# Reproductive Features of Dentaria laciniata and D. diphylla (Cruciferae), and the Implications in the Taxonomy of the Eastern North American Dentaria Complex

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# REPRODUCTIVE FEATURES OF DENTARIA LACINIATA AND D. DIPHYLLA (CRUCIFERAE), AND THE IMPLICATIONS IN THE TAXONOMY OF THE EASTERN NORTH AMERICAN DENTARIA COMPLEX[1]

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ABSTRACT

Reproductive features including ovule development, megasporogenesis, megagametogenesis, microsporogenesis, microgametogenesis, pollen tube growth, embryogeny, and natural seed germination were studied in a single population each of *Dentaria laciniata* Muhl. ex. Willd. and *D. diphylla* Michx. to test for possible agamospermy. The population of *D. laciniata* studied is sexual. The archesporial cell functions directly as the megasporocyte. It undergoes two meiotic divisions, but the micropylar cell of the dyad fails to undergo meiosis II, and a linear triplet of three cells is formed. The chalazal megaspore divides to form an eight-nucleate, seven-celled megagametophyte of the *Polygonum* type. Simultaneous cytokinesis follows the second meiotic division of the microsporocyte yielding a tetrahedral tetrad of microspores. A three-celled pollen grain is formed prior to anther dehiscence. Following apparent fertilization, the *Capsella*-variation of the Onagrad type of embryogeny results in a conduplicate embryo. Endosperm is initially nuclear, but eventually becomes cellular. Seeds readily germinate in nature. Similar events are documented in one population of *D. diphylla* up to the organization of the embryo-sac, which disintegrates before cellularization. These reproductive events and other data indicate that the eastern North American species of *Dentaria* may form a sexual polyploid complex with some sexual populations and some sterile ones.

*DENTARIA LACINIATA* Muhl. ex Willd. and *D. diphylla* Michx. are members of a complex of five species of rhizomatous perennial herbs, commonly referred to as the toothworts, distributed in stable woodland habitats in the eastern United States (Fernald, 1970), including *D. heterophylla* Nutt., *D. maxima* Nutt., and *D. multifida* Muhl. Historically, species have been difficult to delimit due to complex inter- and intrapopulation variability. Some workers have suggested hybrid origins for many of the intergrading members (see Montgomery, 1955, for a summary; Feeny and Rosenberry, 1982). *Dentaria laciniata* is perhaps the most variable member of this complex, and numerous varieties or forms have been named based on vegetative features, as described by de Candolle, 1821; Torrey and Gray, 1838; Schultz, 1903 (the names here described under the illegitimate combination *Cardamine laciniata* [Muhl. ex Willd.] Wood ex Schultz., Bot. Jahrb. Syst. 32: 345. 1903; non *Cardamine laciniata* Muell., Trans. Phil. Soc. Victoria 1: 34. 1855); Fernald, 1908, 1938; Wolden, 1917; Farwell, 1930; and Louis-Marie, 1940. All members of the complex are high polyploids, with chromosome counts ranging from $2n = 64–248$ (Montgomery, 1955; Easterly, 1963; Harriman, 1965). Darlington and Wylie (1956), and Rollins (1966) reported the base number as $n = 8$, a number common in the closely related and possibly congeneric *Cardamine* L. (Manton, 1932; Mulligan, 1965). In addition to high ploidy levels, Harriman (1965) reported multivalents and micronuclei in microsporocyte meioses of *D. diphylla* and *D. laciniata*.

Montgomery (1955) reported that the embryo-sacs of *D. diphylla, D. laciniata,* and *D. maxima* aborted shortly after the "eight-cell" stage was reached, and concluded that the eastern North American species of *Dentaria* formed an "agamic complex" that reproduced mostly, if not entirely, by rhizomes (Grant [1981] refers to this system as a clonal complex, using the term "agamic complex" to designate an aesexual array of microspecies with agamospermy). Braun (1957), however, located populations of *D. laciniata* with viable seeds, and questioned Montgomery's hypothesis, arguing that the relatively slow spread by rhizomes,

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which are active in vegetative propagation in all of the above species, would be insufficient to distribute the species across glaciated areas into southern Ontario, the current northern limit of *Dentaria laciniata*.

