

A STUDY OF HYBRIDIZATION IN DOWNINGIA (CAMPANULACEAE)

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EVERY TAXONOMIC STUDY is tentative, and none is to be regarded as final, for each is, instead, one more laborious step toward a greater understanding of living organisms. Each careful investigation provides a new level from which subsequent workers may move forward in the analysis of the complexities and the synthesis of the information about a taxonomic group. Progression is thus by stages in taxonomy, as in other branches of science, although this seems often to be misunderstood by workers in other fields, and perhaps sometimes even by taxonomists. The genus *Downingia* Torr., a western American group of Campanulaceae-Lobelioideae, comprising about a dozen species of small, semiaquatic annuals with colorful, intricately formed corollas, well illustrates this continuing development.

The first species of *Downingia* was described in 1829 from plants grown from seeds collected by David Douglas in what is now Oregon, but only this and one other species were recognized until 1886. Then, from 1886 to 1895, nine new species were described, largely by E. L. Greene, who studied the genus in the field. Subsequently, in 1922, W. L. Jepson published a revision of the species of *Downingia* in California, recognizing only seven, and, in 1937, R. F. Hoover provided a key to the species in that state, including thirteen.

Eventually, in 1941, one hundred and twelve years after the description of the first species, came the first monographic study, that of Rogers McVaugh, which represents a masterful achievement in blocking out the species of *Downingia* on morphological bases. Working entirely from herbarium materials, in which the characteristic details of form and color-pattern of the corolla are largely obscured, it was necessary for McVaugh to treat the genus "exactly as if it were a genus of grasses or of the Compositae, where the distinguishing features are often technical ones and equally often invisible to the unaided eye." This admirable monograph may be modified in details, but the basic alignments will stand. Later (1943), McVaugh himself reduced one species to varietal status. His taxonomic treatment has been followed by Mason (1957), who provided excellent illustrations of most of the taxa, and by Munz and Keck (1959).

With McVaugh's monograph as a firm foundation, the present study was undertaken in the hope of adding something of the biological nature of the species, their chromosome numbers and genetic relationships, and the nature of the interspecific barriers. Annual, easily cultivated in quantity in a small space, with handsome and characteristic corollas, convenient for hybridization studies, and with a manageable number of well-marked species, *Downingia* offers almost as many advantages for a study of living

plants as it does disadvantages in an herbarium study. Begun in California, most of this work has been carried out in greenhouses in Massachusetts and North Carolina with restricted samples of only eight of the species and with no possibility for further field observations. However, the results confirm the taxonomic treatment of McVaugh and show the existence of a pattern of aneuploidy and of strong genetic incompatibilities between species. Since the pressure of other matters has made it impossible to carry this research further, it is presented here, with full recognition of its limitations and gaps, to clear the way for other workers who will undoubtedly solve many more problems, while posing still others. None of these studies of *Downingia* has been complete and final, but each is a step toward the continuing taxonomic goal of knowledge and understanding of plants.

Begun in the native haunts of *Downingia*, but carried out mostly on the opposite side of the continent, this work was possible only through the interest and help of friends and colleagues. I can not here detail the encouragement, advice, materials, or help which each has generously given, but I want to express my sincere appreciation to all of the following for their assistance: R. C. Bacigalupi, L. Constance, R. W. Holm, C. E. Kobuski, J. Kucyniak, Estella Leopold, C. MacMillan, Phyllis Gardner MacMillan, R. McVaugh, C. T. Mason, L. I. Nevling, Jr., M. A. Nobs, J. E. O'Connell, J. A. Rattenbury, R. C. Rollins, O. T. Solbrig, W. G. Solheim, G. L. Stebbins, Jr., the late A. N. Steward, W. H. Wagner, Jr., A. R. Kruckeberg, and R. L. Wyatt. *Figure 8* was drawn by Margaret F. Schroeder, of Cambridge, Massachusetts, from a Kodachrome slide and from preserved flowers. The manuscript could not have been completed without the able help of Mrs. Gordon W. Dillon.

METHODS

The first collections studied were made in California in the spring of 1949 by friends at the University of California or by myself, and a few hybridizations between wild plants brought into the greenhouses at Berkeley were tried at that time. Subsequently, a number of botanists kindly collected and forwarded seeds of various species. Attempts were made to grow and observe the crosses made in 1949, as well as other available collections, at the Biological Laboratories, Harvard University, Cambridge, Massachusetts, in the spring of 1950 and again in 1951. In the next three years, plantings at the University of North Carolina, Chapel Hill, were successful only in 1953; those in 1952 and 1954 mark unfortunate breaks in the data, for all failed, almost none of the seeds germinating (for causes unknown).

Seeds of *Downingia* were sown in February in 3-inch pots or 6-, 7-, or 9-inch azalea pots or bulb pans, in a more or less standard sand-soil-compost mixture. The sifted, firmly packed soil was covered with a thin layer of very fine soil on which the minute seeds were either scattered or placed

individually. A light dusting of sifted soil was added and the surface lightly tamped. The pots stood in saucers or pans of water and were kept constantly wet.

A large number of plants of *Downingia* can be grown in a single pot, allowing ready comparison within small populations. Under such conditions, individual plants do not reach the size or vigor which may be seen under favorable conditions in the wild or when grown singly in the greenhouse, but limitations of space necessitated the arrangements adopted.

In Cambridge six hours of additional light from two 1000-watt bulbs suspended over the greenhouse bench were used in 1951. The effect of the supplemental light will be seen under each species in the length of time required for flowering in this year in comparison with 1950 and 1953.

Sandwiched between other more pressing work, chromosome counts could not always be made at the proper time and were not obtained for all of the collections grown. The counts recorded, all meiotic, showed a pattern of aneuploidy from 11 pairs to 12 and to 10, 9, and 8. These chromosome numbers are given under the species concerned and are summarized in the crossing diagram and discussion toward the end of the paper. Meiosis occurred when the apparent flower bud (not including the pedicel-like ovary) was about 1 mm. long. The best results were obtained when the first buds were being produced in a population; at that time it was possible, with a little patience over a period of a few days, to obtain satisfactory meiotic material. Later on, after the plants were larger, I found it difficult to select buds which were of the proper age.

The material gave good results with a modified Carnoy's fixative (4 parts chloroform: 3 absolute ethyl alcohol: 1 glacial acetic acid) and propionocarmine. Slides were made permanent with a modification of Bradley's vapor transfer method (Stain Tech. 23: 41-44, 1948). Documenting herbarium specimens are in the Gray Herbarium.

The pollen discharge device of *Downingia* (see below) makes hybridization relatively easy. The ovary of a flower was held between forefinger and thumb and the anther tube grasped carefully with forceps and tweaked off. In most instances a clean separation from the filament tube could be obtained and the anthers pulled away, leaving the protruding style with the rudimentary stigmas. When the stigmas matured, pollination of the emasculated flower was accomplished easily with a spear-pointed needle which was used to depress the anther bristles and to collect the out-pouring of pollen. Pollen was then transferred to the appropriate stigma, the needle dipped in 70 per cent alcohol, the pollinated flowers marked with jeweler's tags, and the fruits allowed to mature. (Since the bright yellow pollen is rather powdery, some stray contamination may have come about through the methods used, for the small size of the plants, their closeness in the pots, and the importance of the ovary in the photosynthesis of the plant all precluded any sort of hermetic bagging. Such seedlings were easily recognized in interspecific crosses but could be a serious source of error in crosses involving races of a species. Nonhybrid seedlings of this kind are given for each of the interspecific crosses.) In *Downingia cuspi-*

data and *D. bicornuta* var. *bicornuta* the capsules tended to dehisce almost immediately upon maturity, necessitating prompt gathering. In other species most capsules on a single plant could be gathered at the same time and placed in individual coin envelopes. Each capsule involving an inter-specific cross was split lengthwise with a needle and the number of seeds and/or the condition of aborted ovules recorded.

More than 900 cross-pollinations were effected between the species assembled, the seeds sown, and the results tabulated. These are summarized under each of the species and in *figure 13*, which shows all crosses attempted and the several categories of results, as well as chromosome numbers.

GENERAL OBSERVATIONS

The twelve species of *Downingia* are all low annuals admirably adapted to the climatic conditions of the western United States, especially that part in which the Mediterranean type of climate, with its wet, cool winters and hot, dry summers, prevails. All are associated with moisture, and most are plants either of vernal pools or vernal wet soils. Growth is slow during the winter. With lengthening days, rising temperatures, and drying of the vernal pools, the rate of growth increases and the plants come rapidly into flower and set and mature seeds before the drying of the soil kills the parental plant. Numerous minute, fusiform seeds (to 150 or more) are borne on axile or parietal placentae in elongate 1- or 2-locular cylindrical capsules, which in some species (e.g., *D. cuspidata*) open almost immediately upon maturity by longitudinal splits, but in others (e.g., *D. ornatissima*) appear to be almost or quite indehiscent. In spite of their small size the seeds of some species, particularly those of habitats with the hottest, driest summers (e.g., *D. concolor*, *D. cuspidata*, *D. ornatissima*, *D. pusilla*) appear to be relatively drought resistant and may retain their germinability over a period of years. Those of cooler and moister habitats (e.g., *D. montana*, *D. yina* var. *major*, *D. elegans*) appear to be viable a much shorter length of time.

In nature, the seeds presumably germinate in fall or winter, either on wet soil or under water. The seedling shows an adaptation characteristic of a number of aquatics or plants of seasonally wet habitats. (Cf. *Drosera*, *Sarracenia*, *Pinguicula*, and many aquatics illustrated in W. C. Muenscher, *Aquatic Plants of the United States* [1944].)

In about five to seven days (in the greenhouse), the hypocotyl pushes out of the micropylar end of the seed and comes into contact with the soil. The radicle remains quiescent until the development of a ring of long root hairs at the base of the hypocotyl provides an anchoring attachment. The radicle then begins to grow, and, as the primary root elongates, the usual root hairs are formed.

The hypocotyl then elongates, becoming upright and green. The linear-lanceolate cotyledons gradually emerge, but remain pressed together, the tips being held within the seed coat. With the beginning of growth of the

plumule, the cotyledons may expand or may be held together permanently by the seed coat. Under favorable conditions growth proceeds rapidly. As with many marsh or aquatic plants, the stems are equipped with large gas channels which extend into the roots. Axillary branches may occur from the cotyledons upward, and adventitious rooting may occur at the nodes. A burst of growth appears in connection with the formation of the flower buds, which are solitary and sessile in the axils of bractlike leaves (the first appearing in the axil of the fifth to tenth or fifteenth leaf above the cotyledon, depending upon the species). These upper leaves and especially the elongated cylindric ovaries (which may easily be mistaken for pedicels) must account for much of the photosynthetic area of the plant.

In culture, flowering may occur in *D. pusilla* (the most reduced species) in about 35 days from the time of planting, or in about 45–56 days in most species, or may require some 70 in *Downingia bicornuta* var. *bicornuta*. The flowers are inverted in anthesis by torsion of the ovary, the apparent lower lip being morphologically the upper one. The flowers, with their elaborate and colorful corollas, obviously are adapted to insect pollination. The “lower” lip is more or less highly modified as a “landing platform” and is marked with characteristic patterns of blue to violet, white, yellow to green, and dark purple. The androecium is equally specialized, reserving the pollen until activated by an insect visitor. Most of the species (with the exception of the diminutive *Downingia pusilla* which seems to be self-pollinated) are normally outcrossing, but are self-compatible and may, under some circumstances (see below), be self-pollinated. Seedlings obtained from self-pollination in *D. insignis* and *D. pulchella* show variation in color pattern, suggesting the heterozygosity associated with outcrossing. No evidence of apomixis was found in the present investigation, and none of the cultures set seeds without pollination.

Androecium. The androecium of *Downingia* is a characteristic and highly specialized one, modified along the same lines as that of many of the members of Campanulaceae–Lobelioideae and resembling especially those of its nearest relative, *Porterella carnulosa* (Hook. & Arn.) Torr. (see Mason, 1957, fig. 344), and of *Isotoma axillaris*, described recently by Melville (1960). The five stamens are united around the style, the filaments forming a tube and the five anthers a more or less cylindric anther tube oriented at various angles to the filament tube. (See figs. 1, 8; also McVaugh, 1941, figs. 1–3, 5, 6, 10, and Mason, 1957, figs. 331–340.) The three larger anthers are uppermost, the tips curving downward and inward to those of the two shorter and smaller lower anthers and forming a mouth-like opening. Each of the two smaller anthers is tipped by a few forwardly directed short hairs and by a conspicuous flexible, flap-like bristle which projects forward at the base and then usually downward. The bases of the two bristles and the tuft cover the opening between the anthers, preventing the loss of pollen which is shed inwardly into the common cavity of the anther tube. When one or both of the bristles are

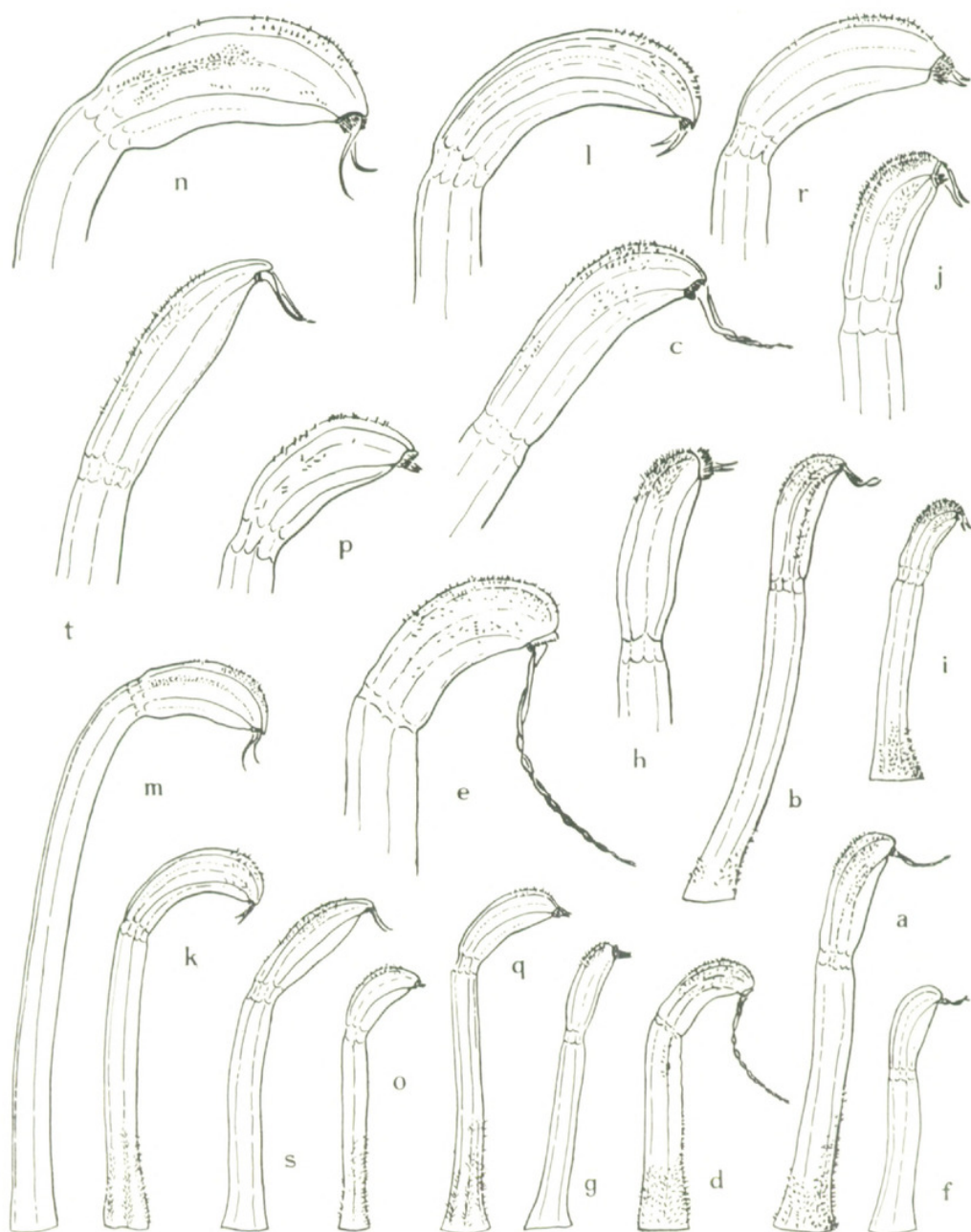


FIG. 1. Stamens of species of *Downingia*, in lateral view to show differences in size, angle of anther tube to filament tube, and length and position of anther bristles. Numerals following the name refer to populations listed under the species. All figures $\times 6$ or 12 . a-c, *D. bicornuta* var. *bicornuta* (30); d, e, *D. bicornuta* var. *picta* (40); f, *D. bicornuta* var. (2674), diagrammatic; g, h, *D. concolor* var. *concolor* (34); i, j, *D. cuspidata* (31); k, l, *D. elegans* var. *brachypetala* (39); m, n, *D. insignis* (14); o, p, *D. ornatissima* var. *ornatissima* (36); q, r, *D. ornatissima* var. *eximia* (28); s, t, *D. pulchella* (26).

pushed backward by a pollinator, the opening is uncovered and a quantity of powdery pollen bursts forth in a stream, dusting the insect. Subsequent displacements produce additional quantities of pollen.

