Table 1. Comparisons of CH (open) and CL (closed) flowers.

Structural features	CH (mean ± S.D.)	CL (mean ± S.D.)
Pollen size (μm)*	50.4 ± 0.09 (N = 5)	46.4 ± 0.9 (N = 6)
Papilla length (μm)*	78.5 ± 13.6 (N = 17)	114.5 ± 17.6 (N = 19)
Style length (mm)*	19.5 ± 1.2 (N = 8)	1.95 ± 0.2 (N = 9)

*CH-CL difference is statistically significant at $P < 0.01$, Mann-Whitney U test.

The stigma and pollen of the two floral types show structural differences (Table 1). The CH stigma lobes are receptive when reflexed (Fig. 1); the CL are receptive only after they have closed up on one another after reflexing (Fig. 2). The CH stigmatic papillae are flask-shaped (Fig. 3) and smaller than those of the CL stigma, which are more digitiform (Fig. 4). The SEM views (Figs. 5 and 6) confirm that CH pollen is larger than CL pollen and establish that differences exist in their exine structures (Figs. 7 to 10). The striate ridges in the sexine are thicker in the CL pollen, and the micropores in the tectum are smaller and fewer. The exine pattern seems less organized in the CL grain. Since the sexine is a storage area for compounds considered important in initial pollen-stigma interaction, these structural differences may be responsible for cross-incompatibility between the two.

Pollen hydrates, germinates, and penetrates the cuticle of the stigmatic papilla in both intra- and intermorph crosses. CH pollen shows a fourfold accelerated growth over that of the CL pollen (Table 2). Because of the tenfold difference in style length, fertilization takes about 3.4 ± 0.6 hours [mean ± standard deviation (S.D.)] in the CH flower and only 1.5 ± 0.3 hours in the CL flower. Intermorph pollinations did not result in fertilization. Growth rates were reduced, and most tubes failed to reach the ovary. Swellings appeared at the pollen tube tips in the CL on CH crosses. The crosses between CL on CH (N = 8) that were done in vivo failed to produce seed.

Collomia grandiflora produces two kinds of flowers, and they are not interfertile. It is not simply a case of genetic controls on incompatibility (*7*). The structural differences seen in the CL and CH flower are reminiscent of those seen in some heterostylous species, where differences in pollen size, exine characters, papilla size and shape, and style lengths characterize the two flower types on genetically different individuals (*3, 7*). Specifically, a reduction in style length and pollen size is correlated here as in the Polemoniaceae in general (*8*) and in many heterostylous species (*3*). However, in *Collomia*, the CL on CH cross

Table 2. Pollen tube growth rates.

Flower type	Pollen	Stigma	Rate (μm/min)
Within*	CH	CH	101.6
	CL	CL	23.2
Between	CH	CL	9.3
	CL	CH	10.8

*CH-CL difference is statistically significant at $P < 0.01$, Mann-Whitney U test.

demonstrates that the smaller CL pollen grain can produce a tube long enough in a CH style to effect fertilization. Another explanation is necessary to account for the incompatibility. Perhaps there are differences in stylar structure and secretions which slow tube growth, and since the growing tube may exert a stimulus on the ovary, the time of arrival of the tube in the ovary after pollination is critical for fertilization to proceed.

In tristylous taxa, two stamen types in one flower exhibit different compatibility relations. In both cleistogamy and heterostyly, differentiation can modify compatibility in a single genome.

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Subtidal Gastropods Consume Sulfur-Oxidizing Bacteria: Evidence from Coastal Hydrothermal Vents

Abstract. *The black abalone (*Haliotis cracherodii*), a commercially important shallow-water gastropod common off White Point, Southern California, is found frequently at subtidal hydrothermal vents within mats of filamentous sulfur-oxidizing bacteria. Foraging vent abalones actively consume the bacteria and confine their nightly feeding forays to bacterial mats surrounding the vents. The growth of abalones consuming the sulfur bacteria exceeds that of control individuals consuming microalgae and is comparable to reported growth rates of abalones consuming macroalgae. Thus, off White Point, the black abalone may derive a portion of its nutrition from the subsidy of geothermal energy.*

It has been suggested that some of the invertebrates inhabiting deep-ocean hydrothermal vents derive nutrition from the direct consumption of bacteria whose metabolic activities are driven by the oxidation of geothermally reduced sulfur compounds, primarily hydrogen sulfide (H_2S) (*1*). Even though hydrothermal vents also occur in shallow coastal waters (*2*), reports of subtidal invertebrates consuming sulfur-oxidizing bacteria have been limited to systems where sulfide is of biological rather than geothermal origin (*3*). Here I present evidence that hydrothermal vent sulfur bacteria can represent a nutritionally important source of energy to subtidal invertebrates.