This current study was initiated to test an alternative hypothesis, that agamospermy, apomixis via seed production, was operative within the group. The relationship between hybridization, polyploidy and meiotic irregularities to agamospermy has long been known (e.g., Gustafsson, 1946-1947; Stebbins, 1950,1971; Grant, 1981). The occurrence of agamospermy in the Cruciferae has been summarized by Greene (1978), and is known or implied to occur in various members of *Arabis, Draba, Erysimium,* and *Parra*. The only known mode of agamospermy in this family to date is in *Arabis holboellii* (Böcher, 1951). This species produces embryo sacs via the *Taraxacum*-type of diplospory (Battaglia, 1963). The first division of the megasporocyte ends in a restitution nucleus, and meiosis II produces a dyad of unreduced cells of which the lower functions as the gametophyte initial and forms an eight-nucleate embryo-sac. The embryo develops from the unreduced egg cell only after discharge of both male nuclei into the embryo-sac, neither of which fuses with the egg or endosperm nuclei.

MATERIALS AND METHODS—Field collections of *D. laciniata* were made in Athens Co., Ohio from 18 March to 23 May 1975, and of *D. diphylla* from 8 May to 10 June, 1975. Flowers in various stages of pre- and post-anthesis were fixed in the field in either Randolph's fluid (Randolph, 1935), or FPA 50 (formalin, propionic acid, 50% ethanol; 5:5:90, v:v:v). Air was evacuated from the tissues in a vacuum chamber, and after 24 hr, flowers were transferred to two changes of 70% ethanol. Dehydration was effected by the n-butyl alcohol series and flowers were embedded in Paraplast Plus (S/P). For embryological observations, Randolph's-fixed material was sectioned at 20 μm, transferred to water, and stained in Stockwell's modification of Flemming's triple stain, using two parts of distilled water (Stockwell, 1934) for 18 hr. Sections were brought to xylene and mounted in Pro-Tex (S/P). Pollen tube growth was observed using FPA 50-fixed pistils following the technique of Ramming, Hinrichs and Richardson (1973). Pollen viability was assessed using aniline blue in lactophenol. Pollen was taken from recently collected dried specimens and stained for 30 min before observation. Population density of *D. laciniata* was determined within two 10-$m^2$ plots with 20 separate 1-$m^2$ plots chosen from a random number table. Flowering plants and seedlings were both counted from these 40 plots. This study is based on an examination of 749 sectioned flowers and fruits of *D. laciniata,* and 106 of *D. diphylla.*

OBSERVATIONS—*Dentaria laciniata*—The initial embryological stages of *D. laciniata* occur in early spring after the plants emerge from the soil, but are still beneath the leaf litter. The archesporial cell functions directly as the megasporocyte (Fig. 1). It is discernable when pistils are ca. 800 μm long, and appears as a large, conspicuously nucleated cell situated directly beneath the nucellar epidermis at the micropylar end and is flanked in its chalazal region by one additional layer of nucellar cells. The megasporocyte enlarges longitudinally, and is obvious by its large size and dark-staining nucleus. When the ovules are ca. 1,500 μm long, it undergoes meiosis I (Fig. 2), producing a dyad (Fig. 3), whose chalazal cell is the larger. At this time, two, two-cell-thick integuments envelop the nucellus, and the ovule assumes a campylotropous form. Only the chalazal cell of the dyad undergoes meiosis II (Fig. 4), resulting in a linear array of three meiotic products. At this stage, the integuments have completely overarched the nucellus. The micropylar non-functional megaspore degenerates, forming a dense stain-positive area in sectioned material (Fig. 5). Megagametogenesis proceeds in