The impetus for the pollen movement is provided primarily by the growth of the style. At the time of shedding of the pollen, the style is about as long as the filament tube, but the two stigmatic lobes are rudimentary and are tightly appressed to each other, the two occupying about the same diameter as the style. Immediately behind them is a ring of several ranks of styler hairs. With the continuing elongation of the style, the hairs act as a plunger, putting the pollen ahead under pressure and pushing it against the tips of the three upper stamens, so that it pours out, as though a valve were opened, when the anther bristles are depressed. A second source of pressure on the pollen may possibly come from the shrinking and drying of the tough outer walls of the anthers. The growth of the style continues, and, when the undeveloped stigmatic lobes push through and beyond the anther tube, they expand rapidly, separating, recurving, and maturing. (See Mason, 1957, fig. 334f.)

The flowers are thus strongly proterandrous, and self-pollination usually cannot occur. Exceptionally, however, in all of the species studied here, the style may fail to push through the anther tube, with the result that the stigmas expand within the tube and self-pollination occurs. In the single collection grown of *Downingia pusilla*, self-pollination of this type appeared to be the rule, for the stigmas were seldom seen to protrude from the anther tube, and, in contrast to the other species, in greenhouse culture plants of *D. pusilla* always set many seeds whether or not artificial pollination was attempted. (Apomixis, with the possible exception of pseudogamy, may be ruled out here, for emasculated flowers of *D. pusilla* did not set seeds.)

In his monograph of *Downingia*, McVaugh found that the androecium furnishes some of the most useful taxonomic characters for the identification of herbarium specimens (in which the corollas are almost always deformed and faded). Although flower size may vary greatly with the size and vigor of the plant, the androecium is relatively constant quantitatively and has a characteristic range in length. It is usually distinctive in shape of the anthers, the angle of anther tube to filament tube, the anther pubescence, the length and shape of the bristles, and the degree of exsertion of the anthers from the corolla tube. Such characteristics, combined with the elaborately formed and colored corollas, suggest specific pollination agents, or at least different sizes of agents. However, only a single field observation is available. (See *Downingia concolor*.)

The pollen discharge device of *Downingia* is more advanced than that of *Lobelia* and some other Lobelioideae in the elaboration and specialization of the two trigger bristles, the bases of which close the anther tube. In several species of *Lobelia* in which I have tested this mechanism, the bristles are lacking and the opening is closed by the two tufts of forwardly directed hairs which also act as the trigger device, releasing the pollen when pushed downward. In the eastern United States, the ruby-throated

hummingbird regularly trips the device of *Lobelia cardinalis* and may be seen with the top of the head yellow with pollen, while honey bees visiting *L. siphilitica* deliberately manipulate the discharge hairs and collect and carry away in their pollen baskets the bluish-gray pollen with which they are then dusted. However, the bristles of *Downingia* and *Porterella* would seem to make for a somewhat more efficient and sensitive mechanism.

OBSERVATIONS ON THE SPECIES

The data given here include notes which have accumulated in the course of growing and attempting to hybridize the eight species. For ease in consultation, the species are arranged in alphabetical order. Under each will be found observations (intended only to supplement those of McVaugh [1941] and the excellent drawings in Mason [1957]) on color patterns and morphology of the flowers, documenting data on the collections grown, summaries of the crosses attempted with other species, chromosome counts, and miscellaneous notes and comments which bear on the biology or taxonomy of the species. In all crosses the seed parent is given first. The hybrids of *Downingia insignis* and *D. pulchella* are treated separately in a following section.

Downingia bicornuta Gray var. *bicornuta*

FLOWER MORPHOLOGY AND COLOR: See fig. 2 for conformation and general color pattern of corolla; see also McVaugh (1941, figs. 4, 5, and p. 27), Mason (1957, p. 755). Lower lip of corolla nearly plane, not concave as in var. *picta*, the outer portion blue-violet (Bradley's violet [Ridgeway]), the central area white with two green to sulfur-yellow spots (these usually confluent as single patch) extending to the mouth of the corolla tube; upper lobes erect or reflexed, divergent or overlapping, blue-violet without and within; corolla tube blue-violet, darker on the upper side, the lateral walls with or without violet veining (this inconspicuous on account of the darker ground color); lower side of tube blue-violet within two-thirds of way from base, then with two elongated green or yellow spots or streaks (these visible on the outside) banded with reddish purple at either end, then with a band of violet, and finally with a deep violet band across the front of 2 of 4 hornlike projections at the mouth of the tube, the deep violet sometimes extending into the tube; hornlike projections shorter than in var. *picta*, erect and not curving outward. Corolla tube 5–6.1 mm. long from base to upper sinus, (3.8–)4.8–6.2 mm. long to lateral sinus in fresh material (*vs.* 2.5–4.5 mm. in dried material studied by McVaugh). Anthers not exerted from corolla tube or only tip exerted; anther tube not at an angle to filament tube; anther bristles twisted, short, 0.6–1.5 mm. long. (See fig. 1a–c.)

COLLECTION GROWN: 30, from Boggs Lake, Lake Co., Calif., *Estella Leopold*, 31 May 1949 (plants pollinated in wild, seeds matured in greenhouse).

CROSSES ATTEMPTED:

- 30 \times *D. concolor* (34, 87): 133 seeds from 5 capsules, 77 white or pale green lethals, 21 nonhybrids; reciprocal, 65 seeds from 25 capsules, 2 white lethals, 45 nonhybrids.
- 30 \times *D. bicornuta* var. *picta* (40): 620 seeds from 9 capsules, green and vigorous hybrid seedlings flowering in about 56 days (1953); reciprocal (20 capsules, seeds not counted) equally successful; F_1 apparently fertile but F_2 lost in 1954.
- 30 \times *D. ornatissima* (28, 36): 14 seeds from 6 capsules, 2 white lethals, 6 nonhybrids; reciprocal (27, 28, 36 \times 30), 235 seeds from 20 capsules, 15 white lethals, 62 nonhybrids.
- 30 \times *D. cuspidata* (33): 3 seeds from 4 capsules, 1 nonhybrid; reciprocal, seeds aborted near maturity, did not germinate or were lethal (in 33 \times 30, no seeds from 15 capsules; but 105 seeds from 1 capsule gave 18 nonhybrid seedlings; in 31 \times 30, 175 seeds from 7 capsules, about one-half of these planted, 16 white or yellowish lethals, 26 nonhybrids).
- 30 \times *D. pulchella* (26): 49 seeds from 5 capsules, no hybrids, 38 nonhybrids; reciprocal, 21 seeds from 10 capsules, no hybrids, 29 nonhybrids.
- 30 \times *D. insignis* (14): 13 seeds from 4 capsules, no hybrids, 12 nonhybrids; reciprocal, 79 seeds from 13 capsules, no hybrids, 38 nonhybrids.
- 30 \times *D. elegans* var. *brachypetala* (39): 8 seeds from 3 capsules, no hybrids, 1 nonhybrid; reciprocal, 24 seeds from 12 capsules, no hybrids, 1 nonhybrid.

CHROMOSOMES: $n = 11$ (metaphase, anaphase II) in population 30; meiosis regular.

Plants developed relatively slowly, beginning to flower in 69 days in 1950, 64–70 days in 1951. The flowers were without the curious odor of var. *picta*. This collection is the rather large-flowered, slow-maturing northern plant which extends from California into Oregon, Nevada, and Idaho.

Hybrids between vars. *bicornuta* and *picta* were green, vigorous, and apparently quite fertile, beginning to flower in 56 days in 1953. The color pattern of the corolla was like that of var. *bicornuta*, but the corolla tube was shorter and the anther bristles longer. An F_2 planted in 1954 failed, along with all other seeds planted that year. See also var. *picta* below.

Downingia bicornuta var. *picta* Hoover

FLOWER MORPHOLOGY AND COLOR: See fig. 2 for conformation and general color pattern of corolla; see also McVaugh (1941, fig. 6 & p. 27), Mason (1957, fig. 335a, b). Outer portion of concave lower lip of corolla violet, central area white with a central yellowish-green spot; upper lobes reflexed or erect, violet, paler at the base; corolla tube conspicuously reticulate within, with deep violet veins on a paler background, the upper side of the tube usually greenish or brownish for a distance of about 1–1.5 mm.; lower side of corolla tube zoned with purple at base, white with two green lines in the median portion, and purple at the distal end, the purple extending onto 4 hornlike projections at the mouth of the tube; exterior

of corolla tube light reddish purple (much paler than in var. *bicornuta*), the reticulations conspicuous, the tube often greenish distally; hornlike projections longer and more conspicuous than in var. *bicornuta*, curving outward, purple, the two central darker because of the extension of the greenish-yellow patch in center of lip over them. Corolla tube 2.4–3.1 mm. long from base to upper sinus, 2.0–2.9 mm. long to lateral sinus (vs. 1.5–2.6 mm. at lateral sinuses in dried material studied by McVaugh). Anthers usually exerted from corolla tube for almost the full length of the anther tube; anther tube at an angle to filament tube. (See fig. 1*d,e*.)

COLLECTIONS GROWN:

- 40, from 2 mi. n. of Sheldon, Sacramento Co., Calif., G. L. Stebbins, Jr., 20 May 1950 (seeds).
46, from 3.5 mi. n. of Snelling on La Grange Road, Merced Co., Calif., R. C. Bacigalupi, 22 April 1951 (seeds).

CROSSES ATTEMPTED:

- 40 × *D. bicornuta* var. *bicornuta* and reciprocal: good seed-set, green and vigorous hybrids of intermediate morphology. (See var. *bicornuta* above.)
40 × *D. concolor* var. *concolor* (34): 22 seeds from 2 capsules, no hybrids, 11 nonhybrids; reciprocal, 61 seeds from 14 capsules, 7 yellowish lethals, 13 nonhybrids.
40 × *D. ornatissima* (28, 42): 26 seeds from 5 capsules, no hybrids, 10 nonhybrids; reciprocal (with 27, 28, 36, 42), 214 seeds from 25 capsules, 7 white lethals, 35 nonhybrids.
40 × *D. cuspidata* (29, 31): 6 seeds from 2 capsules, none germinating; reciprocal (with 31), 397 seeds from 9 capsules, 78 white or very pale green lethals, 106 nonhybrids.
40 × *D. insignis* (14): not attempted; reciprocal, 18 seeds from 5 capsules, no hybrids, 10 nonhybrids.
40 × *D. elegans* var. *brachypetala* (39): 13 seeds from 6 capsules, no hybrids, 5 nonhybrids; reciprocal not attempted.

CHROMOSOMES: $n = 11$ (metaphase I, anaphase I, metaphase II) in population 40; meiosis regular.

Plants developed rapidly, the first flower bud located in the axil of the fifth leaf above the cotyledons, meiosis occurring in about 36 days and flowering in 50 days in 1951, 53 days in 1953, the first flowers opening two weeks ahead of var. *bicornuta* (cf. McVaugh, 1941, pp. 28, 30). The flowers had a strong "mousy" odor quite different from that of var. *bicornuta* or any of the other species grown.

The flowers of *Downingia bicornuta* var. *bicornuta* and var. *picta* are so different, when seen on the living plant, that on morphological grounds one could well deduce that two species are involved. The corollas differ in color pattern, shape of the lobes and length of the tube, and the androecia in length, angle of anthers to filament tube, degree of exertion of anthers, and length of the paired twisted bristles. In addition, var. *picta* begins to flower earlier, an adaptation probably related to its restriction to the Central Valley of California. However, on the basis of the twisted bristles,

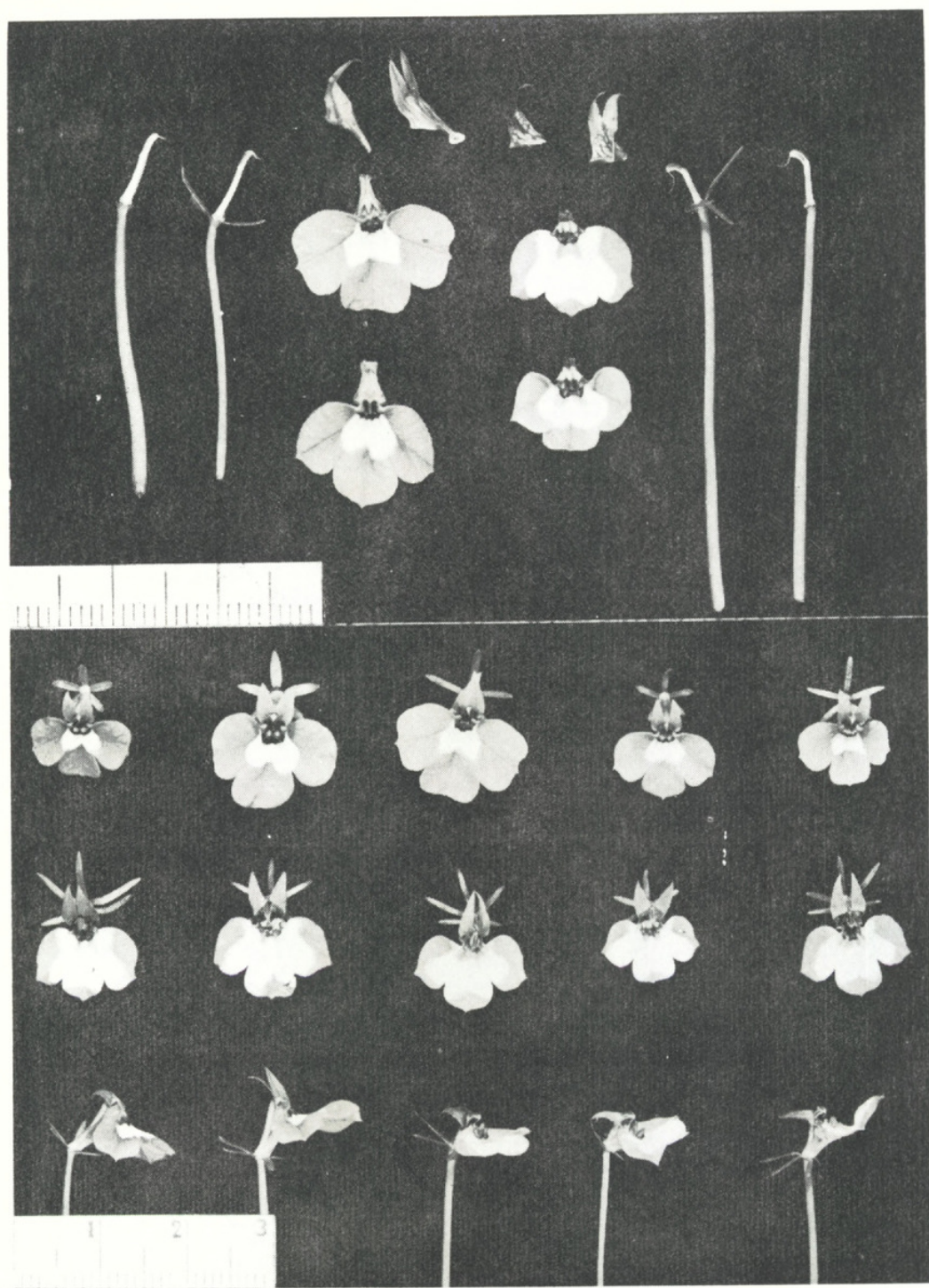


FIG. 2. Flowers of *Downingia bicornuta* vars. *bicornuta* and *picta*. Above: left, var. *bicornuta*, androecium and portion of ovary, upper corolla lobes, lower corolla lobes; right, var. *picta*. Below: top row, var. *bicornuta*; center row, var. *picta*; bottom row, two flowers of var. *bicornuta* (left), three of var. *picta*.

the corolla tube bearded within on the lower side, and the four conspicuous hornlike projections on the lower lip at the entrance to the corolla tube, as well as the occurrence of occasional apparent intermediates in the Sacramento Valley where the ranges of the two appear to overlap, McVaugh treated both as components of one species. The chromosome numbers and crossing evidence are entirely in accord with this disposition.

