Stimulation of Food Species Growth by Limpet Mucus

Abstract. *The trails of mucus secreted by certain species of intertidal limpets serve as adhesive traps for the microalgae that are their primary food resource. In addition, the mucus trails of two solitary homing limpets,* Lottia gigantea *and* Collisella scabra, *stimulate growth of the microalgae that the limpets consume. In contrast, the trails of an aggregating limpet,* Collisella digitalis, *do not stimulate microalgal growth. These results and their possible ecological significance are interpreted in light of the differences in the behavioral repertoires of the three limpet species.*

All gastropods secrete trails of mucus as they crawl over the substrate. In addition to being essential for adhesion, this thin layer of mucus couples the force of pedal muscular contractions to the substrate, thus enabling locomotion (*1*). The energetic cost of locomotion is due primarily to the cost of mucus production, which, depending on the species, ranges from 9 to 26 percent of assimilated energy (*2*). This makes gastropod movement considerably more expensive than other nonburrowing forms of locomotion.

The high cost of gastropod locomotion may be defrayed by other functions of pedal mucus. We report here that mucus trails act as adhesive traps for the limpet's microalgal food source. In addition, two solitary homing species produce mucus trails that stimulate microalgal growth. This is important because limpets frequently retrace their trails, ingesting the mucus and any attached material.

We examined these provendering abilities in marine limpets during 1982 at the Bodega Marine Laboratory, Sonoma County, California. *Lottia gigantea, Collisella scabra*, and *Collisella digitalis* are limpets inhabiting rock faces in splash and upper intertidal zones of the exposed coast of western North America. All three are herbivores, grazing primarily on microalgae and encrusting algal forms (*3*). The specific grazing activities of the three species differ, however. *Lottia gigantea* actively defends discrete territories and maintains a home scar to which it returns at low tide (*4*). *Collisella scabra* is also solitary, maintains a home scar, and has a very restricted home range, but is not territorial (*5*). Because their homing behavior results in restricted home ranges, *L. gigantea* and *C. scabra* frequently retrace, cross, and feed upon their own old mucus trails. In contrast, *C. digitalis* generally does not home, ranges more widely, and frequently forms large aggregations (*6*).

We allowed limpets of each species to traverse glass plates. For each species, mucus from several individuals was collected from the plates, pooled, divided into equal portions (~0.05 cm^3), and spread evenly over one side of 12 Millipore filters (*7*). This procedure resulted in a mucus layer 10 to 50 μm thick, similar to that of feeding trails. We used the mucus-coated filters in laboratory experiments to study the effects of pedal mucus on algal adhesion and growth. Field experiments also were conducted to complement the laboratory results. In all the experiments we estimated microalgal biomass spectrophotometrically by using the chlorophyll analysis technique of Strickland and Parsons (*8*).

To assess the role of mucus as an adhesive trap, we attached the mucus-coated Millipore filters to rigid plastic sheets and suspended them in battery jars filled with circulating microalgal cultures. Filters not coated with mucus were used as controls in all experiments. As another control, coated filters were placed in jars containing filtered seawater (*9*). After 18 hours in the dark (to minimize algal growth), the filters were removed and the amount of microalgae adhering to the filters was determined.

To determine whether mucus acts as a growth stimulant, we applied portions of microalgae of equal biomass to mucus-coated filters after the filters were placed in Plexiglas wells located under plant Gro-lights (General Electric). The filters were removed after 7 days and algal biomass was determined.

Field experiments were conducted to determine the applicability of laboratory results to field conditions. Millipore filters coated with mucus and uncoated filters were attached to Plexiglas and anchored in a surge channel 1.7 m above the mean low water level. The algal biomass attached to the filters was determined from samples removed after 1 and 7 days. For the 1-day experiment it is assumed that differences in the amount of microalgae attached to the filters were due primarily to differences in mucus adhesiveness. The 7-day field experiment combined the effects of mucus acting as an adhesive trap and mucus acting as a microalgal growth stimulant.