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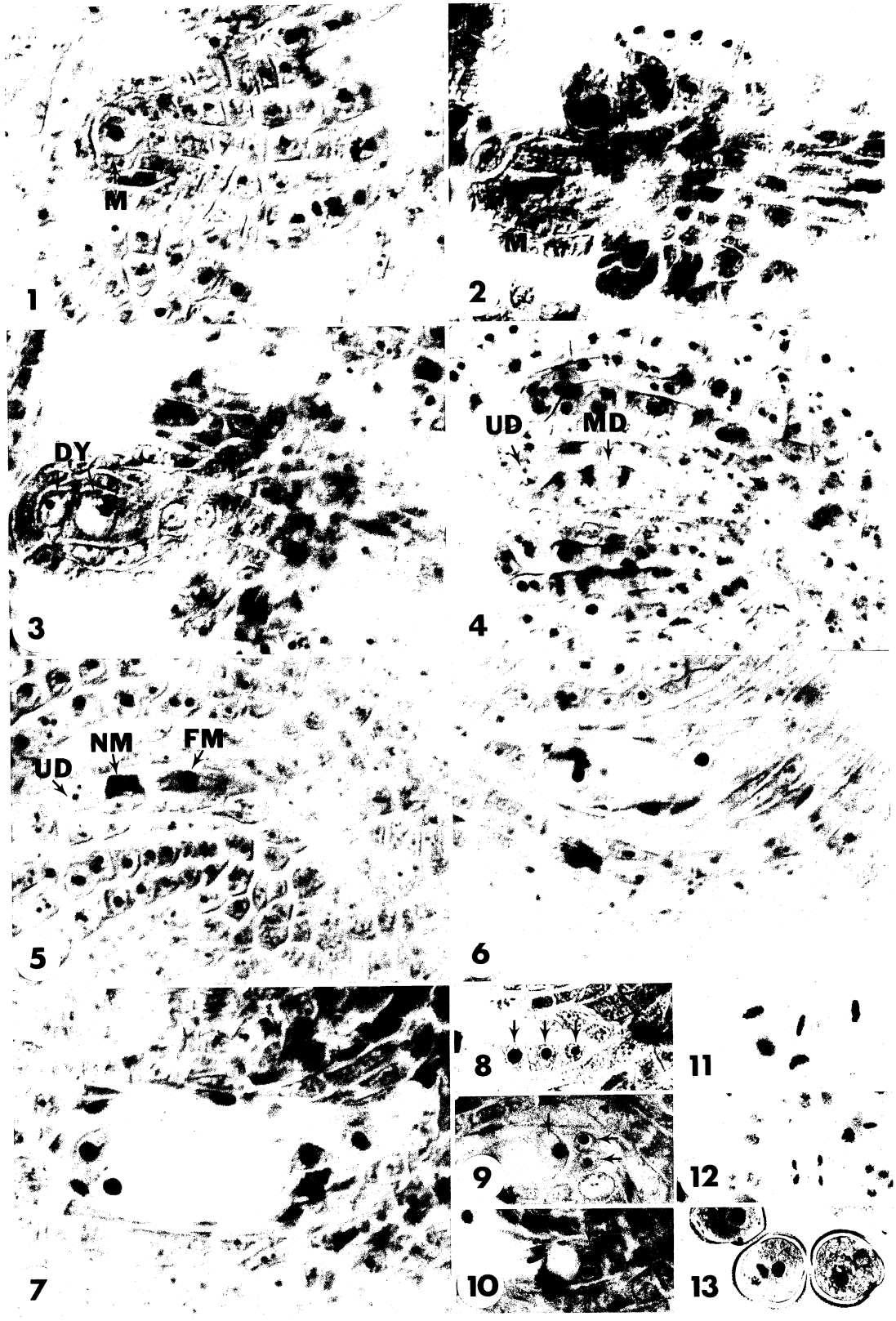
KEY TO LABELING: DY, dyad; E, egg; FM, functional megaspore; M, megasporocyte; MD, cell of the dyad undergoing meiosis II; NM, nonfunctional megaspore; PN, polar nuclei; PT, pollen tube; S, synergid; UD, cell of the dyad that fails to undergo meiosis II. All photomicrographs of ovules are situated with the micropylar area to the left. All figures are of *Dentaria laciniata* except Fig. 21.