Downingia bicornuta var. [with short anther bristles and corolla tube]

FLOWER MORPHOLOGY AND COLOR: Appearing to be a small-flowered form of var. *bicornuta*; sepals and formation and color pattern of corolla as in var. *bicornuta*, but the corolla tube somewhat paler. Corolla tube short, 3.0–4.5 mm. long from base to upper sinus, 2.7–4 mm. long to lateral sinus in fresh material. Anthers as in var. *bicornuta*, with short, straight or twisted bristles, the anther tube not exerted from the corolla tube and not at an angle to the filament tube; filament tube ca. 2.6 mm. long. (See fig. 1f.)

COLLECTION GROWN: in alkaline pools, 2 mi. w. of Tranquility Junction on State Highway 180, Fresno Co., Calif., *Bacigalupi, Wiggins & Ferris* 2674, 29 July 1941 (GH) (flower and fruit).

CROSSES ATTEMPTED: This collection was crossed with vars. *picta* (46) and *bicornuta* (30) in 1953. Seed-set apparently was normal, but the hybrid seed planted in 1954 failed, along with all other seeds sown that year.

CHROMOSOMES: $n = 11$ (metaphase–anaphase I); meiosis regular.

The seeds from which this culture was grown came from a then ten-year-old specimen in the Gray Herbarium. The seeds, which bore faint longitudinal cellular markings, germinated readily in about 8 days. The plants developed rapidly, meiosis occurred about 34 days after sowing and flowering began in about 55 days in 1951 (sown in mid-April) or 50 days in 1953 (sown in late February).

This plant, which indicates the need for further study of variation in *Downingia bicornuta*, is undoubtedly closely allied to var. *bicornuta*, which it resembles in the conformation and coloration of the corolla, in the sepals, in the included anthers, in the straight filament-anther tube, and in the short anther bristles. It differs, however, in the short corolla tube (intermediate in length between the cultures grown in vars. *bicornuta* and *picta*), the filament tube (even shorter than that of var. *picta*), and in the time of flowering. It thus combines some features of vars. *bicornuta* and *picta*, but the uniformity of the population grown, the short filament tube, and the short bristles suggest at least that this is not of immediate hybrid origin. The geographical distribution remains to be determined, but it may be suspected of being a low-altitude, rapidly maturing form of at least the southern part of the Central Valley of California. Collections from the Central Valley which appear to be var. *bicornuta* should be examined critically.

This collection was determined by McVaugh as *Downingia bella* Hoover, a species which was imperfectly known to him and one of which I have seen only scanty herbarium material. (See Mason, 1957, p. 754, for illustration of *D. bella*.) The determination is easily understandable, for, in the herbarium specimen, the details of the corolla are quite obscured and the anther bristles are not the characteristically twisted ones of *D. bicornuta*. However, whatever the identity of *D. bella*, a plant which McVaugh thought to be related to *D. concolor*, the present plant is clearly allied to *D. bicornuta*, with both of the recognized varieties of which it is presumably interfertile.

Downingia concolor Greene var. *concolor*

FLOWER MORPHOLOGY AND COLOR: See McVaugh (1941, fig. 12 & pp. 18–21), Mason (1957, pp. 752, 755). Corolla pale to bright blue, the lower lip with a central white area and, at the mouth of the corolla tube, with a 2-lobed to w-shaped to almost square velvety, dark-violet spot covering two low ridges, each of which sometimes bears a streak of yellow within the mouth of the tube; white of the lip sometimes reduced or lacking, the whole lip then blue, the purple spot then completely obscuring the yellow streaks; corolla tube narrowly funnel-shaped, the lower side reddish violet from base to middle (or the entire length in dark flowers), the distal portion light blue to almost white.

COLLECTIONS GROWN:

- 15, from dry, hard adobe, roadside ditch, ca. 10 mi. s. of Dixon, Solano County, Calif., C. E. Wood 7614, 16 April 1949 (plants in flower; growing with *D. insignis*).
- 20, in vernal pools in meadow, ca. 2¼ mi. ne. of Middletown, Lake Co., Calif., C. E. Wood 7620, 28 April 1949 (plants in flower).
- 34, from near Dixon, Solano Co., Calif., M. Nobs, May 1949 (living plants).
- 87, a population originated in the greenhouse through crossing of 15 × 20 in May, 1949, before any chromosome numbers had been determined.
- 1170, growing in Middletown Meadow (Skaggs Ranch or Bar X Ranch), 3 mi. e. of Middletown on Highway 53, Lake Co., Calif., A. R. Kruckeberg, 2 July 1949.

INTERSPECIFIC CROSSES ATTEMPTED:

- 34, 87 × *D. ornatissima* (27, 35, 36, 42): 47 seeds from 28 capsules, 1 white lethal, 25 nonhybrid seedlings; reciprocal (27, 28, 35, 36 × 34, 87), 129 seeds from 35 capsules (43 from 1 capsule, 23 from another), 14 white lethals, 52 nonhybrids.
- 34, 87 × *D. bicornuta* var. *bicornuta* (30): 65 seeds from 25 capsules, 2 white lethals, 45 nonhybrids; reciprocal, 133 seeds from 5 capsules, 73 white to pale green lethals, 22 nonhybrids.
- 34 × *D. bicornuta* var. *picta* (40): 61 seeds from 14 capsules, 7 very pale, yellowish lethals, 13 nonhybrids; reciprocal, 22 seeds from 2 capsules, no hybrids, 11 nonhybrids.

- 15, 34, 87 \times *D. cuspidata* (29, 31, 33): 37 seeds from 23 capsules, 4 pale green lethals, 22 nonhybrids; reciprocals (31, 33 \times 15, 20, 34, 87), 188 seeds from 27 capsules, 30 white lethals, 64 nonhybrids.
- 34 \times *D. pusilla* (2063): 71 seeds from 2 capsules, 48 weak and nearly white lethals, some surviving almost 4 weeks.
- 15, 20, 34, 87 \times *D. pulchella* (25, 26): 27 seeds from 18 capsules, no hybrids, 17 nonhybrids; reciprocal (25, 26 \times 15, 34, 87), 86 seeds from 16 capsules, 1 white lethal, 67 nonhybrids.
- 34, 87 \times *D. insignis* (14): 32 seeds from 21 capsules, no hybrids, 25 nonhybrids; reciprocal (14 \times 15, 20, 34, 87), 131 seeds from 40 capsules, 3 white or very pale green lethals, 45 nonhybrids.
- 34 \times *D. elegans* var. *brachypetala* (39): 99 seeds from 9 capsules, no hybrids, 90 nonhybrids; reciprocal, 34 seeds from 5 capsules, no hybrids, 28 nonhybrids.

CHROMOSOMES: $n = 8$ (metaphase II) in population 34; $n = 9$ (late diakinesis to metaphase I) in population 1170; meiosis regular in both.

Plants matured rapidly, flower buds appearing in the axil of the seventh leaf above the cotyledons. Meiosis occurred in about 33 days after planting in 1951, and the first flowers opened in 55 days in 1950, 43–49 days in 1951, and 53 days in 1953.

The presence of two chromosome numbers (eight and nine) in two populations (34, 1170) of this species in northern California is of special interest. The meiotic behavior of a hybrid between these two races should provide a valuable clue to the way in which aneuploid repatterning of the chromosome complement has proceeded in the genus. Chromosome counts were not obtained on cultures 15 and 20 which are from approximately the same areas as are the 8- and 9-chromosome cultures (34 and 1170 respectively) and which might well have the same number of chromosomes as those collections. Wild plants from which cultures 15 and 20 were derived were crossed in California in 1949, long before any chromosome counts had been made. The resulting population (87) appeared to be fully fertile, and estimates, confusing at the time, of nine (definite) and eight (tentative) were both obtained, but these data are anything but unequivocal. No meiotic irregularities were seen, and one count of 1000 pollen grains showed 98.1 per cent stainable.

Another relationship which should be investigated is that of *Downingia concolor* var. *brevior* McVaugh, known only from San Diego County, California, and distinguished by its shorter and readily dehiscent capsules, to these more northern plants. Also of interest is the relationship of *D. concolor* to *D. bella* Hoover, which it resembles in a number of features.

This is the only species in which I have observed pollination in the wild. In Lake County, California, a small bee, unfortunately not captured, was seen visiting successive flowers (population 20). Pollen was being ejected on the face of the bee above the mouthparts, suggesting that the positions of the anthers in the various species may result in the deposition of pollen on specific parts of the pollinator's body.

Downingia cuspidata (Greene) Greene ex Jepson

FLOWER MORPHOLOGY AND COLOR: See McVaugh (1941, p. 31), Mason (1957, p. 759). Lower lip of corolla nearly plane, the lobes sky-blue to pale blue (or the blue varyingly restricted, in some plants only the very tips bluish), occasionally violet or pink (or anthocyanin sometimes entirely suppressed), the central area white with a two-lobed yellow spot covering two inconspicuous, low, mound-like ridges at the entrance to the corolla tube; upper lobes correspondingly blue, violet, or pink, usually somewhat darker than lower lobes, erect; corolla tube narrow, pale, unmarked, except for the tip of the yellow spot which extends to within the entrance; mouth of corolla tube narrow, mostly filled by the slightly exerted anther tube.

COLLECTIONS GROWN:

- 29, in one of four vernal pools about 1.5 feet deep with bare (dry) bottom and scattered plants of *Eryngium*, ca. 2 mi. e. of Keystone, Tuolumne Co., Calif., C. E. Wood 7629 & C. T. Mason, 9 May 1949 (only this species present, in fruit; absent in two shallower adjoining pools).
- 31, in very dry, very hard clay in three dry vernal pools in blue-oak grassland, ca. 3 mi. w. of Coarse Gold, Madera Co., Calif., C. E. Wood 7631 & C. T. Mason, 10 May 1949 (most plants with mature seeds, a few, including one pink and one violet, in flower).
- 33, in vernal pools along highway in bottomlands near a creek, ca. 1.2 mi. w. of Chinese Camp, Tuolumne Co., Calif., C. E. Wood 7633 & C. T. Mason, 10 May 1949 (fruiting in a dry pool, but flowering in wet areas).
- 47, from 3 mi. n. of Snelling on La Grange Road, Merced Co., Calif., R. C. Bacigalupi, 22 April 1951 (seeds).

INTERSPECIFIC CROSSES ATTEMPTED:

- 31, 33 \times *D. concolor* var. *concolor* (15, 20, 34, 87): 188 seeds from 27 capsules, 30 white lethals, 64 nonhybrids; reciprocal (29, 31, 33 \times 15, 34, 87), 37 seeds from 27 capsules, 4 pale green lethals, 22 nonhybrids.
- 31, 33 \times *D. ornatissima* (27, 35, 36): 536 seeds from 33 capsules, 136 white lethals, 78 nonhybrids; note especially 31 \times 27, 293 seeds from 6 capsules, 112 white lethals, 48 nonhybrids; reciprocal (with 27, 28, 35, 36), 27 seeds from 41 capsules, no hybrids, 10 nonhybrids.
- 31, 33 \times *D. bicornuta* var. *bicornuta* (30): seeds aborted near maturity, did not germinate or were white lethals (in 33 \times 30, no seeds from 15 capsules, but 105 seeds in one capsule gave 18 nonhybrid seedlings; in 31 \times 30, 175 seeds from 7 capsules, about one-half of these planted, 16 white or yellowish lethals, 26 nonhybrids); reciprocal (33 \times 30), 3 seeds from 4 capsules, no hybrids, 1 nonhybrid.
- 31 \times *D. bicornuta* var. *picta* (40): 397 seeds from 9 capsules, 78 white or very pale green lethals, 106 nonhybrids; reciprocal (40 \times 29, 31), 6 seeds from 2 capsules, none germinated.
- 31 \times *D. pusilla* (2063): 39 seeds from 2 capsules, no hybrids, 20 nonhybrids; reciprocal (with 29, 31), plants not emasculated, 61 seeds from 5 capsules, no hybrids, 47 nonhybrids, two capsules, each with 25 flat and twisted seeds aborted near maturity.
- 31, 33 \times *D. pulchella* (26): 117 seeds from 20 capsules, 5 white or pale green lethals, 33 nonhybrids; reciprocal (26 \times 29, 31, 33), 85 seeds from 37 capsules, 1 white and 9 very pale green lethals, 62 nonhybrids.

- 31, 33 \times *D. insignis* (14): 104 seeds from 24 capsules, 3 white lethals, 14 non-hybrids; reciprocal, 78 seeds from 17 capsules, 1 yellowish lethal, 40 non-hybrids.
- 31 \times *D. elegans* var. *brachypetala* (39): 38 seeds from 2 capsules, no hybrids, 11 nonhybrids; reciprocal (29 \times 39), 12 seeds from 11 capsules, no hybrids, 11 nonhybrids.

CHROMOSOMES: $n = 11$ in population 29 (metaphase I, anaphase II), in population 33 (metaphase I, metaphase II), and in population 47 (diakinesis, metaphase II, anaphase II); meiosis quite regular throughout.

Seeds sown in February germinated readily, and the plants flowered in 45 days in 1951, 48–52 days in 1953. However, seeds sown at Cambridge on Dec. 8, 1949, and cultivated without supplemental light required 80–90 days, with growth proceeding very slowly through the first two months. The first flower bud appeared in the axil of the fifth leaf above the cotyledons. The flowers had a delicate fragrance reminiscent of that of *Syringa vulgaris*.

The hyaline lines on the ovary, which mark the valves of the capsule, are quite conspicuous in the living plant, even being reddish in color in some individuals. The prompt dehiscence of the capsule necessitates special care in harvesting seeds.

The form of the corolla and its lack of purple spots, the capsule splitting at maturity, and the seeds with spiral cellular markings on the seed coat, all features stressed by McVaugh, are characteristic. Although I have seen the species growing only in Tuolumne and Madera counties, California, it was evident there (and from the cultures subsequently grown) that the amount (intensity) and distribution of anthocyanin in the corolla varies greatly both within and between populations. Crosses between populations from this region (29, 31, 33) resulted in good seed-set and green, vigorous seedlings. McVaugh's disposition of *Downingia pallida* Hoover (described from adjoining Stanislaus County) as a synonym of *D. cuspidata* is undoubtedly the correct one.