My study arose from the discovery of black abalones occurring around subtidal zone (1 to 10 m in depth) hydrothermal vents within the eastern cove off White Point, Southern California (33°42′50″N, 118°19′00″W). The substratum immediately surrounding the vent openings is devoid of macroalgae and is covered with conspicuous white bacterial mats averaging 1 to 2 m^2 in area (maximum 14 m^2). The mats are comprised of a diverse assemblage of colorless sulfur bacteria, with large attached filaments of an unidentified bacterium closely resembling *Thiothrix* being the largest and most conspicuous component. *Thiothrix* obtains energy from the oxidation of H_2S and elemental sulfur, although its growth as an obligate chemoautotroph is still open to question (*4*). Individual filaments of this bacterium, containing refractile sulfur inclusions (*5*) (Fig. 1), range from 10 to 100 μm in width and grow to 15 mm in length. The filaments are intermittently bathed with sulfide-rich water of up to 140 μM H_2S (*6*) emitted from the vents.

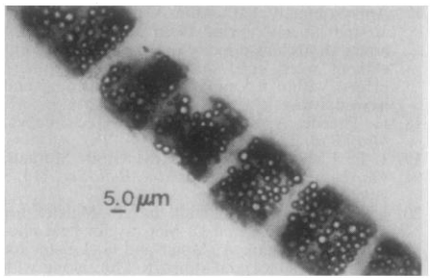

Fig. 1. Bright-field light micrograph of a segment of a bacterial filament showing intracellular sulfur inclusions (*5*).

Although the vent effluent (28°C) is initially warmer than the surrounding ambient water (13° to 18°C), it diffuses rapidly and there is no detectable temperature difference 2 cm away from the most active vent.

Closer observations of the vent abalones indicated that they graze on the bacteria, as evidenced by the characteristic radular scrape marks on the rocks covered with the bacterial mats (Fig. 2). Although intact bacterial filaments were rarely observed within vent abalone gut contents, probably because of the grinding action of the abalones' radular mechanism, filament fragments containing the characteristic sulfur inclusions were evident. In addition, free sulfur granules were observed in the intestinal lumen and contained inside amoebocyte cells within the digestive diverticula. Amoebocyte cells have been shown to eliminate indigestible material from abalones' digestive tracts (*7*). Further evidence of the consumption of sulfur bacteria came from sulfur analyses of vent and nonvent abalone gut contents (*8*). The sulfur content of vent abalones' guts varied between 0.5 and 32.6 percent of the total dry weight of the gut contents, with the highest percentages obtained in the posterior intestine. By contrast, no measurable sulfur was found in the digestive tracts of nonvent abalones collected off White Point.

Nocturnally foraging abalones actively consume the sulfur bacteria. I affixed movement monitors (*9*) to six vent and four comparably sized (50 to 70 mm) nonvent abalones in the late afternoon and returned the following morning to record the length of their nightly foraging movements. Vent abalones moved shorter distances [15.8 ± 6.6 cm, mean ± standard error of the mean (S.E.M.)] in their nightly feeding forays than their nonvent counterparts (235 ± 94.3 cm) and confined their foraging movements to the bacterial mats surrounding the vents. These limited foraging movements by vent abalones may reflect the relative abundance of available food at vent versus nonvent sites. Vent sites showed a higher standing crop biomass than surrounding substratum (*10*), and it has been demonstrated that black abalones display area-restricted foraging behavior when food is abundant (*11*).