Fig. 1–13. **1.** Young tenuinucellate ovule with a megasporocyte. × 480. **2.** Telophase I of the megasporocyte. × 480 **3.** Cells of the dyad in interphase. ×480. **4.** Telophase II of the chalazal cell of the dyad (MD). ×480. **5.** Integuments overarching the ovule, with the functional chalazal megaspore enlarging, the nonfunctional megaspore degenerating (here seen as two darkly stained areas) and the micropylar cell of dyad that has not undergone meiosis II. ×480. **6, 7.** Two- and four-nucleate embryo-sacs with a large central vacuole. ×480. **8–10.** Three different arrangements observed of the antipodal cells. ×480. **11, 12.** Metaphase I, and Telophase II of the microsporocytes. ×480. **13.** Three-celled pollen grains before release from the anther. ×480.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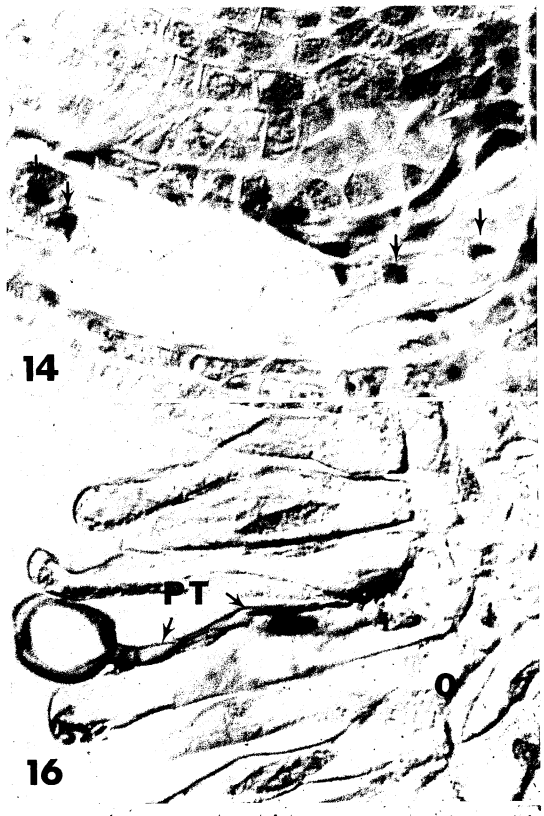
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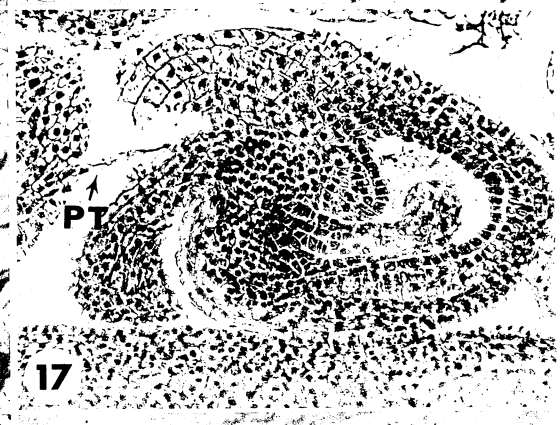
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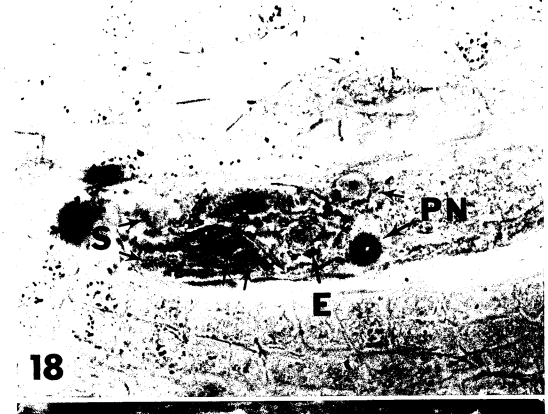
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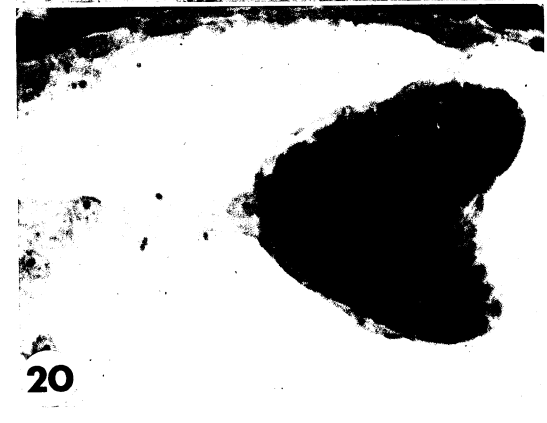
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a normal *Polygonum*-scheme (Maheshwari, 1950) (Fig. 6, 7, 14). Just before apparent fertilization, two synergids, an egg, the central cell (Fig. 15) and three antipodal cells (Fig. 8–10) comprise the embryo sac. Of interest are the variable dispositions of these antipodal cells (Fig. 8–10).

