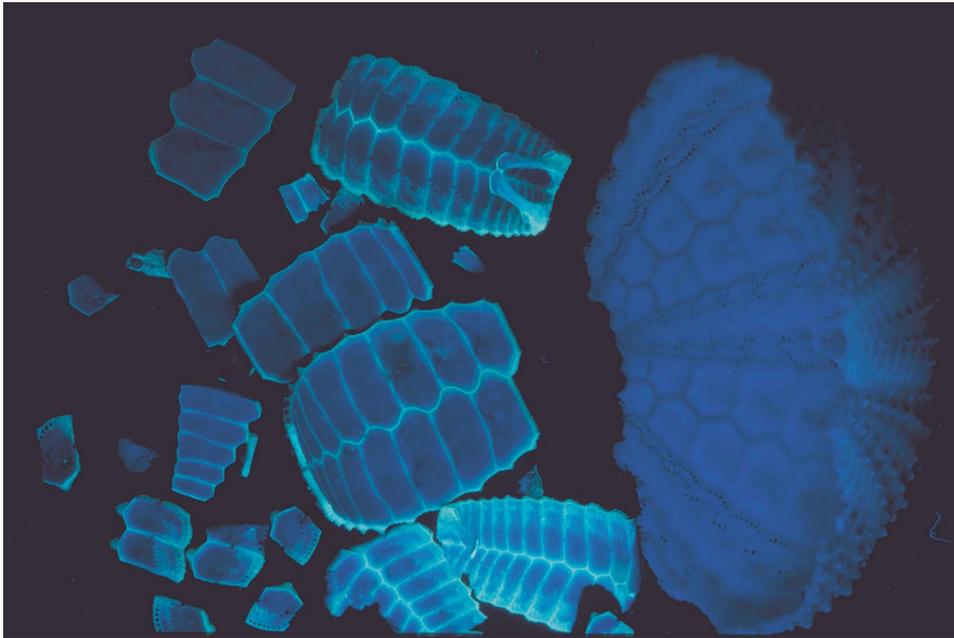


Figure 4. Bleached skeletons of *S. franciscanus* that had been injected with tetracycline label and were fed (left) and unfed (right) for two and a half weeks. Under UV illumination, skeletons of unfed urchins showed no tetracycline-marked lines indicating no addition of calcite whereas fed urchins showed such lines indicating addition of calcite at the plate edges. Fed urchins also disarticulated at plate margins under their own weight, whereas unfed urchins had to be broken to produce the photograph shown (note breaks across plates rather than along sutures).

ing is also seen in field populations where the proportion of loose-sutured urchins in a given size class is related to the growth rate typical of that size class (figure 3). Loose sutures allow the flexibility that is crucial to a mathematical model used to calculate urchin-like shapes from patterns of forces acting on the skeleton (Ellers 1993). Because loose sutures are bound together by sutural ligaments, the ligaments may play a role in growth.

Sutural ligaments presumably lengthen when sutural gaps widen and shorten when sutural gaps narrow. During growth, sutural collagenous ligaments may soften and elongate by creeping, under the influence of coelomic pressure. Coelomic pressure fluctuates above and below zero (Ellers & Telford 1992) and its magnitude is independent of urchin growth. If ligaments creep when pressure is positive and bend or shorten when pressure is negative, a net extension of the sutural ligaments could occur mechanically. Perhaps the material properties of sutural ligaments change such that creep is increased during growth.

Tissues that creep more can be expected to force-relax more and greater force relaxation was seen in growing, loose-sutured urchins (figure 2*b,c*). Loose-sutured urchins are those that, when bleached, would disarticulate under their own weight. Thus, in loose-sutured urchins, force relaxation reflects the material properties of the ligaments, the applied 15 N force being much higher than the force required to disarticulate the plates in the absence of ligaments (Ellers *et al.* 1998). For urchins with tighter sutures, force relaxation may be due to less soft ligaments and/or to some interactions between adjacent calcite projections at the sutures. Notwithstanding this, the skeletal plate–ligament composite force-relaxes more and thus presumably creeps more, in fed, growing urchins with loose sutures.

Growth-associated changes in ligamental material properties do occur in other echinoderm tissues. For example, the dental ligaments of sea urchins and sand dollars soften periodically (Ellers & Telford 1996) allowing teeth to be advanced by dental promoter muscles. Material property changes not associated with growth can also occur, as in echinoderm MCTs. MCTs can change from stiff to compliant states within seconds or minutes and are thought to be under nervous control (Wilkie 1996). Two examples of such tissues are the ligaments at the base of sea urchin spines and the body wall of sea cucumbers. Unlike vertebrate collagenous tissues, MCTs soften when exposed to a lowered calcium concentration. Dental ligaments (Ellers & Telford 1996) and sutural ligaments (*L. pictus* in this paper) also soften in Ca-free solutions. Sea urchin spine and sutural ligaments contain a novel protein, fibrosurfin, which may be unique to echinoderms (Cluzel *et al.* 2001). Because fibrosurfin contains epidermal growth factor-like domains, it might be responsible for some of the Ca-dependence in mechanical properties of MCTs (but see Trotter & Koob 1995). The molecular and Ca-dependency similarities between sutural and spine ligaments strengthen the speculation by Ellers & Telford (1996) that the mutable properties of MCT may have had evolutionary origins in tissues that were mutable during growth.

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ENDNOTES

¹The Tanaka function given in eqn (1) in Russell *et al.* (1998) contains a misprint. For the correct function see eqn (2) of Ebert *et al.* (1999). Furthermore, the correct Tanaka function used with the parameters reported in Russell *et al.* (1998) must be multiplied by a conversion of 10 (to convert cm to mm) to obtain the units for jaw height as shown in their fig. 5. Tanaka function parameters used were: $a = 34.448$; $d = -0.1616$; $f = 38.883$; $c = 11.7$ yr.

²Sample calculation: growth rate of 2.9% per two weeks by weight, if sustained, corresponds to a factor of $1.029^{26} = 2.1$ times per year and, assuming isometry, that fractional weight change corresponds to a diameter change of $2.1^{(1/3)} = 1.28$, or an increase of 28%.

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Errata

Proc. R. Soc. Lond. B 269, 215–220 (7 February 2002)

Sutural loosening and skeletal flexibility during growth: determination of drop-like shapes in sea urchins

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On page 215, the digital object identifier was incorrectly given. It should read 10.1098/rspb.2001.1881.

Proc. R. Soc. Lond. B 269, 455–460 (7 March 2002) (DOI 10.1098/rspb.2001.1909.)

Volatiles from potato plants infected with potato leafroll virus attract and arrest the virus vector, *Myzus persicae* (Homoptera: Aphididae)

Sanford D. Eigenbrode, Hongjian Ding, Patrick Shiel and Philip H. Berger

On page 458, in § 3e, and on page 459, in table 2, the compound listed as ‘cobebene’ should be listed as ‘cubebene’. On page 459, in table 2 and in paragraph 3 of § 4, the compound listed as ‘7-11-dimethyl-3-methyl-dodecatriene’ should be listed as ‘7,11 dimethyl-3-methylene dodecatriene’ or ‘ β -farnesene’.

Proc. R. Soc. Lond. B 269, 2381–2388 (22 November 2002) (DOI 10.1098/rspb.2001.2161.)

The evolution of anisogamy: a game-theoretic approach

M. G. Bulmer and G. A. Parker

On page 2383, below equation (2.8), the first sentence of the new paragraph should read:
We expect the best response function $R(m)$ to have a negative slope (the larger m_2 , the smaller the optimal value of m_1).