Cryptic Self-Fertilization in the Malpighiaceae

Abstract. *Some Malpighiaceae produce minute cleistogamous flowers in addition to showy chasmogamous flowers. Standard techniques fail to reveal how the cleistogamous flowers achieve self-fertilization. Fluorescence in longitudinal sections shows that the pollen germinates inside the indehiscent anther. The pollen tubes then grow down through the filament, into the receptacle, up into the carpels, and into the nucellar beak of the ovule.*

The Malpighiaceae are a predominantly Neotropical family of flowering plants. Most Malpighiaceae have only showy chasmogamous flowers with exposed anthers and stigmas (Fig. 1). In these flowers fertilization is achieved in the usual way for angiosperms; that is, pollen germinates on the stigma, and the pollen tubes grow through the style into the locules of the gynoecium and into the ovules. However, in four small related genera (*Aspicarpa*, *Camarea*, *Janusia*, and *Gaudichaudia*), the chasmogamous flowers are often supplemented by tiny cleistogamous flowers (flowers that never open and are therefore incapable of outcrossing) (Fig. 2). These flowers have been called (*1*) "abnormal"; but in the species that have them they are morphologically constant and produce good seeds, being responsible in most cases for a large part of the total seed crop, far more than the "normal" chasmogamous flowers. I report here the bizarre and intriguing way the cleistogamous flowers achieve self-fertilization: The pollen germinates inside the indehiscent anther and the pollen tubes then grow down through the filament, into the receptacle, up into the carpels, and into the nucellar beak of the ovule.

A cleistogamous flower consists of five eglandular sepals, no petals or one rudimentary petal, one stamen with the anther containing rather few (about 20 to 200) pollen grains, and two uniovulate carpels that usually lack styles. All these structures are tiny, and the entire flower at the time of fertilization is only 1.5 mm or less in diameter (Fig. 3). It was expected at the outset that the anther would be found to dehisce and shed the pollen onto the carpels in the region where the style would develop in a chasmogamous flower, the likeliest region to be "stigmatic." An alternative possibility was that the anther would not dehisce, but the pollen would be found to grow through the wall of the anther directly into a stigmatic region. Either phenomenon would be easy to detect by standard techniques of clearing and staining and bright-field microscopy. However, exhaustive search revealed no evidence of either, and no standard stain for pollen tubes revealed their presence.

This suggested that the tiny flowers might actually be apomictic rather than cleistogamous. The distinction is of fundamental genetic and evolutionary sig-

