Ecology and physiology of mesohyl creep in *Chondrosia reniformis*

Lorenzo Parma(1*), Dario Fassini(1), Giorgio Bavestrello(2), Iain C. Wilkie(3), Francesco Bonasoro(4), Daniela Candida Carnevali(1)

(1) Dipartimento di Biologia “Luigi Gorini”, Università degli Studi di Milano, via Celoria 26, 20133 Milano. Italia. lorenzo.parma@unimi.it
(2) Istituto di Scienze del Mare, Università Politecnica delle Marche, via Brecce Bianche, 60131, Ancona. Italia
(3) Department of Biological and Biomedical Sciences, Glasgow Caledonian University, 70 Cowcaddens road, Glasgow G4 0BA, Scotland

Abstract: *Chondrosia reniformis* is a marine demosponge which consists mainly of a collagenous tissue known as ‘mesohyl’. Mesohyl is a very adaptable material: for example, it reacts to mechanical stimulation by stiffening. It has also been observed in nature that parts of sponges undergo slow elongation and attenuation resulting in the formation of propagules, a process which provides a means of asexual reproduction and dispersal. This phenomenon of mesohyl creep (slow progressive deformation) appears to be initiated by the fragmentation of the substratum to which the sponge is attached, and can be interpreted as a possible response to gravity. The aim of the present work was to provide more information on the creeping phenomenon of *C. reniformis* specimens in nature and to investigate the possible control of the phenomenon by the sponge itself. These aspects have been addressed using an integrated approach which consists of: 1) a field survey; 2) an experimental field study; 3) and an experimental laboratory study. Field survey: the phenomenon was explored in parallel in three different regions of the Italian coasts. The behaviour of specimens from the different areas was correlated with the nature of the substratum, the water temperature and the trophic conditions. Experimental field study: the creeping phenomenon was induced experimentally in the field by attaching weights (2 to 40 g) to the sponges. The consequent elongation, which was similar to the natural phenomenon, demonstrated that gravity is involved in creep. Experimental laboratory study: the effect on mesohyl tensility of changing parameters (temperature, salinity) was tested in physiological experiments. The results confirmed a close relationship between water temperature and mesohyl stiffness.

Keywords: collagenous tissue, creep, mechanical properties, mesohyl, propagule

Introduction

*Chondrosia reniformis* Nardo 1847 is a common demosponge (Lazoski et al. 2001) which lives on shaded rocky cliffs or caves at a depth of 1 to 50 m, and lacks siliceous spicules or the reinforcing spongin fibres present in many other members of the phylum Porifera (Garrone et al. 1975, Harrison and De Vos 1991). Its generic name is due to the cartilage-like consistency of its thick and dense collagenous cortex (ectosome) (Garrone et al. 1975), which is crossed only by branched inhalant channels (Bavestrello et al.1988). The sponge can generate long, attenuated outgrowths which extend from the parental body for up to 3 m (normal sponges being on average 5-20 cm in their maximal diameter) then detach and form a new sponge (propagule). These outgrowths can extend downwards, as if under the force of gravity. Less frequently small attenuated portions extend horizontally (personal observation). This capacity for creep (slow and progressive deformation) and elongation is known also in other species as *Oscarella lobularis* (Schmidt, 1862) (Sarà and Vacelet 1973) and *Chondrilla nucula* Schmidt, 1862 (Gaino and Pronzato 1983), all of which lack an organised endoskeleton of either spicules or spongin fibres.

This creeping phenomenon has been interpreted in different ways: a) It has been regarded as a form of asexual reproduction (Connes 1967, Gaino and Pronzato 1983, Bavestrello et al. 1998). Asexual reproduction by budding occurs in several sponges (see Simpson 1984 for a detailed review), and sometimes is accompanied by the development of long retractile filaments which usually do not detach from the parent body (Fell 1994). b) Bond and Harris (1988) suggested that these dynamic deformations can represent a sort of localised locomotion by parts of the sponge, possibly preceding asexual reproduction. c) They may be responses to environmental changes: the whole sponge body can slowly flatten and slide under compressive stress (Garrone et al. 1975) or stretch out under tensile stress such as develops when part of the substratum to which the sponge is adhering breaks off (Sarà and Vacelet 1973).

Recent studies demonstrated that *Chondrosia reniformis* is able to react to mechanical stimulation by stiffening its collagenous body. In fact when previously undisturbed specimens of *C. reniformis* in the sea or laboratory aquaria...
are touched repeatedly, they feel softer the first time they are touched than on second and subsequent occasions. Morphological studies of *C. reniformis* had not revealed the presence of enough potentially contractile cells to account for this phenomenon (Bonasoro et al. 2001, unpublished observations), so the response to mechanical stimulation is not due to contractile structures, but, as evidenced in other studies, is due to changing in mechanical properties of the extracellular matrix which are under cellular control (Wilkie et al. 2006, Wilkie et al. 2004a).