The results suggest significant differences among the provendering abilities of the mucus trails produced by the three limpet species (Table 1) (*10*). Interspecific comparisons of the effect of pedal mucus on microalgal adhesion indicate that the sticky mucus trails of all three limpet species entrapped microalgae, but to various degrees depending on experimental conditions (*11*). In the laboratory the mucus of *L. gigantea* and *C. scabra* appeared to trap more algae than that of *C. digitalis*. However, the field studies suggest that the mucus of *C. scabra* was not significantly more adhesive than that of *C. digitalis*, although *L. gigantea* trails were still significantly stickier. The laboratory experiments indicate that, in addition to being adhesive, the mucus of *L. gigantea* and *C. scabra* increase the rate of microalgal growth. In contrast, the mucus of *C. digitalis* did not stimu-

Table 1. Effects of pedal mucus on microalgal adhesion and growth. Values (untransformed means ± standard errors) are milligrams of chlorophyll per 25 cm^2. Analysis of variance and mean separation by the Student-Newman-Keuls multiple range test were performed on logarithmically transformed data. For interspecific comparisons in each experiment, values with different superscripts are significantly different ($P < 0.05$). Sample size is indicated in parentheses.

Experiment	*Lottia gigantea* mucus	*Collisella scabra* mucus	*Collisella digitalis* mucus	Control (no mucus)	Control (filtered seawater)
		Laboratory			
Adhesion (18 hours)	78.3 ± 9.7^{a} (12)	67.5 ± 5.5^{a} (12)	26.5 ± 5.8^{b} (12)	18.2 ± 3.6^{b} (12)	0.0^{c} (8)
Growth (7 days)	72.9 ± 5.7^{a} (14)	56.2 ± 5.8^{b} (10)	35.6 ± 2.8^{c} (10)	39.2 ± 2.8^{c} (10)	0.0^{d} (8)
		Field site			
Adhesion (1 day)	26.2 ± 2.8^{a} (9)	14.5 ± 1.5^{b} (10)	9.4 ± 2.7^{b} (9)	5.0 ± 1.1^{c} (9)	
Adhesion and growth (7 days)	3.2 ± 0.41^{a} (12)	3.1 ± 0.48^{a} (11)	$2.2 \pm 0.17^{a,b}$ (10)	1.8 ± 0.28^{b} (9)	

late microalgal growth. Results from the field were consistent with those from the laboratory.

These interspecific differences in food growth stimulation may be understood by considering the interspecific differences in natural history and the energetic cost of mucus production (*12*). *Lottia gigantea* and *C. scabra* invest more energy in mucus per unit of body weight than *C. digitalis* (*13*). Mucus production accounts for approximately 23 percent of the energy budget of an individual *C. scabra*, compared to 20 percent for *C. digitalis* (*13*). This extra investment may be tolerated by *L. gigantea* and *C. scabra* because it brings greater returns by its growth-stimulating action. In order to receive these benefits, however, individuals must be separated and must remain in the same area to allow retracing and ingestion of previously laid trails. *Lottia gigantea* and *C. scabra* meet both conditions. Individual *L. gigantea* defend territories and return at low tide to home scars excavated in the rock (*3*). *Collisella scabra* maintain home scars, move an average of only 12 to 15 cm in a feeding cycle (*13, 14*), and retrace their own trails (*5*). Thus, members of both species may effectively, and often exclusively, benefit from any increase in food caused by their mucus. Production of a nutrient-rich provendering mucus may allow these limpets to restrict their foraging distance to ensure return to their home scars.

In contrast, production of a provendering mucus by *C. digitalis* might not be advantageous because these limpets commonly follow the trails of conspecifics and form large intraspecific aggregations. Aggregations presumably prevent a limpet from exclusively receiving the nutritional benefits derived from nutrient-rich mucus. A "cheater" could produce a low-cost mucus while benefiting from the high-cost mucus produced by others (*12*). *Collisella digitalis* individuals have larger home ranges and are more migratory than the other two limpet species, and thus would not be as effective at retracing their own mucus trails.

These experiments show that pedal mucus, previously thought to be associated primarily with adhesive locomotion, may also play an important role in the feeding biology of herbivorous limpets. The mucus of all species examined acts as an adhesive that traps food species. In addition, a territorial species and a homing species produce mucus that stimulates algal growth; these limpets may be viewed as farming algae for their exclusive use. With the large energetic cost of mucus production in gastropods, it is perhaps not surprising that these secretions serve multiple functions and that some may be specifically tailored to the animal's social and feeding ecology.

