

## Calcium granules in the mucus trails of three littorinid species

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The feet of terrestrial and freshwater snails has been shown to be important in calcium regulation, often secreting granules of  $\text{CaCO}_3$ . This phenomenon has not, until now, been observed in marine snails. Here I report the presence of  $\text{CaCO}_3$  granules in the trail mucus of *Littorina littorea*, *L. saxatilis* (both collected from Rhosneigr, Anglesey, Wales) and *L. obtusata* (collected from Derbyhaven, Isle of Man). Snails were allowed to crawl over "thermonox" (Bio-Rad) plastic coverslips in an aerial environment. The trail mucus produced was immediately fixed by immersing the coverslip in liquid nitrogen and then lyophilising the coverslip overnight. Coverslips were then cut into small pieces, carbon-coated and the mucus trails analysed for elemental composition by X-ray microanalysis in a Cambridge 360 SEM.

Of the single-metal granules observed in the mucus trails the most abundant were of Ca (means : *L. littorea*, 440  $\text{mm}^{-2}$  ; *L. saxatilis*, 401  $\text{mm}^{-2}$  ; *L. obtusata* 348  $\text{mm}^{-2}$ ) followed for each species by Si (maximum mean density : *L. saxatilis*, 120  $\text{mm}^{-2}$ ) and Fe (maximum mean density : *L. saxatilis*, 65  $\text{mm}^{-2}$ ) granules. Single-metal granules of Al, Ti, Mg and P were also found but only in the mucus trails of *L. obtusata*, perhaps reflecting its different collection site from the other two species. The mean size of the Ca granules varied significantly interspecifically (Kruskal-Wallis test :  $H = 66.3$ ,  $p = 0.000$ ) : *L. littorea*, 1.32  $\mu\text{m}$  diameter  $\pm 0.08 \mu\text{m}$ ,  $n = 143$  ; *L. saxatilis*, 1.80  $\mu\text{m} \pm 0.12$ ,  $n = 113$  ; *L. obtusata*, 2.14  $\mu\text{m} \pm 0.09$ ,  $n = 167$ . Granule diameter was taken as the mean of the longest and shortest visible axes. Most Ca granules (*L. littorea*, 80 %,  $n = 35$  ; *L. saxatilis* 57 %,  $n = 113$  ; *L. obtusata*, 69 %,  $n = 167$ ) were attached to or were embedded within microthreads of mucus (up to 1  $\mu\text{m}$  diameter) which tended to run parallel to the direction of locomotion. The significance of this is unknown although it may imply that the  $\text{CaCO}_3$  granules are secreted with the mucus. The density of granules was constant across the width of the mucus trail of each species.

Assuming a mucus trail width of 10 mm, that the  $\text{CaCO}_3$  granules are of calcite with a density of 2.71  $\text{g cm}^{-3}$  and that snails move 2  $\text{m day}^{-1}$ , the loss of Ca in mucus trails is 11.51  $\mu\text{g day}^{-1}$  for *L. littorea*, 26.33  $\mu\text{g day}^{-1}$  for *L. saxatilis* and 39.18  $\mu\text{g day}^{-1}$  for *L. obtusata*. These losses appear too small for pedal mucus to be a significant ionoregulatory route for Ca. The Ca in the trail may therefore perform other functions, for example indicating trail polarity.

Histological examination of the foot sole of *L. littorea* revealed an unidentified granular secretion from "cell type L4" (Shirbhatte & Cook, 1987). This secretion alone also stained positively for Ca. It is therefore likely that this cell type is the source of the Ca granules, although granule function remains unclear.

### REFERENCE

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## Population genetics of *Littorina striata* at a microgeographical scale

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*Littorina striata* is a planktotrophic developer and is therefore, according to the theories of Scheltema (1971) and Crisp (1978), expected to have great dispersal abilities, resulting in a high degree of gene flow between geographic populations. Scheltema (1971) and Crisp (1978) consider gene flow as a homogenizing force, counteracting population differentiation in both genetic and phenotypic traits. However, in contrast to these ideas *L. striata* exhibits a high degree of shell variation and heterogeneity, even on a microgeographical scale. This shell variation can be described by three combinable traits : (1) smooth vs. nodulous shells, (2) shells with or without a white band on the last whorl and (3) eroded or non-eroded specimens. In Ilheu de Vila Franca, a volcanic crater along the south coast of Sao Miguel (Azores), all these shell morphs co-exist, even though there appears to be a clear spatial differentiation : nodulous animals are mainly, but not exclusively, found on the sheltered inside of the crater, whereas the other types are proportionally more common on the wave-exposed outside of the crater.

In order to investigate whether this morphological heterogeneity was correlated with a genetic population substructuring, we surveyed five populations of *L. striata* from Ilheu de Vila Franca and one from Vila Franca on the mainland (Sao Miguel, Azores) by means of polyacrylamide gel electrophoresis of four polymorphic enzyme loci (GPI, PGD, MDH and MPI).

The genotype proportions in the six populations showed no deviations from Hardy-Weinberg equilibrium expectations. Interpopulation allele frequency heterogeneity, as tested by means of a contingency chi-square table analysis (Workman & Niswander, 1970), showed a significant ( $p < 0.05$ ) differentiation at MPI between populations from the inner and outer side of the crater, as well as between the outside populations only. Yet, no allozyme heterogeneity could be detected between any of the morphotypes. Similarly, no sex-linked differences were observed. Finally, Wright's F-statistics revealed no population differentiation ( $F_{st} = 0.14$ ) and accordingly, gene flow, as estimated from  $F_{st}$ , was high ( $N_m = 17.06$ ).

Hence, these preliminary results do not provide evidence for a correlation between morphological and allozyme variation in *L. striata*. However, many more morphological, physiological and genetic data are needed before the nature of the shell polymorphism of *L. striata* may possibly be understood.

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## Mate searching in a marine prosobranch, *Littorina littorea* : trail following and fractal dimension of movement

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We studied mate search behaviour in a quantitative way of the gastropod *Littorina littorea* in laboratory experiments during parts of their non-mating season (November 1992) and during their mating season (April-May 1993). Snails were sampled at one boulder shore on the north west coast of Sweden.