The interspecific data show the strong genetic barriers characteristic of other species of the genus. However, it is noteworthy that when *Downingia cuspidata* is used as the seed parent in crosses with *D. bicornuta* vars. *bicornuta* and *picta*, *D. ornatissima*, and *D. concolor*, albino seedlings are produced, whereas the reciprocal crosses usually fail even to set seeds. A single count of 1000 pollen grains from a plant of population 31 showed 99.3 per cent stainable grains.

Downingia elegans var. *brachypetala* (Gandoger) McVaugh

FLOWER MORPHOLOGY AND COLOR: See *fig. 3* for conformation of corolla and general color pattern; see also McVaugh (1941, p. 55). Corolla tube broadly funnellform, the lateral sinuses much deeper than the dorsal, the two upper lobes reflexed, often divergent; lower lip concave, about equalling the upper lobes in length, merging gradually with the tube, the three lobes parallel; corolla light blue, the tube pale without, the lower lip with a cen-

tral area of white, lacking ridges or yellow spots in the throat; lower side of corolla tube within pale, with an ill-defined, more or less diffuse bluish-violet spot on either side of the bluish-violet midvein (other veins sometimes bluish violet and visible through the corolla).

COLLECTION GROWN: 39, from the Calapooya River, Linn Co., Oregon, *L. Constance*, 5 Aug. 1950 (seeds).

INTERSPECIFIC CROSSES ATTEMPTED:

- 39 \times *D. concolor* var. *concolor* (34): 34 seeds from 5 capsules, no hybrids, 28 nonhybrids; reciprocal, 99 seeds from 9 capsules, no hybrids, 90 nonhybrids.
 39 \times *D. ornatissima* (36, 42): 11 seeds from 6 capsules, 1 pale green (probably nonhybrid); reciprocal (with 28, 36), 80 seeds from 16 capsules, no hybrids, 43 nonhybrids.
 39 \times *D. bicornuta* var. *bicornuta* (30): 24 seeds from 12 capsules, no hybrids, 1 nonhybrid; reciprocal, 8 seeds from 3 capsules, no hybrids, 1 nonhybrid.
 39 \times *D. bicornuta* var. *picta* (40): not attempted; reciprocal, 13 seeds from 6 capsules, no hybrids, 5 nonhybrids.
 39 \times *D. cuspidata* (29): 12 seeds from 11 capsules, no hybrids, 11 nonhybrids; reciprocal (with 31), 38 seeds from 2 capsules, no hybrids, 11 nonhybrids.
 39 \times *D. pulchella* (26): 38 seeds from 9 capsules, no hybrids, 20 nonhybrids; reciprocal, 37 seeds from 10 capsules, no hybrids, 14 nonhybrids.
 39 \times *D. insignis* (14): 23 seeds from 10 capsules, no hybrids, 11 nonhybrids; reciprocal, 30 seeds from 9 capsules, no hybrids, 11 nonhybrids.

CHROMOSOMES: $n = 10$ (metaphase II) in population 39; meiosis regular.



FIG. 3. Flowers of *Downingia elegans* var. *brachypetala* (top row and bottom row, right) and of *D. insignis* (center row and bottom row, left).

Seeds sown at the end of February, 1951, grew through a relatively long vegetative period. The first buds appeared in the axil of the tenth to fifteenth leaf above the cotyledons in about 45 days, meiosis occurred in about 50 days, and the first flowers opened in about 60 days. Seedlings of this species were easily distinguished from all others grown by the lanceolate, completely entire leaves.

This plant appears to represent *Downingia elegans* var. *brachypetala*, with which it agrees in respect to conformation of corolla and stamens (fig. 1*k*) and habit. It does not have, however, the two yellow spots in the white area on the lower lip which are characteristic of var. *elegans*. The chromosome number ($n = 10$) is distinctive, and, in addition, the plant is completely isolated genetically from the seven other species grown: in no instance was it possible to obtain hybrid seeds in interspecific crosses. In view of these results, McVaugh's suggestion (1941, p. 41) of a hybrid origin for atypical specimens of *Downingia elegans* collected with *D. insignis* (Malheur Co., Oregon, *Cusick 1261* [F]) appears to be an unlikely possibility. The developmental differences between the unilocular ovary with parietal placentation and the bilocular ovary with axile placentation would also seem to be such incompatible ones that hybrids between these species could hardly be expected.

Although this collection of *Downingia elegans* var. *brachypetala* was the only representative which was studied in any detail of the species which are characterized by unilocular ovaries with parietal placentation, it was possible to grow a few plants of *D. yina* var. *major* McVaugh (*D. willametensis* M. E. Peck), from Corvallis, Benton Co., Oregon, in the spring of 1955. In the latter plants, which began to flower in 68 days, the corolla tube was slender (ca. 5 mm. long), the upper lobes erect, and the anther tube not at an angle to the filament tube. The lower lip was concave, as in *D. elegans* var. *brachypetala*, and the corolla markings were similar, with two light purple dots in the mouth of the corolla tube and no yellow markings on the lip. McVaugh (1941, p. 57) found anomalous collections of var. *brachypetala* which seemed to combine features of *D. yina* var. *major* and suggested that "it is not impossible that var. *brachypetala* is of hybrid origin." Certainly further study of the group composed of *D. montana* Greene, *D. yina* Applegate vars. *yina* and *major*, and *D. elegans* vars. *elegans* and *brachypetala* is in order. Regardless of their interrelationships, however, it is probable that these five taxa with unilocular ovaries comprise a distinctive genetic, as well as morphological, group within the genus.

Downingia insignis Greene

FLOWER MORPHOLOGY AND COLOR: See figs. 3, 7 for conformation and general color pattern of corolla; note broadly funnelform throat, parallel, ascending upper lobes (with an angle of 20–50° between them), concave lower lip which is not at an angle to the tube; see also McVaugh (1941, p. 40), Mason (1957, p. 749). Corolla sky-blue (to violet-blue, probably sometimes pink or white), the veins darker, the lower lip with a central

white area; white area with two triangular-oblong green or yellow-green spots each extending onto a low fold which ends in a yellow protuberance at the mouth of the corolla tube; green and yellow spots interrupted by a dark violet-purple bandlike spot across each of the folds, the two spots often with diffuse color between or confluent as a band and truncating the central white area; corolla tube within with midveins of the three lower corolla lobes with violet streaks from base to mouth and with 6 or more small reddish-violet spots near the mouth (2 flanking each of the yellow protuberances), a streak of reddish violet extending downward from each spot. See also *figs. 1, 7* (as well as McVaugh, 1941, *fig. 1*; Mason, 1957, *fig. 332c* [*332d* is not characteristic]) for androecium.

COLLECTIONS GROWN:

- 14, from dry, hard adobe, roadside ditch, ca. 10 mi. s. of Dixon, Solano Co., Calif., *C. E. Wood 7614*, 16 April 1949 (in flower; growing with *Downingia concolor*).
 44, on eastern outskirts of Stockton along State Highway 4, San Joaquin Co., Calif., *R. C. Bacigalupi*, 21 April 1951 (seeds).

INTERSPECIFIC CROSSES ATTEMPTED:

- 14 × *D. concolor* var. *concolor* (15, 20, 34, 87): 131 seeds from 40 capsules, 3 white or very pale green lethals, 45 nonhybrids; reciprocal (with 34, 87), 32 seeds from 21 capsules, no hybrids, 25 nonhybrids.
 14 × *D. ornatissima* (27, 35, 36): 75 seeds from 28 capsules, no hybrids, 65 nonhybrids; reciprocal (with 27, 28, 36), 35 seeds from 22 capsules, 2 white lethals, 1 greenish yellow, 26 nonhybrids.
 14 × *D. bicornuta* var. *bicornuta* (30): 79 seeds from 13 capsules, no hybrids, 38 nonhybrids; reciprocal, 13 seeds from 4 capsules, no hybrids, 29 nonhybrids.
 14 × *D. bicornuta* var. *picta* (40): 18 seeds from 5 capsules, no hybrids, 10 nonhybrids; reciprocal not attempted.
 14 × *D. cuspidata* (31, 33): 78 seeds from 17 capsules, 1 yellowish lethal, 40 nonhybrids; reciprocal, 104 seeds from 24 capsules, 3 white lethals, 14 nonhybrids.
 14 × *D. pulchella* (25, 26): See below. Abundant seed-set, hybrids very pale green, weak, many inviable, others slow-growing, but if flowering, fertile; F₂ partially lethal, backcrosses to either parent vigorous and fertile.
 14 × *D. elegans* var. *brachypetala* (39): 30 seeds from 9 capsules, no hybrids, 11 nonhybrids; reciprocal, 23 seeds from 10 capsules, no hybrids, 11 nonhybrids.

CHROMOSOMES: $n = 11$ (metaphase II) in population 14; meiosis regular.

Seeds planted in February germinated readily, the plants flowering in 51–55 days in 1950, 46–52 days in 1951, and 54–55 days in 1953. Pollen 94.0 to 99.7 per cent stainable. (See TABLE I.)

Although *Downingia insignis* was long confused with *D. elegans*, McVaugh pointed out the numerous differences between the two species and correctly oriented the relationships of *D. insignis* toward *D. pulchella* instead. With *D. laeta* (Greene) Greene (of which I have not seen living

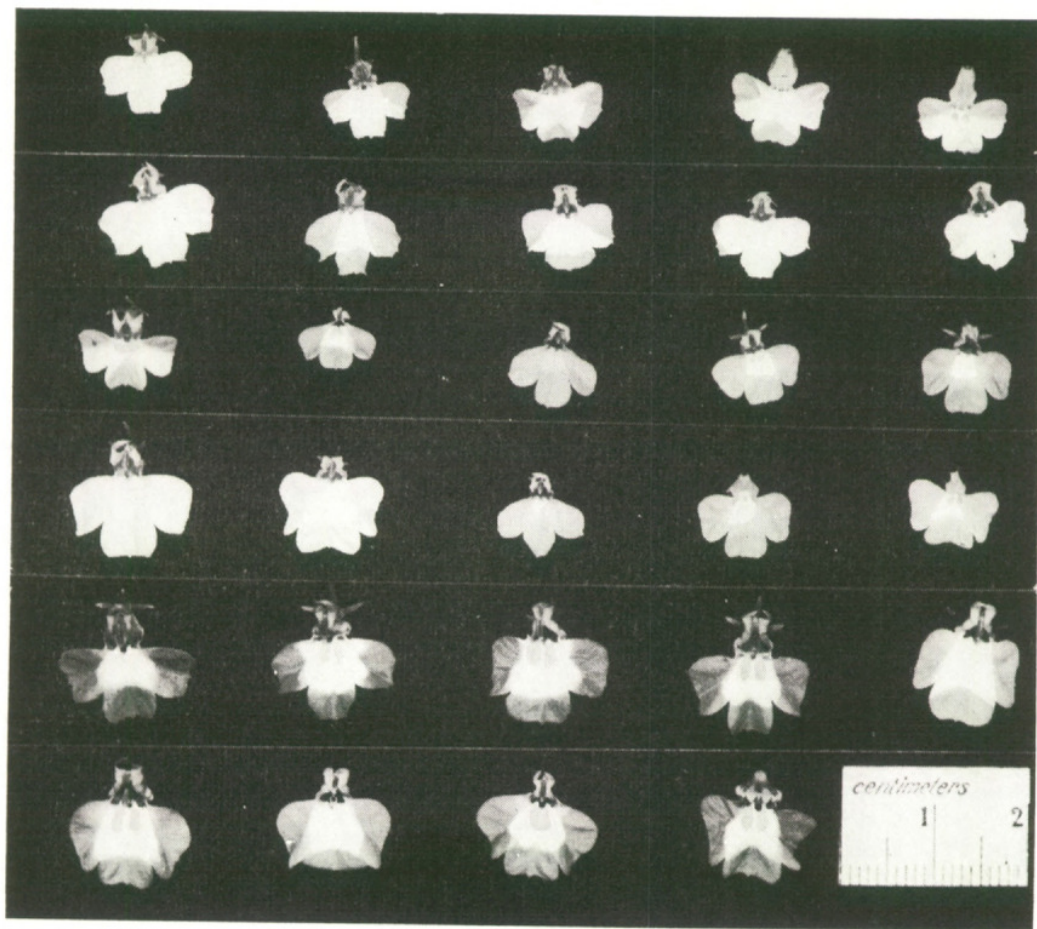


FIG. 4. Flowers of three populations of *Downingia ornatissima* to show geographical variation. Two top rows: Population 35 (var. *ornatissima*), from Tehama County, California. Two center rows: Population 41, from Sacramento County. Two bottom rows: Population 27 (var. *eximia*), from Stanislaus County, California.

material) the two form a group of morphologically related species with strong genetic incompatibilities with other species of the genus, although the chromosome numbers and crossing data show more of a relationship to other species with bilocular ovaries (*D. bicornuta*, *D. concolor*, *D. cuspidata*, *D. ornatissima*, et al.) than to those with unilocular ovaries (*D. elegans* var. *brachypetala*, et al.). The large, concave lower lip not forming an angle with the broadly funnelform corolla tube, the striking color pattern, and the anther tube at right angles to the long filament tube suggest pollination by some fairly large bee.

Downingia ornatissima Greene

FLOWER MORPHOLOGY AND COLOR: See McVaugh (1941, p. 23), Mason (1957, p. 753). Corolla deep blue, violet-blue or violet to pale lilac or nearly white (with only the tips of the lower lobes tinged with blue), with a squarish white central area with two greenish-yellow to bright yellow



FIG. 5. Upper corolla lobes of two populations of *Downingia ornatissima* to show range of variation in position. Above: Variation in population 36 (var. *ornatissima*), from Tehama County, California. Below: Variation in population 27 (var. *eximia*), from Stanislaus County, California. Hairs omitted from drawings; first four types of first row of population 36 with one or two scattered hairs on each corolla lobe; all members of population 27 with hairs on corolla lobes. Numerals show number of individuals of each type.

spots (sometimes confluent) which extend onto two nipple-like folds at the mouth of the corolla tube; corolla tube with three deep violet to very pale (in pale-flowered forms) spots or streaks alternating with the nipple-like folds, otherwise unmarked; upper lobes and tube concolorous with lower lip but usually paler (white in the palest forms).

COLLECTIONS GROWN:

- 27, in vernal pool in grassland, ca. 1 mi. sw. of Knight's Ferry, Stanislaus Co., Calif., C. E. Wood 7627 & C. T. Mason, 9 May 1949 (only this species present, most plants in fruit).
- 28, in dry vernal pool on Route 120 at Stanislaus Co. line, Tuolumne Co., Calif., C. E. Wood 7628 & C. T. Mason, 9 May 1949 (only this species present; plants in fruit).
- 35, in dry vernal pool with *Eryngium* in rolling grassland on Route 99W, ca. 8 mi. s. of Corning, Tehama Co., Calif., C. E. Wood 7635, L. Constance, R. Holm & L. Bonar, 20 May 1949 (seeds; only this species present).
- 36, in dry roadside vernal pools with *Eryngium* and *Lythrum* just n. of Corning, Tehama Co., Calif., C. E. Wood 7636, L. Constance, R. Holm & L. Bonar, 20 May 1949 (seeds; only this species present).
- 41, w. of Sloughouse, Sacramento Co., Calif., G. L. Stebbins, Jr., 13 May 1950 (seeds).

- 42, from low spots in grain field, 3 mi. n. of Sheldon, Sacramento Co., Calif., *G. L. Stebbins, Jr.*, 30 May 1950 (seeds).
 45, growing 2 mi. e. of Stockton on State Highway 4, *R. C. Bacigalupi*, San Joaquin Co., Calif., 21 April 1951 (seeds).
 48, on Hornitos Road, 2.5 mi. s. of Merced Falls, Merced Co., Calif., *R. C. Bacigalupi*, 22 April 1951 (seeds).