The sulfur bacteria appear to be of nutritional value to abalones. Maricultured juvenile (17 to 25 mm) green abalones (*Haliotis fulgens*) (*12*), whose growth rates have been documented (*13*), were used in growth experiments along with young adult (41 to 48 mm) black abalones at vent and control sites. The abalones were marked with a triangular file on their anterior shell margin and placed in each of two polyvinyl chloride pipes (15 cm in diameter by 35 cm long) which had been capped with plastic screens and conditioned for 2 weeks prior to the experiment at the study sites. The vent pipe was positioned so that light incident on its interior and resultant photosynthetic activity were minimized, thereby maximizing the contribution of sulfur bacteria to any subsequent abalone growth. The control pipe, situated approximately 5 m away from the vent in an area with the same depth (12 m) and temperature (13° to 15°C), was similarly positioned to account for diatom settlement or any minimal photosynthetic activity that might occur. By the onset of the experiment, bacterial filaments had covered the inner and outer surfaces of the vent pipe, and a thin layer of pennate diatoms and other microalgae coated the control pipe. After 30 days, all abalones were retrieved and their new growth was measured from the score mark to the new shell margin. The vent abalones grew faster than the nonvent controls (Fig. 3), and some showed growth rates exceeding those reported for laboratory (green) and field (black) grown abalones consuming macroalgae (*14*). Although the relatively rapid growth of the vent abalones may have been partially supported by the consumption of plankton or detritus adherent on the bacterial mats, the major portion of the vent abalones' gut contents appeared to consist of the sulfur bacteria. In any case, the growth data indicate that the sulfur bacteria may be nutritionally sufficient for abalones when macroalgae are unavailable.

Fig. 2. Black abalone inhabiting a shallow-water hydrothermal vent. White filaments of sulfur-oxidizing bacteria grow attached to the abalone's shell and to the surrounding substratum. Radular scrape marks are visible on the substratum where the abalone has grazed the bacteria. The abalone pictured here is approximately 9 cm in length.

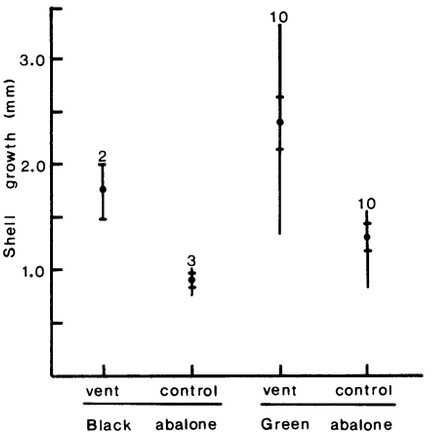

Fig. 3. Monthly (30-day) shell growth of abalones on diets consisting primarily of sulfur-oxidizing bacteria (vent) and microalgae (control). Data are presented as means ± S.E.M.; vertical bars represent ranges. For growth differences from the control groups, green vent abalone showed a $P < 0.001$ (Mann-Whitney U test); black vent abalone showed a $P < 0.2$.

Adult black abalones normally consume attached or drift macroalgae (*15*), and it is possible that a shortage of this food source may induce them to frequent the bacterial mats surrounding the vents. Abalones occur frequently at vents in areas of barren, rocky substratum, where densities of the purple sea urchin (*Strongylocentrotus purpuratus*), a trophic competitor (*16*), often exceed 100 per square meter. Urchins occur rarely at vent openings, and a preliminary experiment (*17*), along with evidence that echinoderms are less tolerant of H_2S than are gastropods (*18*), indicated that urchins may avoid vent areas. Thus, in foraging within the bacterial mats, the abalones appear able to exploit a re-

source not available to the urchins and thereby may benefit by minimizing diet overlap with a potential competitor.

Among the macroinvertebrates found at White Point, only the gastropods commonly occur at the vents. Besides the black abalone, the California sea hare (*Aplysia californica*), the giant keyhole limpet (*Megathura crenulata*), and the Norris top snail (*Norrisia norrisii*) graze around the vents. In addition, several species of limpets appear to graze the bacterial mats surrounding intertidal zone vents. A survey of other shallow-water vent sites may provide additional examples of this type of trophic relationship. Ciliates, zooflagellates, and insect larvae have been reported to ingest sulfur-oxidizing bacteria at terrestrial vent systems (*19*), but this is the first account of marine invertebrates consuming this unconventional food source at coastal hydrothermal vents.