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References and Notes

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4. Many limpet species form snug depressions on the substrate and return to them after each foraging bout. When on this home scar, they are more resistant to desiccation and are more protected from predation, sand scouring, and dislodgment by waves.
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7. Millipore filters were used as the substrate to minimize error during chlorophyll extraction and determination. The use of any other substrate would require microalgal collection by scrubbing the substrate and then filtering the resulting suspension.
8. J. D. H. Strickland and A. Parsons, *Bull. Fish. Res. Board Can.* **167** (1968).
9. The microalgae used in the laboratory experiments were primarily a mixture of diatoms (*Fragilaria, Navicula, Coscinodiscus, Nitzschia, Achnanthes, Skelatonema, Synedra*, and *Melosira*) and two unidentified species of unicellular green algae collected directly from the substrate of the same field sites where the *Collisella* species were collected. Diatoms are the primary food resource of both *Collisella* species, and all the diatom species included in the culture are consumed by the limpets. *Lottia gigantea* ingests diatoms, but also consumes significant amounts of blue-green or encrusting red algae; diet appears to vary with geographic location.
10. Interspecific comparisons of the effect of pedal mucus on microalgal adhesion and growth stimulation are based on an analysis of variance and mean separation (Student-Newman-Keuls multiple range test) performed on logarithmically transformed data.
11. Microalgal biomass was higher after 1 day of exposure than after 7 days. This is because the 1-day experiment was conducted during a phytoplankton bloom and the 7-day experiment was not. Limpets can utilize phytoplankton as an additional food resource.
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15. We thank R. Jensen and J. Allen for collecting *L. gigantea* and K. Rice, D. Phillips, J. Crowe, B. Johnson, and two anonymous reviewers for commenting on the manuscript. Supported by research grants from Sigma Xi and the University of California, Davis.
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O-Acetylation of Disialoganglioside GD_3 by Human Melanoma Cells Creates a Unique Antigenic Determinant

Abstract. *Monoclonal antibody Mab D1.1 recognizes on human melanoma cells a ganglioside antigen characterized by an alkali-labile* O*-acetylated sialic acid residue. Immunochemical analysis showed that this molecule is an* O*-acetylated product of the neuroectoderm-associated disialoganglioside GD_3. Controlled chemical* O*-acetylation of purified GD_3 resulted in the generation of this same epitope. Lysates of human melanoma cells were found to contain* O*-acetyltransferase activity capable of generating the antigenic epitope recognized by Mab D1.1. Thus, the addition of a single* O*-acetyl group to a common cell surface–associated ganglioside can create a potentially tumor-specific antigen.*

Gangliosides (sialic acid–bearing glycolipids) on the surface of normal and transformed eukaryotic cells have received attention in recent years because of their putative role in cell surface recognition phenomena (*1*). Monoclonal antibodies have been of great value in defining these carbohydrate structures and in investigating their role as tumor cell markers. Monoclonal antibodies that specifically recognize gangliosides associated with melanoma (*2, 3*), neuroblastoma (*4*), and colon carcinoma (*5*) have been reported. Levine *et al.* (*6*) described the monoclonal antibody Mab D1.1, which recognized a fetal rat neuroectoderm–associated antigen present on a ganglioside. We used Mab D1.1 to screen various human adult, fetal, and tumor tissues and showed that its reactivity in human tissues was restricted to melanomas (*7*).

We now report that the antigen specifically recognized by Mab D1.1 is the alkali-labile *O*-acetylated product of the neuroectoderm-associated disialoganglioside GD_3 (*8*). The first indication that Mab D1.1 recognized an *O*-acetylated ganglioside came from our original studies, indicating that alkali treatment of this melanoma-derived ganglioside caused a decrease in its migration on thin-layer chromatography (TLC) (*7*). To extend these observations, we isolated small amounts of the ganglioside and found that it migrated on TLC as a doublet between the monosialylated ganglioside standards GM_1 and GM_2. When