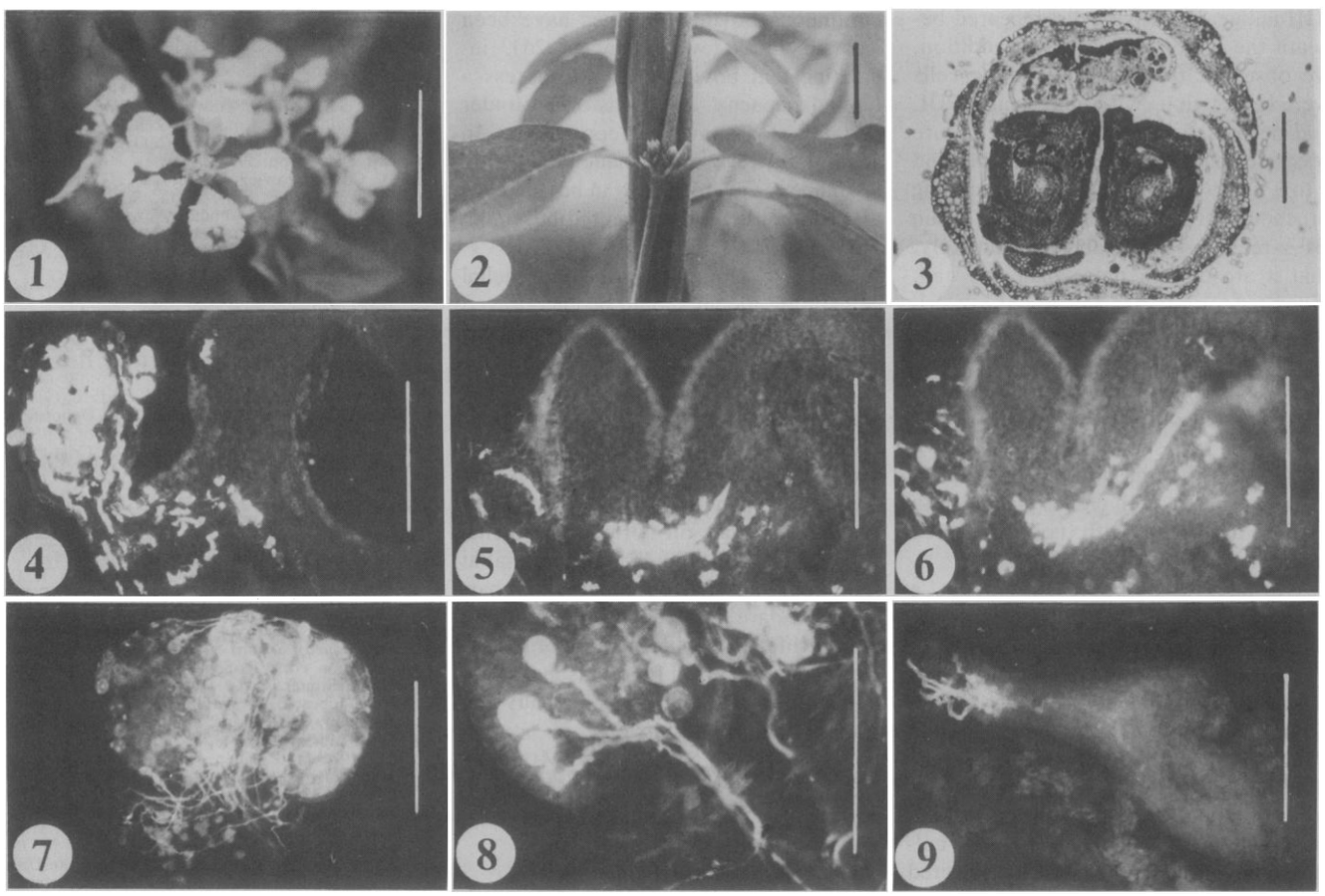

Fig. 1. Chasmogamous flowers of *Gaudichaudia*; scale, 1 cm. Fig. 2. Cleistogamous flowers of *Gaudichaudia*, in axils of leaves; scale, 1 cm. Fig. 3. Cross section of cleistogamous flower of *Gaudichaudia*, showing five sepals, one petal (lower left), one anther (top), and two carpels (center); scale, 0.4 mm. Figs. 4 to 6. Longitudinal sections of a single cleistogamous flower of *Janusia guaranitica*, showing fluorescing pollen tubes descending from the anther (Fig. 4), traversing the receptacle (Fig. 5), and ascending to the nucellar beak of the ovule (Fig. 6); scale, 0.4 mm. Fig. 7. Whole stamen from cleistogamous flower of *Gaudichaudia*, defined by fluorescing pollen grains and basipetal pollen tubes; scale, 0.4 mm. Fig. 8. Isolated pollen grains and pollen tubes from stamen in Fig. 7; scale 0.4 mm. Fig. 9. Ovule of cleistogamous flower of *Gaudichaudia*, with integument removed, showing several pollen tubes in nucellar beak and one penetrating to embryo sac; scale, 0.4 mm.

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nificance. In apomixis, sexual recombination is bypassed and the genotype of the progeny is identical to that of the parent. Apomictic plants are often unable to complete normal meiosis and may be highly heterozygous. Their breeding system is an escape from sterility and retains heterozygosity. Cleistogamy is an extreme form of inbreeding, with normal meiosis and recombination, and as in all autogamous plants the genetic consequence of the breeding system is a rapid shift toward homozygosity. I eventually rejected apomixis as unlikely for these reasons: (i) The anther is always present and always contains pollen that stains well with aniline blue. Development proceeds normally from pollen mother cells to tetrads to small but otherwise typical pollen, and in one case normal meiosis was observed. (ii) Development of the embryo sac is likewise perfectly normal, from spore mother cell to egg. (iii) Embryogenesis is also just what one would expect in a normally sexual plant.

A better method for discovering the path of the pollen tubes was clearly needed. Since pollen tubes often contain callose, a substance which can be made to fluoresce in the presence of aniline blue, cleistogamous flowers were embedded and sectioned longitudinally at 17 μm, then stained with a dilute buffered solution of aniline blue (*2*). This temporary preparation was examined with a Nikon Apophot microscope equipped with a 200-watt mercury burner for fluorescence, a BG 12 exciter filter, and a yellow (OG 530) barrier filter. Under these conditions, callose in the pollen grains and pollen tubes fluoresces bright yellow or yellow-green. Photographs were taken with a Nikon AFM camera using Kodak Tri-X pan film for black and white and high-speed Ektachrome, exposed and push-developed at ASA 400, for color slides (Figs. 4 to 6).