Meiosis of the microsporocytes (Fig. 11, 12) is initiated when the ovules are approximately 1,000 μm long. Cytokinesis follows only the second division, resulting in a tetrahedral tetrad of microspores. The microspores separate from the tetrad, and two successive mitotic divisions produce a three-celled pollen grain (Fig. 13). After release from the anther, pollen viability was 96–100% ($\bar{x}$ = 99%).

Open flowers of *D. laciniata* are visited and apparently pollinated by honeybees (*Apis mellifera*). Following apparent pollination, the pollen grains germinate on the stigma (Fig. 16) and pollen tubes grow through the septum of the ovary to the ovules (Fig. 17). Tracing the growth of these tubes is easily accomplished due to the periodic deposition of darkly stained callose "plugs" in the pollen tubes. Although fertilization was not observed, it was implicated by the presence of three structures observed within one synergid cell (Fig. 18); see discussion. Following apparent fertilization, the zygote grows into the embryo-sac as the synergids and antipodals quickly degenerate (Fig. 19). Embryogeny conforms to the *Capsella*-variation of the Onagrad type (Johansen, 1950). Endosperm is initially nuclear, but becomes cellular when the embryo reaches a cordate-shaped stage (Fig. 20). Asymmetrical growth of the cotyledons results in a conduplicate embryo.

In approximately 5% of the flowers examined, two megasporocytes underwent simultaneous meiotic divisions in one ovule. At approximately the same frequency, two laterally adjacent embryo sacs were encountered. Although one of these embryo sacs could possibly have been produced by somatic apospory from a nucellar cell, this was not observed, and the two embryo sacs probably originated from two megaspores.

Seedlings of *D. laciniata* were common in the study area, with a density of 0–8 ($\bar{x}$ = 2.4)/m$^2$. The density of flowering individuals was 3–31 ($\bar{x}$ = 11.7)/m$^2$; in addition, there were many nonflowering individuals. The plants in this population were extremely variable, with a number of the forms identifiable as the formally named varieties.

*Dentaria diphylla*—Gametophytic development in *D. diphylla* resembles that in *D. laciniata* from the formation of a triad of meiotic products to the production of an eight-nucleate embryo-sac and three-celled pollen grain. Development of more than one megaspore in an ovule was not observed. Pollen viability was 69–80% ($\bar{x}$ = 74%). Apparent pollination by honeybees also occurs, but emerging pollen tubes fail to penetrate the style. Embryo sacs fail to organize and eventually abort. In one case, a 16-nucleate embryo sac was observed (Fig. 21).