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9. A 1-m length of monofilament (20-pound) line was threaded through the ratchet end of a plastic cable-tie that was cemented to the substratum adjacent to an abalone. The line was oriented so that it could be pulled through the cable-tie only in the direction of abalone movement. It was then attached to the abalone's shell at the third or fourth respiratory pore. In this way, the maximum nightly foraging radius could be determined by measuring the length of line pulled through the cable-tie. Foraging distances exceeding 1 m were estimated from the abalone's point of origin to its displaced position the following day (straight line).
10. Epilithic biomass was scraped and suctioned from a 15.3-cm^2 quadrat sampled at intervals along a transect line originating from a vent. Standing crop biomass (measured as ash-free dry weight) declined from 1.3 mg/cm^2, 10 cm from the vent opening, to 0.02 mg/cm^2, 50 cm from the vent opening.
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Somatic Activation of *ras*^{K} Gene in a Human Ovarian Carcinoma

Abstract. *A tumor isolate from a patient with serous cystadenocarcinoma of the ovary contained an activated* ras^K *gene detected by transfection of NIH/3T3 cells. In contrast, DNA from normal cells of the same patient lacked transforming activity, indicating that activation of this transforming gene was the consequence of somatic mutation in the neoplastic cells. The transforming gene product displayed an electrophoretic mobility in sodium dodecyl sulfate–polyacrylamide gels that differed from the mobilities of* ras^K *transforming proteins in other tumors, indicating that a previously undescribed mutation was responsible for activation of* ras^K *in this ovarian carcinoma.*

Transfection experiments with DNA have led to the identification of activated cellular transforming genes in a wide variety of human tumor cell types (*1*). In many cases, these genes are related to the transforming genes of Harvey and Kirsten murine sarcoma viruses, *ras*^{H} and *ras*^{K}. In particular, a human homolog of *ras*^{H} is responsible for the transforming activity of DNA from single human bladder and lung carcinoma cell lines (*2–5*). Activation of a human homolog of *ras*^{K} seems to be more common, however, since this gene accounts for the transforming activity detected in DNA from human carcinoma cell lines of the colon, lung, gallbladder, and urinary bladder and from an acute lymphocytic leukemia cell line (*2, 6–9*). Furthermore, primary tumor isolates of human lung, colon, and pancreatic carcinomas as well as a rhabdomyosarcoma contain an activated form of this gene (*6*). Finally, activation of a third member of the human *ras* gene family, *ras*^{N}, also occurs in many tumor cell types including neuroblastoma, fibrosarcoma, leukemia, and lymphoma cell lines, as well as primary tumor isolates of a colon carcinoma and of an acute myelogenous leukemia (*9, 10*). However, *ras* gene activation has been detected in only about 20 percent of neoplasms, in contrast to other transforming genes, such as *Blym*-1 and *Tlym*-I, which are activated in most (80 to 100 percent) lymphoid tumors of specific cell types (*11, 12*). This suggests that *ras* activation may contribute to the development of neoplasia in a fraction of many types of tumors, but may not be a necessary event for the development of any particular form of cancer.

Ovarian carcinoma is one of the most prevalent forms of cancer in women. Of the many histological subtypes of this disease, the most common is serous cystadenocarcinoma of the ovary, which appears to represent neoplastic transformation of the ovarian surface epithelium. Previous experiments (*6, 13*) in which a limited number of ovarian carcinoma samples were screened failed to detect the presence of activated transforming genes. However, since *ras* activation occurs in only a small percentage of tumors of a given type, we screened DNA from additional samples of ovarian carcinoma for transforming activity in DNA transfection assays.

Ascites fluid was collected from five patients with serous cystadenocarcinoma of the ovary. Morphological examination revealed that these samples contained from 29 to 98 percent neoplastic cells. In one case, designated OVCA-1, the transfection of high molecular weight DNA extracted from ascites fluid cells induced transformation of NIH/3T3 cells. Since many human carcinomas contain activated *ras*^{K} genes, DNA's