A well studied model showing a similar feature is the “mutable” collagenous tissue (MCT) of echinoderms, the variable tensility of which is neurally modulated and which is involved throughout the phylum in the energy-sparing maintenance of posture and in the rapid detachment of anatomical structures at autotomy (Trotter et al. 2000, Wilkie 2001, 2005). Recent evidence suggests that connective tissue mutability is not a unique feature of echinoderms but is an adaptive strategy present also in primitive animals such as sponges (Wilkie et al. 2004b).

The aim of the present work is to provide more information on the creeping phenomenon of *C. reniformis* specimens in nature in order to identify underlying mechanisms and how these are controlled. These aspects are being investigated by means of an integrated approach, which consists of: 1) a field survey; 2) an experimental field study; 3) and an experimental laboratory study.

**Materials and methods**

Field survey: The general morphology of the specimens of *C. reiniformis* and the creeping phenomena in natural condition were observed at two Italian locations (Trave Central Adriatic sea, Paraggi Eastern Ligurian sea) characterized by different trophic and edaphic conditions. In these places several specimens were photographed in different periods using a digital photocotocamera (Nikon Coolpix 8400 in Ikelite housing). The area and the outline of the specimens were calculated.

Experiments on intact animals: in the same areas and in Bergeggi, Western Ligurian sea, the creeping phenomenon was artificially induced by attaching either lead weights (5-40 g), (see Fig. 1 for attaching methods) or floats (about 4 g of buoyancy, rather spherical fishing float), or by dislodging part of the substratum to which the sponge was attached.

Experiments on isolated tissue samples: specimens of *C. reniformis* were collected by SCUBA divers at Bergeggi on the Italian Ligurian coast, then transported to the University of Milan and maintained in 50 l tanks of artificial seawater at 14-16°C. Beam-shaped samples 2.5 x 2.5 x 15 mm in size were cut from both the ectosome and choanosome regions and attached to a glass coverslip using cyanoacrylate cement, with exactly 10 mm protruding from the edge of the coverslip. A lead pellet (weighing 0.056 g) was attached to the free end of some samples using cyanoacrylate cement. The samples were left untouched overnight, then placed vertically in different test solutions, so that the lead pellet subjected them to a tensile force (Fig. 2).

**Results**

Specimens at Paraggi (Ligurian sea) live on a compact rocky cliff (maximum depth 20 m) and have compact and regular forms each covering an area ranging from 3 to 65 cm² (30.23±20.18 cm²; n=7). During the observation period they showed insignificant variation in either shape and size.

Specimens at Trave (Adriatic sea) live on a substratum mostly constituted by a bed of mussel shells (maximum
depth 8.5 m) and have complex, lobated shapes and cover areas from 200 to 400 cm$^2$ (mean 337.17 ± 152.66 cm$^2$; n=5), during the experimental time they showed a great changing in shape, often making difficult to recognize specimens from one month to the next.

Specimens at Bergeggi live on a substratum constituted of both solid rock and a more organic and loose material (maximum depth 5 m). Their sizes and shapes are similar to those of the Paraggi specimens (covering area 16.7 to 62 cm$^2$; mean 40.1 ± 17.14; n=7). Though the variation in both shape and size during the experimental time was insignificant, they showed more dynamicity than the Paraggi specimens, 3 to 7 new creeping phenomena being observed every months.

All ongoing creeping phenomena slowed down as water temperature fell and attenuated regions of sponges shortened by 50 - 66% by the time the temperature reached the minimum value (Fig. 3). During Spring, as the water temperature rose, all the attenuated regions started to elongate again.

Experiments on intact animals

In Paraggi part of the substratum to which the sponges were attached was dislodged, leaving 5 specimens with a part of the body subjected to the force of gravity. All 5 specimens underwent the creeping phenomenon, though at different rates (from 11 cm elongation in 3 months to 95 cm in 1 month).

The application of a lead weight always induced creep. The phenomenon was influenced by the weight of the lead pellet, the distance between the two attachment points of the sponge to the substratum and the diameter of the line. Results very similar to the natural creeping phenomenon were observed using a small weight (about 5 g) and an elastic strip (instead of a line) with a width of about 1 cm. The application of a weight accelerated the creeping process. The formation of the attenuated region and the final detachment of the propagule occurred generally over a few days (Fig. 4).

A weight greater than 15 g caused the line to pass through the sponge body without, however, separating the sponge into two parts, since the wound closed up soon after the passage of the line, leaving a visible scar (Fig. 5).

The application of a float induced the same creeping phenomenon but directed upwards.

Experiments on isolated tissue samples

The behaviour of the isolated samples was comparable to that of whole animals (Fig. 6). Samples without an attached lead pellet did not change in length over 3 days, whereas weighted samples elongated by up to 3 times their original length (all samples reached the maximum value in the experimental apparatus (Fig. 7).

Weighted samples treated with distilled water showed no elongation (Fig. 8). There was a positive correlation between the amount of elongation of weighted samples and temperature (Fig. 9). Removal of the lead pellet from weighted samples that had undergone elongation was followed by reshortening of the samples.