INTERSPECIFIC CROSSES ATTEMPTED:

- 27, 28, 35, 36 \times *D. concolor* (34, 87): 129 seeds from 35 capsules (43 from 1 capsule, 23 from another), 14 white lethals, 52 nonhybrids; reciprocal (34, 87 \times 27, 35, 36, 42), 47 seeds from 28 capsules, 1 white lethal, 25 nonhybrids.
 27, 28, 36 \times *D. bicornuta* var. *bicornuta* (30): 235 seeds from 20 capsules, 15 white lethals, 62 nonhybrids; reciprocal (with 28, 36), 14 seeds from 6 capsules, 2 white lethals, 6 nonhybrids.
 27, 28, 36, 42 \times *D. bicornuta* var. *picta* (40): 214 seeds from 25 capsules, 7 white lethals, 35 nonhybrids; reciprocal (with 28, 42), 26 seeds from 5 capsules, no hybrids, 10 nonhybrids.
 27, 28, 35, 36 \times *D. cuspidata* (31, 33): 27 seeds from 41 capsules, no hybrids, 10 nonhybrids; reciprocal (with 27, 35, 36), 536 seeds from 33 capsules, 136 white lethals, 78 nonhybrids (note especially 31 \times 27: 293 seeds from 6 capsules, 112 white lethals, 48 nonhybrids).
 27, 28, 36 \times *D. pulchella* (26): 45 seeds from 23 capsules, 2 white lethals (27 \times 26), 30 nonhybrids; reciprocal (with 27, 35, 36), 30 seeds from 18 capsules, no hybrids, 28 nonhybrids.
 27, 28, 36 \times *D. insignis* (14): 35 seeds from 22 capsules, 2 white lethals, 1 greenish yellow, 26 nonhybrids; reciprocal (with 27, 35, 36), 75 seeds from 28 capsules, no hybrids, 65 nonhybrids.
 28, 36 \times *D. elegans* var. *brachypetala* (39): 80 seeds from 16 capsules, no hybrids, 43 nonhybrids; reciprocal (with 36, 42), 11 seeds from 6 capsules, 1 nonhybrid.

CHROMOSOMES: $n = 12$ in populations 28 (diakinesis) and 42 (metaphase I, metaphase II), the divisions apparently regular. In population 35 no unequivocal count was obtained, but one slide showed two cells at anaphase I with a chromosomal bridge, in one with an accompanying fragment.

Seeds planted in February germinated promptly; the plants flowered in 54–59 days in 1950, 50–52 days in 1951, 51–55 days in 1953. The first flower bud appeared in the axil of the seventh leaf above the cotyledons. In population 35, two counts of 900 grains showed 99.3 and 99.5 per cent stainable grains, and a count of 1000 grains from a plant of population 28 showed 99.4 per cent stainable grains.

Capsules of this species are very tough and apparently indehiscent. In Tehama County, California, in late May, 1949, the shiny, spindle-shaped seeds were being released and scattered by grasshoppers feasting upon the dry, erect capsules of dead plants which carpeted the bottom of a dried-up vernal pool.

Downingia ornatissima is not to be confused with any other member of the genus. As McVaugh pointed out (1941, p. 26), it is readily recognized

by the small projection at the base of the sinus between the two upper corolla lobes, by the corolla tube sparsely hairy within, by the filament tube which is slightly curved backward and which is longer than the dorsal side of the corolla tube, by the anther tube at an angle to the filament tube, and by the bulbous-tipped hairs on the back of the anthers. It is also unique (for the present, at least) in its chromosome number (12).

The species varies from a plant of the northern part of the Central Valley in which the flowers are pale and small and the upper corolla lobes glabrous within and the tip of each often curled backward into a ring, to one at the south of the range in which the flowers are bright (violet to blue) and larger, and the upper lobes divergent and pubescent within near the tips. (See *figs. 4, 5.*) The two extremes were treated as vars. *ornatissima* and *eximia* by McVaugh, who regarded them as "rather formal varieties, and arbitrarily separated as such." He further realized that "as a result of such arbitrary segregation, a single collection will sometimes contain plants of both varieties" and found "this to be true to the greatest degree in collections from Merced and Stanislaus Counties."

In the three collections of var. *eximia* (27, 28, 48, from Stanislaus, Tuolumne, and Merced counties) studied here, all of the individuals had the upper corolla lobes pubescent, but showed much variation in the divergence or curling of the lobes; the flowers were relatively large and intensely colored (deep blue to violet). Those representing var. *ornatissima* (35, 36, from Tehama County), were paler, smaller-flowered (many with the blue restricted to the tips of the corolla lobes), and in nearly all the upper corolla lobes were glabrous (a few with scattered hairs) and with the lobes curled backward in a ring, although some were merely divergent. Collections from the center of the range (*e.g.*, 41, 42 from Sacramento County) were intermediate in flower-size and -color, with the upper lobes divergent or curled, and glabrous or with a few hairs. In a cross between the northern and southern plants (36×28), 335 seeds were obtained from 8 capsules (19–75 per capsule). Only about 130 germinated (perhaps due to the two-year lapse between maturity of seeds and planting), but these were green and vigorous with flowers of intermediate color and size. A clinal situation would appear to be present, but this matter should be pursued, particularly in connection with the possibility of incipient genetic barriers within the species. Taxonomically, however, McVaugh's original conclusion seems to hold, the two geographical varieties being only arbitrarily separable.

Downingia pulchella (Lindley) Torrey

FLOWER MORPHOLOGY AND COLOR: See *figs. 6, 7* for conformation and general color pattern of corolla; note divergent upper lobes (with an angle of $85\text{--}120^\circ$ between them) and the nearly plane lower lip; see also Mc-

Vaugh (1941, p. 37), Mason (1957, p. 750). Corolla bright, deep blue (sometimes violet-blue, pink, or white, the last with all anthocyanin suppressed), the lower lip with a central white area; white area with two roughly triangular or wedge-shaped yellow spots, each covering a low, mound-like fold at the base of the lip (the apex at the mouth of the corolla tube) and with 3 dark-purple spots alternating with the folds; purple spots sometimes confluent across the folds, forming a band and interrupting the yellow area; yellow spots sometimes diffuse, the entire central white area being covered; corolla tube narrowly funnel-shaped, dark reddish purple within (purple without), except for a small white area on the lower side at the mouth. See also below for an exceptional variant.

COLLECTIONS GROWN:

- 25, from wet, muddy soil, Yolo By-pass, e. of Davis, Yolo Co., Calif., *M. Nobs*, May 1949 (in flower).
 26, in heavy, gray, mucky clay around borders of a small pool on Route 50 ca. 2 mi. e. of Dublin, Alameda Co., Calif., *C. E. Wood 7626 & C. T. Mason*, 9 May 1949 (plants very abundant, all in flower).

INTERSPECIFIC CROSSES ATTEMPTED:

- 25, 26 \times *D. concolor* var. *concolor* (15, 34, 87), 86 seeds from 16 capsules, 1 white lethal, 67 nonhybrids; reciprocal (with 15, 20, 34, 87), 27 seeds from 18 capsules, no hybrids, 17 nonhybrids.
 26 \times *D. ornatissima* (27, 35, 36); 30 seeds from 18 capsules, no hybrids, 28 nonhybrids; reciprocal (with 27, 28, 36), 45 seeds from 23 capsules, 2 white lethals (27 \times 26), 30 nonhybrids.
 26 \times *D. bicornuta* var. *bicornuta* (30): 21 seeds from 10 capsules, no hybrids, 29 nonhybrids; reciprocal, 49 seeds from 5 capsules, no hybrids, 38 nonhybrids.
 26 \times *D. cuspidata* (29, 31, 33): 85 seeds from 37 capsules, 1 white and 9 very pale green lethals, 62 nonhybrids; reciprocal (with 31, 33), 117 seeds from 20 capsules, 5 white or pale green lethals, 33 nonhybrids.
 26 \times *D. insignis* (14) and reciprocal: See below. Abundant seed set, hybrids very pale green, weak, slow-growing, many inviable, others flowering and fertile; F_2 partially lethal, backcrosses to either parent vigorous and fertile.
 26 \times *D. elegans* var. *brachypetala* (39): 37 seeds from 10 capsules, no hybrids, 14 nonhybrids; reciprocal, 38 seeds from 9 capsules, no hybrids, 20 nonhybrids.

CHROMOSOMES: $n = 11$ (metaphase II) in population 26; meiosis regular.

Seeds planted in February developed rapidly, the plants flowering in 46–54 days in 1950, and 46–51 days in 1951. Pollen counts showed 90.9 to 99.6 per cent stainable pollen. (See TABLE I.)

With its showy and distinctive corollas 1.5 cm. across and its characteristically exserted filament tube and tapered anther tube, *Downingia pulchella* is not to be confused with any other member of the genus. On morphological bases, McVaugh thought *D. pulchella* to be most closely related to *D. insignis*, a conclusion which is borne out on the basis of chromosomal and genetic data, the two producing weak but fertile hybrids.

Also of this relationship is *Downingia laeta* (Greene) Greene, which ranges from northeastern California, western and central Nevada, and Utah, north to southern Saskatchewan, Montana, southern British Columbia, and western Oregon, to the north and east of *D. pulchella* and *D. insignis*, both of which are primarily Californian. This small-and-pale-flowered species has an androecium and a general corolla pattern most reminiscent of *D. pulchella* and suggestive of derivation from a similar type.



FIG. 6. Variants of *Downingia pulchella*. Upper row: left, the common blue-flowered form referred to in text as "wild type"; right, form with pink corolla lobes. Lower row: left, a small, dark-flowered form; right, form with white flowers, all anthocyanin suppressed; center, a white, small-flowered form from the F_2 of a cross between the two preceding. Flowers seen from above, stamens removed.

An unusual small-flowered mutant form of *Downingia pulchella* which was found in Alameda County, California (see population 26) is of interest in connection with the development of corolla forms and color patterns in the genus. The colony consisted of thousands of densely packed plants, the entire ground appearing to be carpeted with the flowers. Most of the plants bore flowers of the usual blue type described above, but with considerable variation in flower size and in the development of the three spots on the lower lip. Scattered patches of violet, pink, very pale blue, and white plants occurred, with a very few in which the blue anthocyanin was restricted to the tips of the corolla lobes, and at least four in which the white area of the lower lip was strongly suffused with yellow. In addition to these, there were two areas of scattered plants in which the

corollas were small (ca. 1 cm. across) and the lower lip much reduced, all five corolla lobes appearing to be of approximately equal size. (See fig. 6.) The corolla was blue, the white central area was completely lacking, a deep violet area extended from the sinuses between the three lobes toward the base of the lip, and the two yellow areas at the mouth of the corolla tube were restricted as shown in the photograph. On the basis of the corolla alone, these plants were so aberrant that one would hardly suspect that they represented *D. pulchella*; yet it was the only species present in this locality and the stamens were the characteristic ones of *D. pulchella*.

A few simple intraspecific crosses were made later with one of these small, dark "mutants" transplanted from the wild. When crossed with a "wild-type" blue-flowered plant (from population 25 to avoid the possibility of heterozygosity), all of the F_1 plants were of the wild type in respect to both color pattern and corolla conformation. An F_2 obtained by selfing one of these plants was composed of 66 of the wild type and 25 of the mutant type. The small, dark mutant crossed onto one of the white-flowered plants from the same colony also produced an F_1 with flowers of the wild type. The F_2 of 84 individuals from a capsule pollinated from the same plant included 47 of the wild type, 17 white, 16 of the small dark mutant, and 4 of a plant with small white flowers (see lower center, fig. 6). The expected results from a cross involving two recessive genes would be in the ratio of 47: 16: 16: 5 for a progeny of 84 plants. The two forms thus segregate independently, and the small, dark mutant behaves as though controlled by a single recessive gene which changes both corolla shape and color pattern. As to whether a gene mutation or some larger chromosomal change or rearrangement is represented, I would not guess, especially since the cytology of these plants was not studied.

So different is the corolla size, the form of the lower lip, and the color pattern, however, that the question arises as to whether a "mutation" of this kind (whatever its nature) might not lead to differential selection by the insect pollinators of this group in which the corolla conformation and color pattern and the anther position are so highly specific.

The general conformation of the corolla is suggestive of *Downingia laeta* (see McVaugh, 1941, figs. 8, 9; Mason, 1957, fig. 340) and, in fact, of *D. pusilla*, the other species with reduced flowers. The color pattern of the former is quite different, but that of the latter is very similar to the pattern of the double recessive obtained above. One is tempted to postulate the derivation of *D. laeta* from a plant similar to *D. pulchella* and of *D. pusilla* from a plant similar to *D. cuspidata* through changes of this kind.

Downingia pusilla (G. Don) Torrey

FLOWER MORPHOLOGY AND COLOR: See McVaugh (1941, p. 35), Mason (1957, p. 761). Sepals 4–5 mm. long. Corolla limb 4–5 mm. across, the lobes divergent from the tube, the upper and lower lips in about the same plane; lower lip much reduced, the lobes 1.5–2 mm. long, the sinuses extending almost to the mouth of the corolla tube; upper corolla lobes parallel or spreading, about equalling the lower; corolla tube narrow, ca. 2 mm. long; corolla white, unmarked except for a yellow spot at the sinus on either side of the lowermost lobe, the two spots confluent across the lobe at the mouth of the tube.

COLLECTION GROWN: 2063, from 5 mi. n. of Snelling, Merced Co., R. F. Hoover 2063, 4 May 1937 (GH) (fruit).

INTERSPECIFIC CROSSES ATTEMPTED:

2063 \times *D. concolor* var. *concolor* (34): not attempted; reciprocal, 71 seeds from 2 capsules, 48 weak and nearly white lethals, some surviving almost 4 weeks. 2063 \times *D. cuspidata* (29, 31): stigmas protruding from anther tube, flowers not emasculated, 61 seeds from 5 capsules, no hybrids, 47 nonhybrids, 2 capsules each with 25 flat and twisted seeds which aborted near maturity; reciprocal (with 31), 39 seeds from 2 capsules, no hybrids, 20 nonhybrids.

CHROMOSOMES: $n = 11$ (metaphase I, $2n = 11$ bivalents). The plants and buds were so small at the time of meiosis that only a single bud in which only one clear cell was countable was found.

The adaptation of this diminutive species to a fleetingly moist habitat is reflected in the longevity of its seeds and the rapidity of its development. Seeds from Hoover 2063 in the Gray Herbarium germinated readily fourteen years after collection. Flower buds were visible in 21–28 days when the plants were only 1–1.5 cm. high; meiosis occurred in 26–30 days; and the plants began to flower in 39 days in 1951 or 36 days in 1953. At time of flowering the plants were approximately 4 cm. tall, and most of the photosynthetic area of the plant resided in the ovaries and sepals.

Downingia pusilla appears to be self-pollinated, for the most part, all plants in culture setting abundant seeds, in contrast to other species. Some stigmas protrude, but many expand within the anther tube. Although the possibility of pseudogamy has not been ruled out, other types of apomixis appear unlikely, for when the flower was emasculated or the stigma removed completely seeds were not set. Relatively little pollen was produced by these tiny flowers, and it was difficult either to obtain sufficient amounts for hybridization experiments or to remove the anther tube from this species.

The few crosses attempted with other species are inconclusive, but the chromosome number, the corolla, and the spirally marked seeds all seem to corroborate the relationship of *D. pusilla* to *D. cuspidata* postulated by

McVaugh. *Downingia pusilla*, the most reduced species of the genus, represents one of the end points in the evolution of the group, though one attained without change in chromosome number.

Mason (1957, p. 761) has suggested the possibility of two races of this species, noting that plants from north of San Francisco Bay have white flowers and a corolla tube longer than the lobes, while plants of the Central Valley have blue corollas, a tube shorter than the lobes, and the lower lip conspicuously spreading and often with a distinct palate. *Hoover 2063* should belong with the latter group, but it had, however, no trace of blue in the flowers; the tube and corolla lobes appeared to be approximately equal.

DOWNINGIA INSIGNIS \times PULCHELLA, A WEAK BUT FERTILE HYBRID

Crosses were made between *Downingia insignis* and *D. pulchella* in 1949 and in 1951. Seed-set ranged from about 30 to 100 per capsule and germination rates from 14 per cent in one of the least successful combinations to about 88 per cent in the most successful.