DISCUSSION—This study was initiated to test an hypothesis of agamospermic reproduction for the eastern North American species of *Dentaria*. Evidence to support this hypothesis includes a diverse range of high polyploidy, strong implication of hybridization between the species, possible meiotic irregularities, and sterility of some of the populations. The study demonstrates the occurrence of sexuality in one population of *D. laciniata*. Evidence supporting this conclusion includes cytologically documented megasporogenesis, *Polygonum*-type megagametogenesis, microporogenesis, microgametogenesis, high pollen viability, pollen tube growth, and apparent fertilization.

Fertilization was not observed, but was implicated by the presence of three dark-staining structures within one of the synergid cells (Fig. 18). Similar structures, classically referred to as "X-bodies" (Maheshwari, 1950), recently have been identified in *Blandfordia nobilis* Smith, *Epidendrum scutella* Lindl., *Gossypium hirsutum* L., or *Hordeum vulgare* L. as remnants of sperm cell cytoplasm, a pollen tube vegetative nucleus, or a degenerative synergid nucleus (Jensen and Fisher, 1968; Fisher and Jensen, 1969; Cocucci and diFulvio, 1969; Cass

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Fig. 14–21. **14.** Simultaneous mitotic divisions (at arrows) of a four-nucleate embryo-sac. ×480. **15.** Two synergids, the egg, and two nuclei of the micropylar region of the central cell. ×480. **16.** Pollen grain with pollen tube penetrating the stigma and style. ×480. **17.** Pollen tube traversing the gap between the septum of the ovary (at left) and the micropyle of an ovule. ×115. **18.** Micropylar region of the embryo-sac. The synergid cell at the bottom has three dark-staining structures (unlabeled arrows), possibly representing a degenerative synergid nucleus, a pollen tube nucleus, or remnant sperm cytoplasm (see text). ×480. **19.** Expanding one-celled zygote. The endosperm is still nuclear. ×480. **20.** Cordate stage of the embryo. ×200. **21.** Unorganized 16-nucleate embryo-sac of *Dentaria diphylla* (not all of the embryo-sac nuclei are visible here). ×480. See KEY TO LABELING above.

and Jensen, 1970). Similarly, Russell (1982, 1983) has demonstrated post-fertilization remnant sperm cell cytoplasm and a pollen tube vegetative nucleus in between the egg and central cell of *Plumbago zeylanica,* a species lacking synergids.

The failure of one of the cells of the dyad to undergo a second meiotic division occurs frequently in the angiosperms (Davis, 1966), however, this is the first report for the Cruciferae. *Polygonum*-type megagametogenesis is the only type known in sexually reproducing Cruciferae (Davis, 1966), and the *Capsella* variation of the Onagrad-type of embryogeny is common in this family (Johansen, 1950). Polyembryony was not observed, but the occasional double embryo sacs could possibly form two embryos. Polyembryony is common in angiosperms, and has been reported for a number of genera in the Cruciferae (Davis, 1966). Sterility of one population of *D. diphylla* was demonstrated via abortive embryo-sac development and failure of pollen tubes to penetrate the style.

Agamospermy may yet be present in other populations of *D. diphylla* and *D. laciniata.* Indeed, even groups thought to be obligately apomictic have been found to have traces of sexuality (Asker, 1979). However, agamospermy has yet to be demonstrated in this study or in Montgomery's (1955) study.

The above reproductive features are also correlated with sexual polyploid complexes (e.g., Stebbins, 1950, 1971; Grant, 1981), and in light of the above new data this is a more likely explanation for the variability within the group. Due to the possible hybrid nature of many populations, and demonstration of sexuality in *D. laciniata,* it is very possible other members of the complex are sexual when seed is produced and that hybridization is a contemporary and ongoing process in many of these species. The scattered sterile populations possibly have unbalanced genome combinations with reduced sexuality. Alternatively, some populations may be clonal with self-incompatibility. Further embryological and cytological studies are needed to confirm more widespread sexuality and survey the ploidy levels of this complex.

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