Fig. 3: Attenuated region of one specimen. The creeping phenomenon seemed to vary with sea temperature.

Fig. 4: Timelapse photographs of induced creep and propagule detachment.

Fig. 5: Scar (arrow) left after cord has passed through body.
Discussion

Specimens of *C. reniformis* from Trave and Paraggi showed completely different characteristics, both in terms of size and shape, and in terms of frequency of natural creeping.

Specimens at Paraggi are smaller and show a more compact shape while those observed at Trave are larger and lobate. During the observation time the former showed no dynamicity, whereas the latter showed much changing in shape, often making difficult to recognize specimens from one month to the next.

In these two regions the type of substratum is different. At Paraggi the cliff is solid and compact, while at Trave the substratum consists mostly of mussel shells. The natural instability of the latter type of substratum could at least

---

**Fig. 6:** Creeping of isolated samples weighted with 0.056 g lead pellets. **A.** time 0; **B.** 24h.

**Fig. 7:** Mean elongation after 3 days of isolated samples (n=5). **A.** samples weighted with 0.056 g lead pellets; **B.** samples with no attached weight; ec, ectosome; ch, choanosome.

**Fig. 8:** Mean elongation after 24 h of isolated samples weighted with 0.056 g lead pellets (n = 5; bars = standard deviations). asw, artificial seawater; dw, distilled water; ec, ectosome; ch, choanosome.
facilitate the frequent creeping shown by the Trave specimens. Regarding differences in size, it may be relevant that Paraggi has an oligotrophic environment with a low sedimentation rate, while at Trave the environment is eutrophic with a high sedimentation rate.

The observation that the large sponges of Trave are more dynamic than the small sponges of Paraggi suggests that the size of the sponge can influence the probability that creeping will take place. It appears that the Paraggi specimens are not intrinsically less dynamic than those at Trave, since fast creeping was observed in 5 Paraggi specimens after dislodgement of the substratum beneath them. It is feasible that the adaptive significance of creeping is that it reduces the size of large animals, large size being a disadvantage possibly because it increases the chance that a sponge will inhale its own waste products, which could have a deleterious effect on its metabolism. Such waste products could conceivably be the signal that triggers the onset of creeping in large animals (Fry 1979). However, in Bergeggi, where the substratum comprises both solid and loose rocks, and there were frequent creeping events (3 to 7 new per month), there was no correlation between the size of the sponge and its dynamicity. Most of the sponges at this site, which occur at a depths down to 3 m, are exposed to strong wave action which frequently causes the detachment of part of the sponge from the substratum. Thus the frequency of the creeping phenomenon may be more dependent on environmental factors than on intrinsic factors, such as size.

Both detachment of part of the substratum under a sponge, which imposed a tensile force on the “freed” part of the sponge, and the attachment of weights or floats, which also applied tension, resulted in creeping phenomena. Though such creep could be an entirely passive response to extraordinary tensile forces, we suspect that the animal may be able to exert some level of control over the phenomenon (such as modulation of the rate of creep), since there is strong evidence that the stiffness of the collagenous mesohyl of C. reniformis is variable and under direct cellular control (Wilkie et al. 2004a, 2006). We provided evidence for the latter in these experiments, since distilled water completely blocked the elongation of weighted tissue samples. This treatment kills cells by osmotic lysis, possibly resulting in the release of a factor that directly stiffens the collagenous mesohyl (Wilkie et al. 2006).

Our observations are consistent with the idea that temperature is positively correlated with the rate of creep. This may explain the evidence that creeping events in C. reniformis are more frequent in summer than at other times of the year (personal observation). We do not know yet whether water temperature affects directly the mechanical properties of the extracellular matrix or if the observed differences are due to a cell-mediated mechanism.

We also showed that removal of the lead pellet from weighted tissue samples that had elongated resulted in shortening of the samples. This is also shown by the attenuated “filament” of intact sponges once the propagule is detached. Since there are unlikely to be enough contractile cells in the mesohyl to explain such tissue retraction (Bonasoro et al. 2001), it appears to represent a passive elastic mechanism the molecular basis of which needs to be investigated, although the possible active involvement of cells cannot be dismissed completely.

The creeping phenomenon is just one manifestation of the mechanical adaptability of the collagenous mesohyl, which makes up the bulk of the body of C. reniformis. This tissue can also undergo rapid and reversible changes in stiffness, which depend on the direct cellular control of interactions between components of the extracellular matrix of the mesohyl, such as the collagen fibrils and the molecules that interconnect these fibrils (Wilkie et al. 2004a, 2006). At this stage the relationship between creeping and variable stiffness is not clear. Both, however, may depend on what is likely to be a primitive connective tissue feature, which is the absence of strong chemical bonds between the molecules responsible for cohesion between the collagen fibrils, a feature that the mesohyl shares with the mutable collagenous tissue of echinoderms (Wilkie et al. 2004b).

References