Seedlings were albino to pale yellowish green. Many died in varying lengths of time, but some grew slowly. Those surviving to the time of initiation of flower buds became more vigorous, the ovaries and sepals being green in all instances, presumably adding substantially to the photosynthetic efficiency of the hybrids. From 82 to 108 or more than 118 days (the longest period for which records were kept) were required from time of planting to time of production of the first flowers.

The various crosses between the two species are summarized below.

Downingia insignis \times *pulchella* (F_1):

- 92 (14 \times dark, small-flowered mutant of 26): ca. 125 seeds from 2 capsules, ca. 65–75 pale green hybrid seedlings, about 40 surviving six weeks, one producing a very small and pale flower in 100 days, this and other plants later succumbing to attacks of white flies and mites.
- 95 (14 \times white 26): ca. 50 seeds from 1 capsule, 44 pale green seedlings, 37 surviving at time of first flower (82 days after planting), 20 plants surviving to flower. (See below.)
- 96 (14 \times 25): ca. 90 seeds from 1 capsule, 8 hybrid seedlings all pale and spindly, none surviving more than 40 days.
- 99 (14 \times 25): ca. 80 seeds from 1 capsule, 17 very pale and weak hybrids, none reaching flowering, 1 nonhybrid.
- 100 (14 \times pink 26): ca. 35 seeds from 1 capsule, 7 very pale green hybrids, no survivals.
- 153 (14 \times 26): 50 seeds sown, 38 hybrids, 1 nonhybrid; all hybrids pale green and weak: 3 lasting 65 days, 1 almost reaching flowering (108 days), but succumbing to an attack of *Botrytis*.
- 157 (14 \times 26): 104 seeds (of ca. 335 from 2 capsules) sown, 70 per cent germinated; 64 white or pale green hybrids, 9 nonhybrids; 4 hybrids still alive after 118 days with buds but as yet no flowers.

Downingia pulchella \times *insignis* (F_1):

- 93 (26 white \times 14): 75 seeds from 1 capsule; 11 very pale and spindly hybrids, only one surviving to flower; 1 nonhybrid [white-flowered *D. pulchella*].
- 94 (26 white \times 14): ca. 75 seeds from 1 capsule; 22 pale and spindly hybrids, none of which survived more than 50 days, nor flowered; 2 nonhybrids (white-flowered), in 56 days.
- 98 (25 \times 14): ca. 100 seeds from 1 capsule; ca. 25 pale and spindly hybrids, none of which survived to flower; 4 nonhybrids (blue-flowered, one with 3 spots at base of lip confluent in a band).
- 101 (25 \times 14): ca. 200 seeds from 2 capsules; ca. 75 spindly, pale green seedlings, most dead within 50 days (one surviving to flower); 6 nonhybrids (the first flowers in 48 days).
- 154 (26 \times 14): 100 seeds sown; 58 pale hybrids, none surviving more than 63 days, none flowering.



FIG. 7. Flowers of *Downingia pulchella* (above, left, and lower row of five flowers) and of *D. insignis* (above, right, and upper row of five flowers).



FIG. 8. Flowers of first-generation hybrid (population 95) of *Downingia pulchella* and *D. insignis*: a, c, flowers of two plants to show form and color pattern of corolla; b, flower with markings omitted, to show androecium and conformation of upper and lower lips. Compare with figs. 1m, s and 7.

- 155 (26×14): 29 seeds from 1 capsule: 24 pale green hybrids, 6 surviving 70 days, but none surviving to flower (a heavy growth of *Vaucheria* on the soil surface perhaps partly responsible).
 156 (25×14): 100 seeds planted; 52 pale yellowish-green hybrids; 1 nonhybrid (removed); 5 hybrids (all pale and spindly) surviving to flower after 103 days.

Population 95, a cross between the blue-flowered *Downingia insignis* and a white-flowered plant of *D. pulchella*, was the most successful of the hybrids. In this population, sown 17 February 1950, germination was 88 per cent, proceeding over a three-week period. The seedlings were all pale yellowish green and very slender and grew slowly, the largest being only 1.3 cm. tall after seven weeks. When the first flowers opened, 82 days after sowing, 37 plants from 0.4 to 14 cm. tall still survived. Twenty plants lived to flower by May 20. The stems of all of these remained very pale green, reddish near the base. The leaves produced up to the time of flower-bud initiation were pale, but those with axillary flower buds were bright green, and the ovaries of all flowers were green.

The two parental species differ in a whole series of floral characteristics, many of which will be immediately apparent from an inspection of fig. 7. Among those of the corolla are the shape, length, and diameter of the tube; the form and concavity of the lower lip and its angle to the corolla tube; the angle between the upper lobes and their angle to the tube; and the markings of the lower lip and the corolla tube. Features of the androecium include the length of the filament tube; the length, shape, and color of the anther tube and its angle to the filament tube; and the length and shape of the hairs on the anther tube.

The flowers of the F_1 hybrids were intermediate in most respects (see fig. 8), in shape more suggestive of *Downingia insignis* but with a much

narrower corolla throat, a somewhat flatter lower lip and more divergent upper lobes. The color pattern was also intermediate: the white area had the general form of that of *D. pulchella*, but showed the restriction evident in *D. insignis*, and the three dark spots at the base of the lip varied from separate, but larger than usual in *D. pulchella*, to more or less confluent in a narrow band across the two low yellow folds, undoubtedly reflecting the heterozygosity of the parental plants. (Self-pollination of wild plants of *D. pulchella* produces offspring with considerable variation in the shape of the corolla and in the spotting of the lip, sometimes with much larger spots and these sometimes confluent across the low ridges as above.) The

TABLE I. Pollen Fertility in *Downingia insignis*, *D. pulchella*, and Hybrids

| POPULATION | TOTAL GRAINS COUNTED | PER CENT STAINABLE POLLEN | PER CENT ABORTED POLLEN |
|---|-------------------------|---------------------------------|-------------------------------|
| <i>D. insignis</i> | | | |
| 14 | 1600 | 94.0 | 6.0 |
| 14 | 2100 | 99.4 | 0.6 |
| 14 | 1800 | 98.9 | 1.1 |
| 14 | 1500 | 98.3 | 1.7 |
| 14 | 1800 | 99.4 | 0.6 |
| 14 | 2000 | 99.7 | 0.3 |
| <i>D. pulchella</i> | | | |
| 26 | 2000 | 99.6 | 0.4 |
| 26, white, selfed | 1200 | 90.9 | 9.1 |
| 25 | 1600 | 98.0 | 2.0 |
| 25 | 1600 | 98.1 | 1.9 |
| <i>D. insignis</i> × <i>pulchella</i> (F ₁) | | | |
| 95 | 1100 | 94.2 | 5.8 |
| 95 | 1500 | 93.7 | 6.3 |
| 95 | 1800 | 98.8 | 1.2 |
| 95 | 850 | 94.6 | 5.4 |
| <i>D. pulchella</i> × <i>insignis</i> (F ₁) | | | |
| 93 | 975 | 95.2 | 4.8 |
| 93 | 930 | 92.9 | 7.1 |
| 101 | 1350 | 95.8 | 4.2 ^a |
| <i>D. insignis</i> × <i>pulchella</i> (F ₂) | | | |
| 203 (95 × 95) | 1000 | 98.7 | 1.3 |
| 203 | 1100 | 95.4 | 4.6 ^b |
| 203 | 1200 | 90.8 | 9.2 ^c |
| 203 | 1000 | 97.2 | 2.8 ^d |
| 203 | 1100 | 99.1 | 0.9 |

^a Including three giant grains, and many grains slightly smaller than usual; greater variation in pollen size than usual.
^b Considerable variation in size; pollen grains appearing much below normal counted as aborted although contents present.
^c No size variation; all grains clearly aborted.
^d Considerable variation in size, including grains larger and smaller than normal.

androecium is also intermediate: the filament tube in length and the anther tube in shape, angle to the filament tube, and marking (slightly darker than the dirty white anthers of *D. insignis* but lighter and less pronouncedly blue-gray striped than in *D. pulchella*). The hairs on the anther tube were slender and intermediate between the short, stubby hairs of *D. insignis* and the relatively long, slender ones of *D. pulchella*. Aside from the variation in the dark spots at the base of the lower lip, the flowers of the twenty plants of this population were remarkably uniform. Although the flowers generally were somewhat smaller in size than those of either parent, this, as in many annuals, was related to the vigor of the plant rather than to other factors, the most vigorous of the hybrids also bearing the largest flowers. The ovaries ranged from 2.5 to 3 cm. in length and the capsules from 2.5 to 3.7 cm.

Chromosome counts were not attempted on the hybrids because of the small number of surviving plants and their general lack of vigor. Pollen counts, however, showed a range of pollen stainability (and presumably fertility) of 92.9 to 98.8 per cent, approximately the same as that of the parental species. (See TABLE I.) Seed set by the hybrids and in backcrosses bore out this supposition of fertility. However, the pollen variations seen in the surviving plant of population 101 suggest at least a low frequency of meiotic irregularities.

Backcrosses. A number of backcrosses from populations 93 (a single surviving plant) and 95 were made to each of the parents. The hybrid was used as the pollen parent in all except a single instance because of the general weakness of the hybrid plants and the desirability of using the relatively few flowers for the production of a second hybrid generation. The results are summarized below:

BACKCROSSES OF F_1 TO *Downingia pulchella*:

- 205 (26 white \times 95): 85 seeds from one capsule, 77 green seedlings, 1 albino lethal; all greens vigorous, the first flowers in 52 days.
- 206 (95 \times 26 white): ca. 75 brown seeds plus an approximately equal number of nearly white seeds from 1 capsule; ca. 95 germinated, ca. 63 white or pale lethals, 22 pale and weaker, 10 vigorous and green which flowered after 52 days. (Possible contamination with F_1 pollen? Cf. F_2 below.)
- 207 (26 \times 95): 260 seeds from 3 capsules, 1 albino lethal, 210 green and vigorous, the first flowers in 47 days.
- 208 (26 \times 95): 70 seeds from 1 capsule, 65 green and vigorous seedlings, the first flowers in 45 days.
- 209 (26 \times 95): 100 seeds from 1 capsule, good germination (number not recorded), all seedlings green and vigorous, the first flowers in 45 days.
- 210 (25 \times 93): 100 seeds from 1 capsule, 1 white lethal, numerous green and vigorous seedlings (not counted), the first flowers in 47 days.

BACKCROSSES OF F_1 TO *D. insignis*:

- 211 (14 \times 93): 157 seeds from 2 capsules, 1 white lethal, numerous green and vigorous seedlings (not counted), first flowers in 47 days.
- 212 (14 \times 95): 649 seeds from 9 capsules (ca. $\frac{1}{2}$ sown), 1 pale lethal, numerous green and vigorous seedlings (not counted), the first flowers in 47 days.

213 (14×95): ca. 120 seeds from 1 capsule, numerous green and vigorous seedlings, the first flowers in 50 days.

With the exception of the possibly contaminated cross (206) in which the hybrid was used as the seed parent, in each of the combinations above the seed-set and excellent germination indicate full fertility and vigor. The seedlings were green and vigorous, grew rapidly, and flowered in 47–52 days, the period required for the parental species. As would be expected, the variation ranged roughly from the F_1 to the backcross parent. Populations obtained from backcrosses in each direction are shown in *figs. 9* and *10*.

Only one plant of population 209 was examined cytologically. Numerous divisions at anaphase I and metaphase II were seen. All divisions appeared to be regular, except for two cells at anaphase I showing traces of a lagging chromosome or bridge and one at metaphase II with one plate of eleven chromosomes, the other of eleven plus a fragment.

The Second Generation. Various combinations of the single survivor in population 93 (*Downingia pulchella* \times *insignis*) and those in 95 (*D. insignis* \times *pulchella*) were tried in 1950. The results are summarized below:

Downingia insignis \times *pulchella* (F_2):

- 200 (93 selfed): 181 seeds from 3 capsules from a single plant, 59 white or pale green lethals, 117 varying from weak and pale, to vigorous and green, most plants green eventually but some definitely more vigorous than others; first flowers in 52 days.
- 201 (93×95): 142 seeds from 2 capsules (with 12 and 130 seeds), 110 seeds germinating, 55 white or very pale lethals, 55 pale green, yellowish-green, or green seedlings showing great variation in vigor, 0.9–6.5 cm. tall after 42 days; first flowers in 50 days.
- 202 (95×93): 2 seeds from each of 2 capsules, 1 white lethal, 1 pale green (died in 30 days), 2 slow-growing pale greens flowering after 60 days.
- 203 (95×95): 454 seeds from 8 capsules, 114 white or pale green lethals, 85 yellowish-green to green survivors with much variation in vigor (0.5–4.4 cm. tall after 42 days), first flowers after 52 days.
- 204 (95 selfed): 68 seeds from one capsule, 31 white or pale lethals, 23 of various shades of green surviving, with wide range of vigor (0.6 to 1.8 cm. tall after 42 days), no record of flowering.

Seed set averaged 50 to 70 (extremes 2–130) per capsule and germination was generally excellent. Thirty to fifty per cent of the F_2 seedlings were albino or pale green lethals, while the remainder ranged from pale green to vigorous, bright green plants, many of which survived to maturity. In contrast to the F_1 generation, flowering began in about 52 days (approximately the same period required for the flowering of the parents). Pollen stainability (see TABLE I) ranged from 91 to 99 per cent in five plants of population 203.

Obviously many segregant combinations were eliminated in the F_2 . As

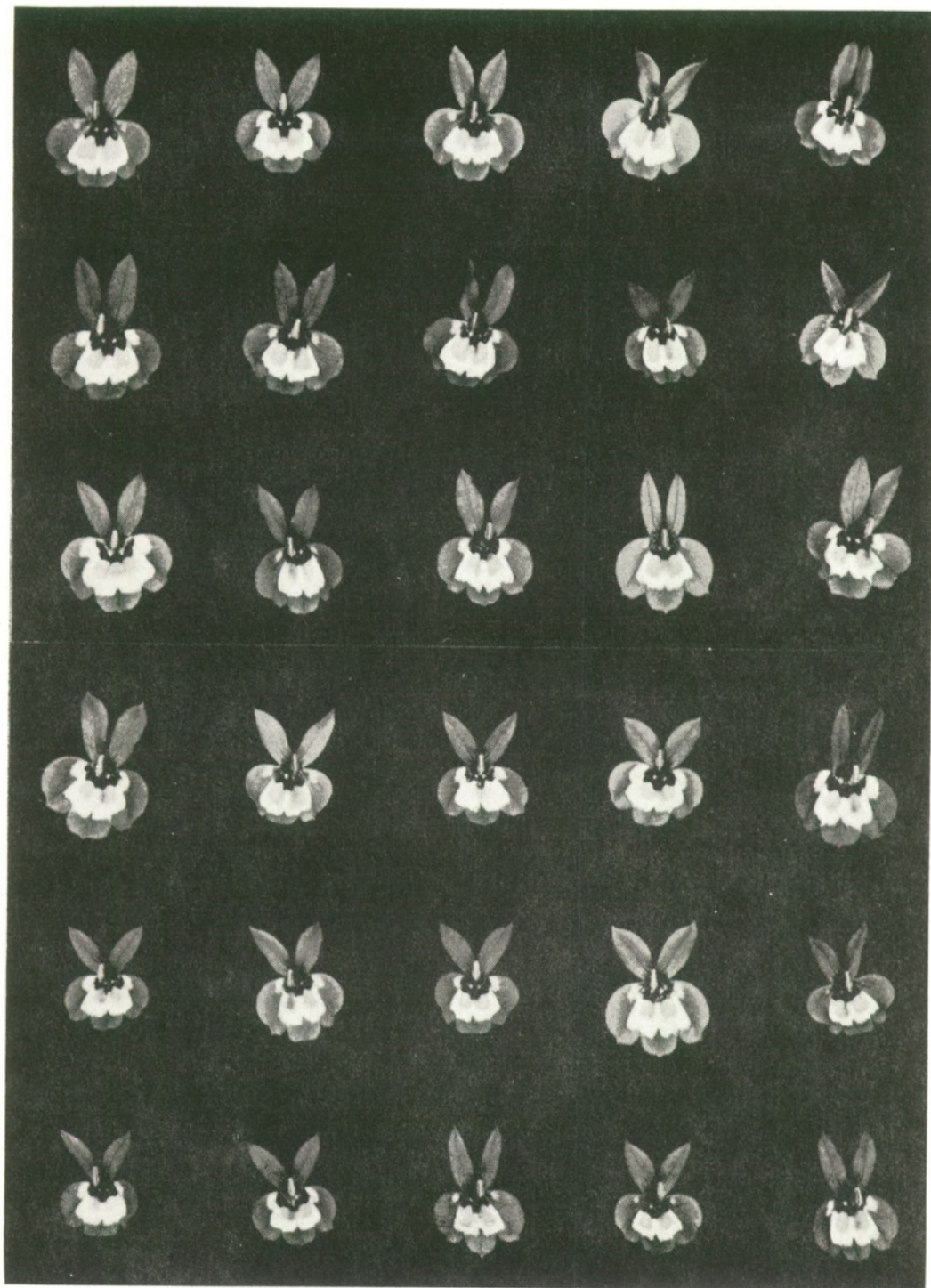


FIG. 9. Flowers of thirty plants of a backcross, population 211: *Downingia insignis* (14) \times (*D. pulchella* \times *insignis*, population 93). Natural size.

