Reports

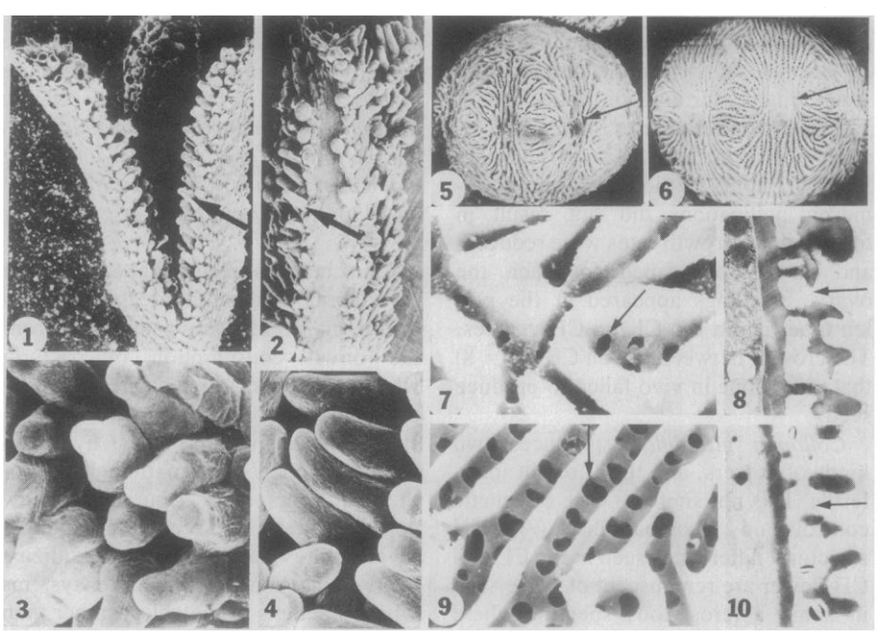
Incompatibility Between the Dimorphic Flowers of *Collomia grandiflora*, a Cleistogamous Species

Abstract. *The cleistogamous species* Collomia grandiflora *produces dimorphic cross-incompatible flowers on a single plant. The open or chasmogamous flower has smaller, flask-shaped stigmatic papillae, larger pollen grains, longer styles, and faster pollen tube growth rates down the style than the closed or cleistogamous flower. In intermorph crosses, pollen tube growth rates are greatly decreased and fertilization does not occur.*

Cleistogamy, which occurs in at least 58 families of the angiosperms, represents a mixed breeding system in which the CL (closed) flowers enforce inbreeding and the CH (open) flowers provide possible outbreeding (*1, 2*). Both CL and CH flowers are self-compatible. In contrast, heterostyly, occurring in 24 families, enforces outcrossing since intramorph pollinations are incompatible and all flowers on a plant are of the same morph (*3*). Little structural work has been done on the pollination biology of this self-compatible system in which the dimorphic flowers show a direct relation to function in the breeding system. Previous research suggests that the pollination process may be different for the two flower morphs of a single plant. In some cases, pollen grains never reach the stigma; instead, they germinate inside the reduced closed anthers in the CL flower and penetrate the wall with their tubes which grow to the nearby stigma (*2*). Anderson (*4*) detected another system of fertilization in four genera of the Malpighiaceae, in which pollen tubes grew directly to the ovary via filament and receptacle tissues. Unsuccessful attempts to cross-pollinate the two floral morphs of separate plants in *Salpiglossis sinuata* (*5*) were attributed to morphological differences in the styles of the two flower types. However, attempts to cross-pollinate the floral morphs on a single plant remain untested.

In the annual *Collomia grandiflora* (Polemoniaceae), the two flower types are produced sequentially in the inflorescence. The CL flower is characterized by reduced androecia, corolla, and style lengths; there are fewer and smaller pollen grains as well as precocious stigma receptivity, anther dehiscence, and hence accelerated seed production compared to the CH flower (*6*). We now describe our study of the pollen and stigma forms and behavior in the pollination process of *Collomia grandiflora*. We attempted intermorph pollinations to establish whether compatible crosses could be made between these two floral morphs produced within an individual.

Plants were grown in a greenhouse in Riverside, California, and watered biweekly with half-strength Hoagland's fluid. Pollen and stigmas were fixed in Formalin–acetic acid, dehydrated in acetone, then dried (critical point) with carbon dioxide, coated with gold palladium, and examined by scanning electron microscopy (SEM) (JEOL-35). For transmission electron microscopy (TEM), pollen fixation was done in 2.5 percent glutaraldehyde in 0.025*M* phosphate buffer in 8 percent sucrose, and then fixed in 2 percent osmium tetroxide. Tissue was embedded in Spurr's epoxy medium, sectioned, stained with uranyl acetate and lead citrate, and viewed by TEM (Phillips). Pollinations were performed on flowers placed on a 1 percent agar medium. Flowers were emasculated prior to anther dehiscence, and intramorph pollinations were done with self- or cross-CH pollen. Between-morph self- and cross-pollinations were performed in vitro for CH pollen on CL stigma and both in vivo and in vitro for CL pollen on CH stigma. Timed pollination series were done to establish rates of tube growth down the style. Gynoecia were fixed in a mixture of 100 percent ethanol and glacial acetic acid (3:1), softened for 20 minutes in 1*N* sodium hydroxide, and squashed in 0.1 percent aniline blue in 0.1*N* potassium phosphate. These preparations were viewed on a fluorescence microscope equipped with a Zeiss filter set 48 77 05. Pollen tube lengths were measured with an eyepiece micrometer.



Figs. 1 and 2. Stigmas of chasmogamous (Fig. 1) and cleistogamous (Fig. 2) flowers of *Collomia*; the arrows indicate receptive papillae. Note differences in positioning of lobes (×43). Figs. 3 and 4. Flask-shaped papillae of chasmogamous (Fig. 3) and digitiform-shaped papillae of cleistogamous (Fig. 4) stigmas (×257). Figs. 5 and 6. Cleistogamous (Fig. 5) and chasmogamous (Fig. 6) pollen grains of *Collomia*; the arrows indicate germination pores. Note the difference in grain size (×644). Figs. 7 and 8. Exine of pollen of cleistogamous flowers of *Collomia*; the arrows indicate micropores in the tectum. Note the thickness of sexine ridges and micropore size. The magnification in Fig. 7 is ×6435, and that in Fig. 8 is ×4010. Figs. 9 and 10. Exine of pollen of chasomogamous flowers of *Collomia*; the arrows indicate micropores in the tectum. The magnification in Fig. 9 is ×6435, and that in Fig. 10 is ×4010.

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.

E. M. Lord
K. J. Eckard
Department of Botany and Plant Sciences, University of California, Riverside 92521

References and Notes

1. C. Darwin, *The Different Forms of Flowers on Plants of the Same Species* (Appleton, New York, 1897).
2. E. M. Lord, *Bot. Rev.* **47**, 421 (1981).
3. F. R. Ganders, *N.Z. J. Bot.* **17**, 607 (1979).
4. W. R. Anderson, *Science* **207**, 892 (1980).
5. C. W. Lee, H. T. Erickson, J. Janick, *Physiol. Plant.* **43**, 225 (1978).
6. T. C. Minter and E. M. Lord, *Am. J. Bot.* **70**, 1499 (1983).
7. D. Lewis, *Sexual Incompatibility in Plants* (University Park Press, Baltimore, 1979).
8. U. Plitmann and D. A. Levin, *Evolution* **37**, 957 (1983).
9. Supported by NSF grant PCM 8308 619.

25 October 1983; accepted 17 November 1983

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.