The pollen grains germinate inside the indehiscent anther and fill it with pollen tubes. Many pollen tubes grow down through the short filament and into the receptacle of the flower (Fig. 4). From there they turn upward (Fig. 5) and grow into the locule of each carpel, through the space between the ovule and the wall of the carpel, and into the nucellar beak of the ovule (Fig. 6). After the basic pathway was thus discovered, whole mounts of stamens (Figs. 7 and 8) and ovules (Fig. 9) were cleared with NaOH and then studied with fluorescence. In Fig. 9 several pollen tubes may be seen in the nucellar beak of the ovule, with one (fluorescing only faintly) penetrating deep into the ovule and presumably effecting fertilization. Since there are only two ovules in the flower, one per carpel, the relatively few pollen grains are more than ample. Since the whole flower is so small, the seemingly tortuous path of the pollen tubes is actually quite short, much shorter than that of a pollen tube that grows through the style of a chasmogamous flower, which is often 3 mm long.

Given the topography of these flowers, it is very difficult to get a single section that shows the complete path of pollen tubes from the anther locule to the nucellar beak; it is usually not all in one plane. However, Figs. 4 to 6 are photographs of sections of a single flower and together they show the path quite clearly. Many preparations have shown identical results in several species of *Janusia*, *Gaudichaudia*, and *Camarea*; *Aspicarpa*, which has similar cleistogamous flowers in some species, is expected to have the same method of fertilization.

The unusual and cryptic self-fertilization found in the cleistogamous flowers of the Malpighiaceae is quite unlike anything previously reported for angiosperms and suggests some interesting evolutionary questions. These include the way in which such a radical reorientation of the pollen tubes arose; the nature of the intermediates that bridged the evolutionary gap between chasmogamous flowers with styles and dehiscent anthers and cleistogamous flowers with no functional styles and indehiscent anthers; and how independent the genetic systems are that control the development of the two types of flowers, which are often produced simultaneously. It is worth mentioning that the single stamen in the cleistogamous flowers is one of the six present in the chasmogamous flowers in the more primitive members of this group (tribe Gaudichaudieae), but in the more advanced species that stamen has been lost from the chasmogamous flowers while remaining in the cleistogamous flowers. This suggests some evolutionary divergence in the parts of the genome controlling production of these flowers.

Whether this mechanism is limited to the Malpighiaceae remains to be seen. Perhaps other plants in which apomixis has been inferred but not rigorously demonstrated should be reexamined for the possible presence of some form of cryptic self-fertilization.

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References and Notes

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3. I thank B. Gates, G. Rogers, and S. Costanza for technical assistance. The University of Michigan Botanical Gardens provided space and assistance in growing plants, and a Faculty Research Grant from the Horace H. Rackham School of Graduate Studies paid for the fluorescence equipment. Fieldwork was made possible by NSF grant GB-37314 to the New York Botanical Garden.

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Ala-Gly- and Val-Asp-[Arg^8]-Vasopressin: Bovine Storage Forms of Arginine Vasopressin with Natriuretic Activity

Abstract. *Extracts of fresh-frozen bovine neurohypophysis were purified by chromatographic techniques to isolate and characterize the components that produce natriuresis in nondiuretic dogs. Two compounds with natriuretic properties similar to those of synthetic arginine vasopressin accounted for most of the natriuretic activity and appeared to be the prevalent vasopressin-like molecules in the extract. These peptides were Ala-Gly-[Arg^8]-vasopressin and Val-Asp-[Arg^8]-vasopressin; the natriuretic potency of each appeared to be similar to synthetic arginine vasopressin and could be observed with doses in the range of 50 picomoles. In the dog the most conspicuous difference between synthetic arginine vasopressin and the new vasopressin peptides was the smaller pressor responses to natriuretic doses of the new compounds.*

Evidence suggests that a humoral factor may contribute to the natriuresis (urinary excretion of sodium) produced by the expansion of extracellular volume (*1*, *2*). We have attempted to determine the sites of origin of such natriuretic substances, assaying their activity in nondiuretic male mongrel dogs (*3*). Selective natriuretic activity was observed in extracts prepared from bovine posterior pituitary lobes (*4*). These results provided the impetus for our further evaluation of the substances that produced this response. The absence of pressor activity in some of our early partially purified fractions made it unlikely that the natriuretic activity was produced entirely by arginine vasopressin, a known natriuretic substance (*5*).

We used extraction procedures similar

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