will be seen from *figs.* 11 and 12 which illustrate individuals of populations 200 and 203, the recombinations in no instance duplicate the parental types exactly, and most resemble the F_1 . Although the *Downingia pulchella* parent in the original crosses was white-flowered, the few white-flowered

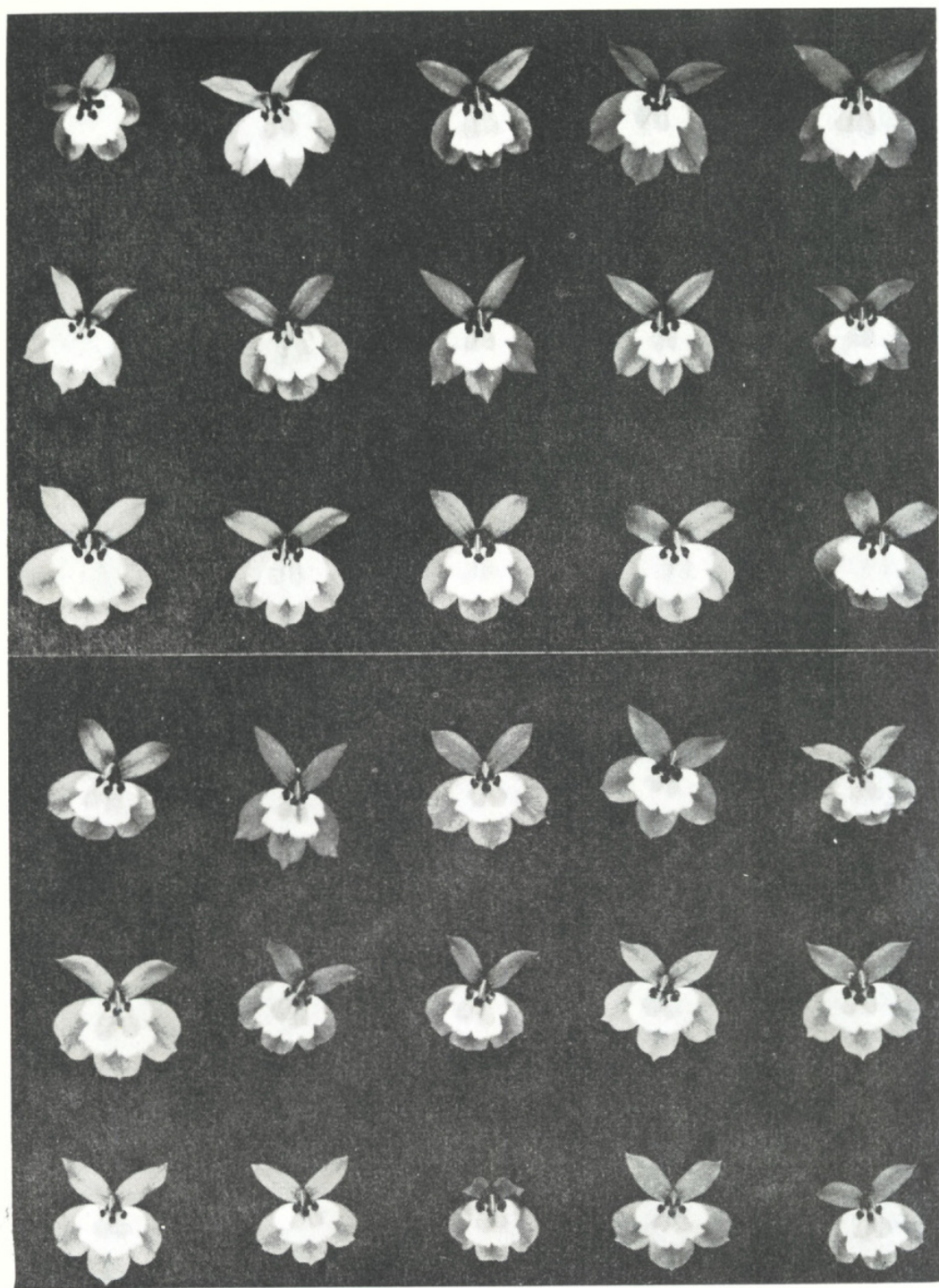


FIG. 10. Flowers of thirty plants of a backcross, population 207: *Downingia pulchella* (26) \times (*D. insignis* \times *pulchella*, population 95). Natural size.

segregants in the F_2 were more like *D. insignis*, although with divergent corolla lobes. In one bizarre individual of this population the corolla and androecium were suppressed altogether, but the stigma was well developed, and apparent stigmatic surfaces were found on the tip of each of the sepals.

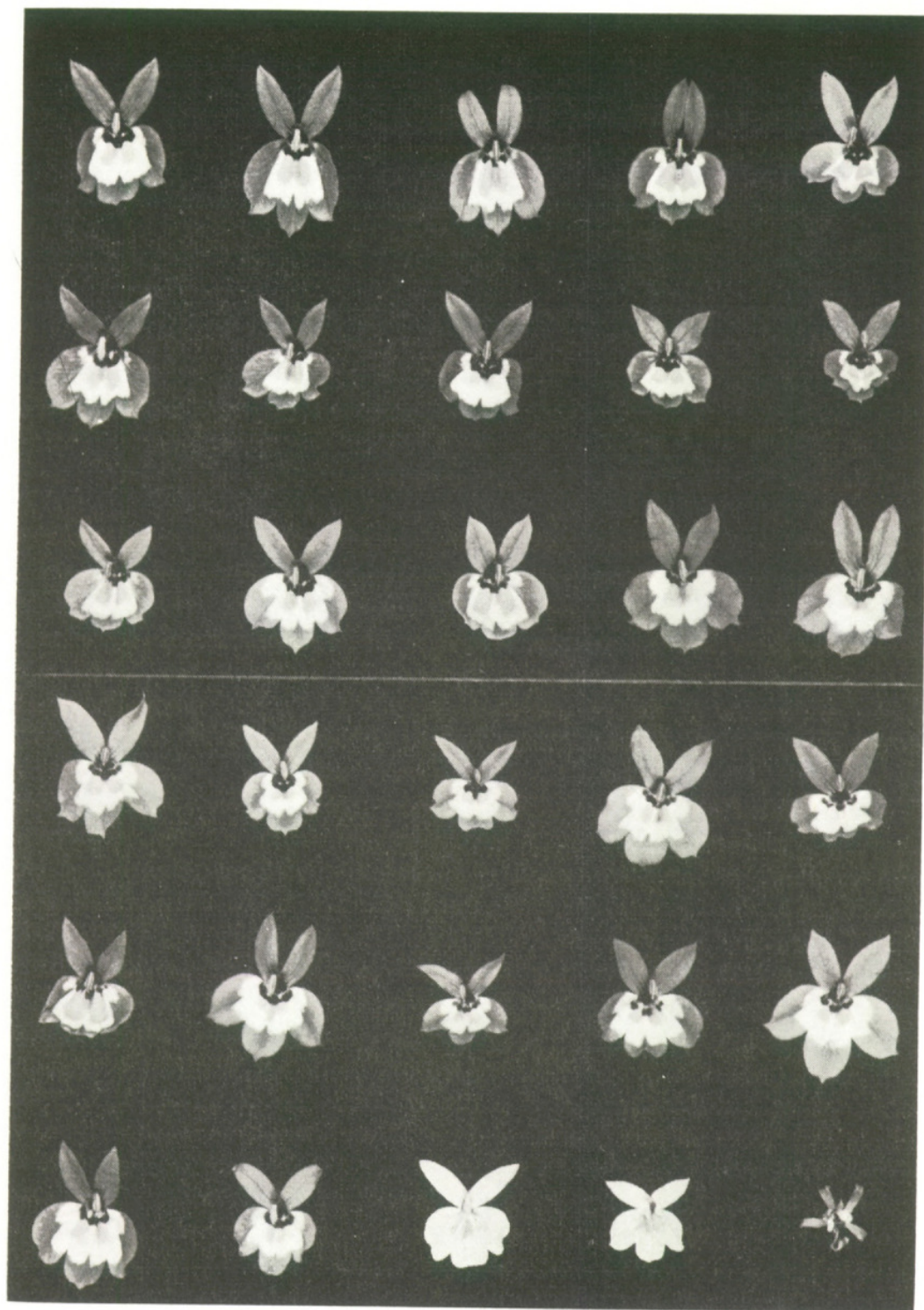


FIG. 11. Flowers of thirty plants of second-generation hybrid of *Downingia pulchella* \times *insignis*: population 200 from self-pollination of the single survivor of F_1 population 93. Plant at right in bottom row lacked corolla and stamens and had apparent stigmatic lobes on tips of sepals. Natural size.

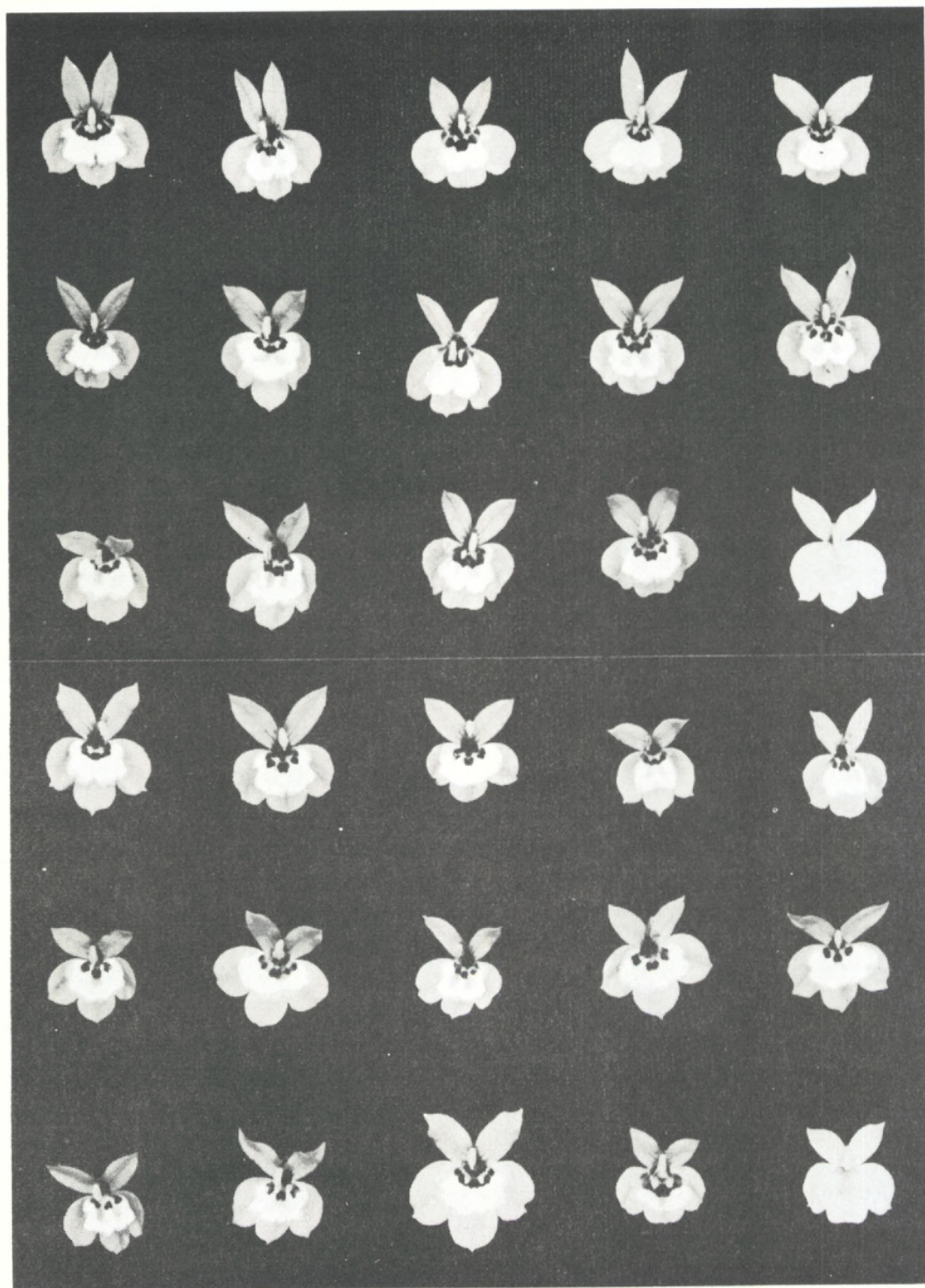


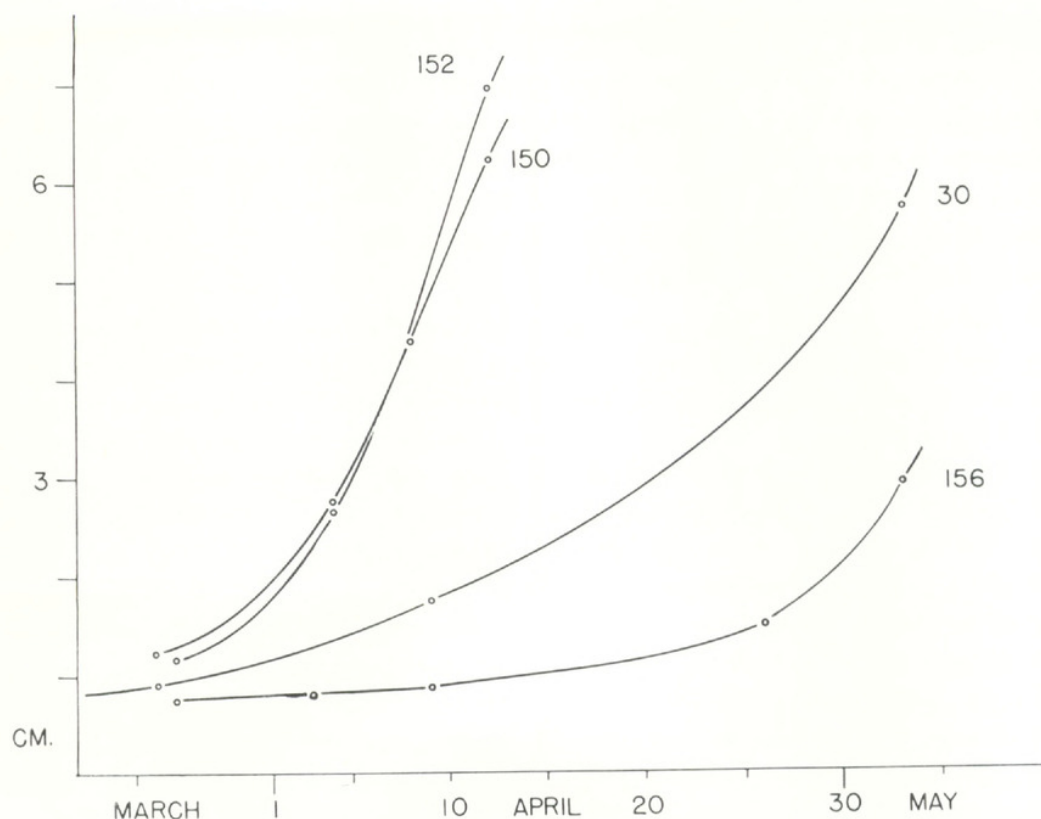
FIG. 12. Flowers of thirty plants of second-generation hybrid of *Downingia insignis* \times *pulchella*: population 203 from 95 \times 95. The *D. pulchella* parent in this and in fig. 11 was a white-flowered form. Natural size.

The Third Generation. Most of an F_3 generation was lost at Chapel Hill in 1952, a year in which all seeds sown failed. In 1953, four sowings, each derived from a single capsule and involving a cross within population 200, the F_2 of 93 (*D. pulchella* \times *insignis*), were made. Seeds ranged from sixteen to sixty per capsule, but only two to fifteen per capsule germinated, perhaps in part because of the two-year delay in planting. Seedlings were yellow to moderately green, but none was very vigorous. All grew very slowly, and none survived to flower.

From the above data it is evident that hybrids between *Downingia insignis* and *D. pulchella*, both of which have 11 pairs of chromosomes, are easily produced artificially and that germination of hybrid seeds is good. The interaction of the two gene complements produces physiological difficulties, however, and the hybrids are albino or chlorotic, inviable or weak, requiring (when surviving at all) very much longer than the parental species to flower. They are fertile, however, setting abundant seed with other hybrids or producing green and vigorous offspring in backcrosses. The second generation includes many genetically unbalanced plants, including a high proportion of albinos and chlorotic plants which do not survive. However, some of this generation are more vigorous than the first and flower in about the same length of time as the parental species. Although the data on a third generation are quite inconclusive, physiological weakness seems to continue.

In spite of the weakness of the first generation hybrid, there is the potentiality for gene exchange between the parental species, especially by backcrossing. It seems very unlikely, though, that such ever occurs in the wild populations in which *Downingia insignis* and *D. pulchella* grow together (as they sometimes do). No field data are available, but several factors which may work against any genetic exchange are immediately apparent. Insect pollinators of the two species have not, to my knowledge, been studied. The highly specialized flowers suggest, however, the possibility of a barrier to interspecific pollination due to differences in pollinators of the two species, or to differences in the preferences of a single pollinator for a particular color pattern, or to the mechanical barrier of the position of anthers and stigma. Assuming, though, that pollination may occur, the genetic chlorosis of the hybrids and the consequent (?) weakness and slowness of growth would result in the elimination of hybrid plants from wild populations before they could backcross with either parent or mature seeds themselves. The hybrids are either (1) too weak to survive the rigors of the mild winter or of the vernal pool habitat in general, or (2) cannot compete with the parental or other species, or (3) grow so slowly that they are out of synchronization with the drying up of the vernal pools and the flowering of the parental species and may not even be able to flower at all. Any or all are likely.

In the spring of 1951, a number of greenhouse experiments were essayed to test the survival potentialities of hybrids in competition with the parental species. As controls, fifty seeds of each of the parental populations (*D. pulchella* 26, *D. insignis* 14) and of three hybrid crosses (154,



Partial growth-curves of *Downingia insignis* (150), *D. pulchella* (152), their F_1 hybrid (population 156), and the slowest growing of the species, *D. bicornuta* var. *bicornuta* (30). Based on means of heights of varying numbers of plants, graph is intended only to give approximate comparisons of vigor and rapidity of maturation, not statistically accurate growth rates and ranges of variability. Measurements for 150 and 152 were taken respectively from 20 to 36 plants; those for hybrid began with 47 plants but drop to 41, 37, 11, and finally 5 survivors; for *D. bicornuta* involve only two plants grown separately. All seeds sown 28 February 1951; flowering of 150 and 152 began on 12–14 April, of hybrid on 5 June, and of *D. bicornuta* on 6 May.

155, 156; see F_1 above) were planted in 6-inch azalea pots. Ten combinations of hybrid and parental seeds totalling 60 to 300 seeds to each 6-inch pot and in proportions of hybrids to equal numbers of parental seeds of 1:2 to 5:1 were also sown at the same time. Even when grown alone in the controls (see F_1 above), these particular hybrids were hardly successful, and progressed slowly, only five of population 156 surviving to flower after 103 days. In none of the competitions did any hybrids survive to flower, whereas, no matter how crowded the pot, many of the nonhybrids flowered in 45–50 days. Unless combinations of *D. insignis* and *D. pulchella* other than the few tried here produce very much more vigorous hybrids, it seems that a seedling hybrid of these two species stands little chance of survival in the wild.

DISCUSSION

The species of *Downingia* form an aneuploid series with 8, 9, 10, 11, and 12 pairs of chromosomes. Eleven (which occurs in five species) appears

to be the basic number, especially since the less specialized *Porterella carnulosa*, the closest relative of the genus, also has eleven pairs (Carlquist, Madroño 13: 206. 1956).^{*} In addition, both *D. elegans* var. *brachypetala* (10 pairs) and *D. ornatissima* (12 pairs) show morphological specializations indicative of derivation; and the occurrence of eight or nine pairs in different populations of *D. concolor* suggests both specialization and continuation of the aneuploid trend. Hybrids between the two chromosomal races of *D. concolor* should yield evidence from their meiotic behavior of the manner in which aneuploidy has occurred in the genus, and somatic chromosome morphology should provide additional clues.

Chromosome numbers and hybridization data are summarized in fig. 13, in which the species have been arranged on the basis of morphological and genetical similarities. The diagram includes reciprocal interspecific crosses in all possible combinations, except some involving *Downingia pusilla* and those between *D. bicornuta* var. *picta* and *D. insignis* and *D. pulchella*, none of which was made.

It is clear from the crossing results that species of *Downingia*, at least as represented by these populations, are well separated genetically, with strong barriers to hybridization. Interspecific crosses fail to produce embryos; the seeds abort near maturity or the hybrid seedlings are albino; or, exceptionally, the hybrids are weak and slow growing but fertile. These categories mark three genetic groups into which the eight species fall.

The first comprises only *Downingia elegans* var. *brachypetala* which stands in complete isolation, for not even partially developed seeds were obtained in any of the hybrid combinations attempted.

A second group, including *Downingia bicornuta*, *D. concolor*, *D. cuspidata*, *D. ornatissima*, and *D. pusilla*, shows very strong interspecific genetic incompatibilities, but the production of more or less numerous albino seedlings in various combinations of these species provides evidence of closer relationships within the group than outside it. The differences found in most reciprocal crosses between these species (e.g., the production of relatively numerous albino seedlings when *D. cuspidata* is used as the seed parent, but the failure to set seeds when this species is the pollen parent) suggest a prefertilization barrier to hybridization, in addition to the strong incompatibilities of the gene complements in the hybrids.

The third includes *Downingia pulchella* and *D. insignis* which are isolated from the other species grown, producing only an occasional albino seedling with some members of the preceding group. The two species are potentially capable of interbreeding, but their hybrids are albino or chlorotic, weak and slow growing. Those surviving to flower, however, are

^{*} McVaugh's earlier report (1943, p. 25) of $n = 12$ for this species (McVaugh 6282, Lake County, Oregon) was overlooked when the manuscript was being prepared. Carlquist's count was based on *Mason 14439* (uc), from Sierra County, California. In spite of the apparent aneuploidy in this widespread species ("northwestern Wyoming to southeastern Oregon, south in the mountains to northern Utah, Coconino County, Arizona, northern Nevada, and Tulare County, California"), eleven would still seem to be the basic number in *Downingia*.

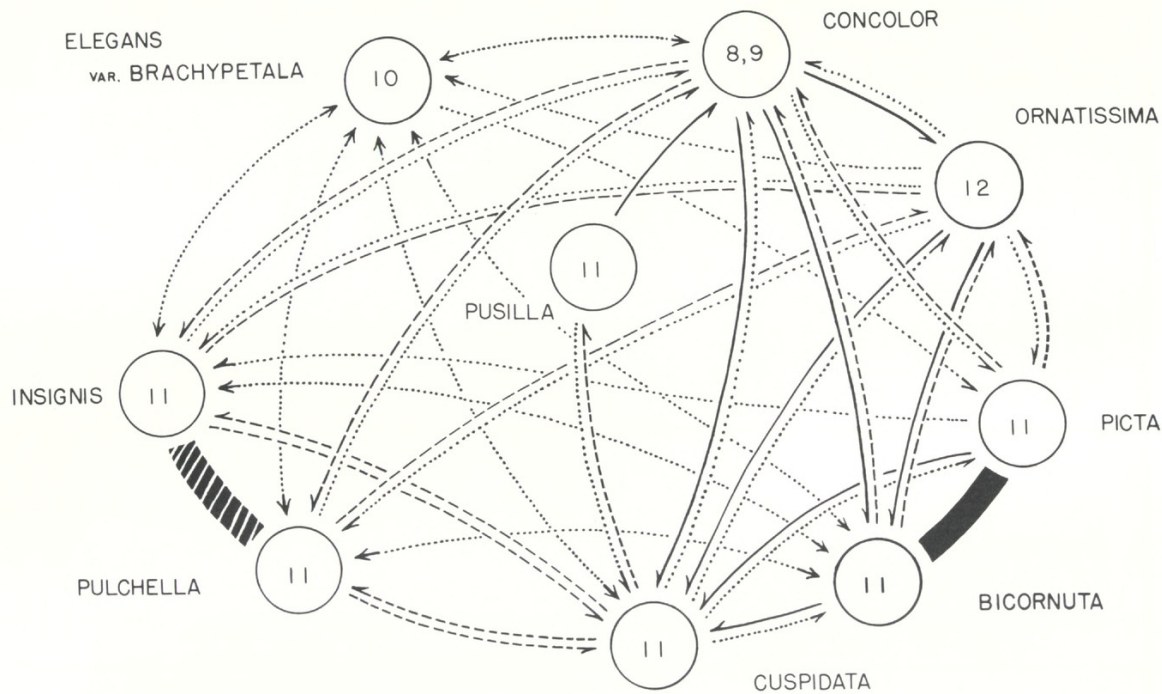


FIG. 13. Crossing relationships and chromosome numbers of eight species of *Downingia*. All crosses attempted are shown. Arrows indicate direction of cross; those unsuccessful in both directions are shown by a single dotted line with arrow at both ends. Dotted line, no trace of hybrid seeds; dashes, production of an occasional albino lethal (averaging less than one per capsule); thin solid line, frequent hybrid lethals; broad striped band, albino or chlorotic but fertile hybrids; broad black band, vigorous fertile hybrids. See text.

as fertile as either parent and produce green and vigorous offspring when backcrossed to the parents. Although they sometimes grow together, the weakness of the first-generation hybrid probably precludes any possibility of gene exchange in the wild, even if hybrid seeds are produced.

In contrast to interspecific crosses, those within species (although limited in number) produced vigorous seedlings. The two recognized varieties of *Downingia bicornuta* (vars. *bicornuta* and *picta*), strikingly different in morphology, geography, and physiology, yield a vigorous and apparently fertile hybrid of intermediate morphology, confirming the taxonomic status accorded them. It is to be expected, however, that further studies will show, in addition to the aneuploidy found in *D. concolor*, other stages in speciation, such as the development of genetic barriers to interbreeding.

Data are lacking for four species, but the chromosome numbers and the genetic results corroborate both McVaugh's taxonomic treatment of the eight species grown and his arrangement of these in three informal morphological groups (which correspond to the genetic categories above). (See McVaugh, 1941, p. 10.) *Downingia bella*, *D. bicornuta*, *D. concolor*, *D. cuspidata*, *D. ornatissima*, and *D. pusilla*, all with bilocular ovaries, form a morphologically and genetically interrelated group of species, while *D. pulchella*, *D. insignis*, and *D. laeta* form another. *Downingia yina* (including *D. willamettensis*) and *D. montana*, with unilocular ovaries, were treated by McVaugh as still another line, while *D. elegans*, also unilocular, was placed in an isolated position, even though the question of hybridization between this species and *D. yina* was raised. *Downingia elegans* var. *brachypetala* is indeed isolated genetically, morphologically, and chromosomally from the other species grown, but it is in many ways similar to *D. yina* var. *major* (*D. willamettensis*), and it is possible that these three species with unilocular ovaries may be more closely related than appeared at first.

Although strong genetic barriers to hybridization now exist, and, in the absence of these, differences in chromosome number might produce at least partial barriers to interbreeding of the species, a number of other barriers may have been important in the past and may be at least partially operative at the subspecific level now. Geographical, ecological, temporal, and mechanical barriers are possible. (See Stebbins, 1950.)

Occurring in both California and Chile, *Downingia pusilla* is the most spectacular example of spatial division of a species; but *D. concolor* var. *concolor*, of Lake to Monterey counties, California, is separated from var. *brevior*, of San Diego County, and *D. cuspidata* seems to be divided into several disjunct areas. *Downingia montana*, of the Sierra Nevada, is separated ecogeographically from its closest relative, *D. yina*, and the same is true of *D. pulchella* and *D. laeta*.

Both ecogeographical isolation and differences in flowering time may play a part in the separation of *D. bicornuta* vars. *bicornuta* and *picta*, which in cultivation flower about two weeks apart. However, subspecific variation in this species is more complex than has been thought and needs further study. *Downingia insignis* and *D. elegans*, perfectly distinct but

long confused taxonomically, also show differences in time of flowering.

Field studies will be necessary to determine whether real ecological differences exist between otherwise sympatric species. Some species do occur together, such combinations as *D. concolor*–*D. insignis*, *D. elegans*–*D. insignis*, *D. bicornuta*–*D. cuspidata*–*D. concolor*, and *D. pulchella*–*D. insignis* having been found. Eight species are reported from Stanislaus County, California, but no further information is available as to their distribution within this center of concentration.

Mechanical barriers to crossing are among the most intriguing possibilities. The variation in length and position of the stamens, their peculiar pollen discharge mechanism, and the wide range in size and coloring of the elaborate corollas strongly suggest specific insect pollinators. It does not seem likely, for example, that the same bee which has its face dusted with pollen from the short, included stamens of the modest flowers of *D. concolor* could pollinate nearby *D. insignis* with its large and showy lower lip, broad corolla tube, and strongly exerted stamens. Careful field observations are needed on pollinators and their behavior, not only in respect to the species, but to mutations in the form and color of the corolla. (See *Downingia pulchella* above.)

These eight species of *Downingia* emerge, then, as distinctive, highly specialized semiaquatic winter annuals, well adapted to a transient habitat. A large number of individuals may occur within a small area, but because of the nature of the habitat the populations are discontinuous. Although self-compatible, the plants are adapted for insect pollination and are out-crossing (with the apparent exception of the most reduced species). As a result, individuals are highly heterozygous. The chromosome numbers form an aneuploid series centering around 11, with 8, 9, 10, and 12 as derived numbers. The presence of occasional chromosomal bridges in meiotic divisions and of different chromosome numbers within a single species show that chromosomal repatterning continues within the genus. Poorly to highly differentiated geographical races attest to a whole series of subspecific levels of development. However, the species are well separated, both morphologically and genetically. Although spatial, ecological, temporal, and mechanical barriers may be operative below the specific level, strong genetic barriers exist between species, which, although presumably evolved allopatrically, can now exist sympatrically without gene exchange.

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