1965

The Evolution of a Pair of Sibling Allotetraploid Species of Cobwebby Gilias (Polemoniaceae)

Alva Day
University of California, Davis

THE EVOLUTION OF A PAIR OF SIBLING ALLOTETRAPLOID SPECIES OF COBWEBBY GILIAS (POLEMONIACEAE)[1]

ALVA DAY

Department of Genetics, University of California, Davis

I. INTRODUCTION

The Cobwebby Gilias *(Gilia* section *Arachnion)*, named for the characteristic pubescence of the herbage which is made up of long, fine, intricately-tangled trichomes, are low-growing annual plants. They occur often in dense profusion in sandy or rocky habitats of the deserts and mountains of the southwestern United States and adjacent areas of northern Mexico. Due to the small size of the plants and flowers of many of the species, and the frequent occurrence of a number of superficially similar species in a single locality the group has presented a taxonomic puzzle.

To construct a system of classification of the existing array of species which will reflect insofar as possible their true biological relationships has been an objective of myself and my collaborator, Verne Grant, for a number of years. A systematic treatment of the Cobwebby Gilias was presented in 1956 (Grant & Grant, 1956), bringing the taxonomy up to date with our first seven years of taxogenetic research on the group. The complexity of the problem was recognized at that time and the necessity for further detailed work on the small-flowered species was noted.

The present study is an analysis of the interrelationships between five of these small-flowered species, chosen because they comprise a unit of phylogenetic significance which, being small in numbers of species, may be dealt with in the detail necessary. The group consists of the following: *Gilia aliquanta* A. & V. Grant, *G. clokeyi* Mason, *G. malior* Day & Grant, *G. minor* A. & V. Grant, and *G. transmontana* (Mason & Grant) A. & V. Grant.

A description of the general nature of the biosystematics of the Cobwebby Gilias may be worthwhile as a background for this presentation. The North American species fall into two series with respect to chromosome number, diploids ($n = 9$) and tetraploids ($n = 18$) (Grant, Beeks & Latimer, 1956).

The diploid species of Cobwebby Gilia have been placed in three species groups, the *G. ochroleuca*, *G. tenuiflora*, and *G. brecciarum* groups, according to their relationships as established by the combined information obtained

---

[25]

from morphological and cytogenetic studies (Grant & Grant, 1960). These are groups of species characterized by contrasting extremes as regards a number of characteristics, a list selected from among all the species of each group and not necessarily characterizing completely any one taxon. A few devients within each group were noted to be "bridging" species, resembling in some ways one of the opposing species groups.

Over a large part of the distributional area of the Cobwebby Gilias, the diploid species are observed to occur sympatrically in various combinations. Correspondingly, natural hybridization takes place in various combinations, in spite of the presence of strong internal barriers. This may occur between species belonging to the same species group or between members of different species groups. In the case of those diploid species which are large-flowered and outcrossing, the hybridization has led to introgression. This is believed to be the explanation for the presence of bridging species among the diploids. Among the small-flowered and autogamous diploid Cobwebby Gilias, on the other hand, the hybridization has resulted in allotetraploidy (Grant, 1956).

The tetraploids tend to be intermediate between their diploid progenitors but they may also resemble one or the other diploid rather closely. Since allotetraploidy has occurred between as well as within the species groups, the three evolutionary lines of development which remain fairly clear for the diploids, are quite blurred at the tetraploid level. This was noted in the taxonomic revision of Section *Arachnion*: "Usually the tetraploid members do not fit as easily as the diploid members into a species group." (Grant & Grant, 1956, p. 219).

From a preliminary survey of the known tetraploids of the Section *Arachnion* it appears that the minimum number of different tetraploid taxa derived by hybridization between members of the *Gilia tenuiflora* group and the *G. diegensis* group is three; between the *G. tenuiflora* group and the *G. ochroleuca* group, five; and between species within the *G. ochroleuca* group, two.

Before the matter of chromosome number had come into consideration the taxonomist was at a loss for morphological gaps in this very diverse assemblage of small-flowered plants—the *Gilia inconspicua* problem of old. Now the problem can be approached with at least more understanding, if not immediate solution, as the picture appears to consist of a network of diploid extremes interconnected by tetraploid bridges. The possibilities of such interconnections are numerous, being limited by the degree of genomic differentiation of a given pair of diploid species, and by their respective geographical and ecological distributions.

The group of species to be discussed herein comprise a segment of this network of small-flowered Cobwebby Gilias.

Evidence is presented in support of the hypothesis that the closest living relatives of the diploid species from which *Gilia transmontana*, a tetraploid, has been derived are *G. minor* and *G. clokeyi*, and that the closest living relatives of the diploid species from which *G. malior*, also tetraploid, has been derived are *G. minor* and *G. aliquanta*. Habit silhouettes of the species are arranged in fig. 1 to illustrate this hypothesis.

The supporting evidence will be drawn from the following lines of investigation: (1) geographical distribution and ecology of the species; (2) morphological comparisons of the species; (3) karyotype analysis; (4) the cytology of

artificial hybrids and of synthetic allotetraploids derived from diploid hybrids; (5) morphological comparisons between the tetraploid species and the corresponding synthetic allotetraploids; and (6) the cytology of hybrids between the tetraploid species and the synthetic allotetraploids.

It is hoped that this study will verify in some degree our assumption as to the nature of the origin of the network-like pattern of relationships in the larger complex which has been outlined above.

II. ACKNOWLEDGMENTS

III. THE GEOGRAPHICAL DISTRIBUTION
AND ECOLOGY OF THE SPECIES

Gilia minor occurs often in abundance and over large areas in the fine sandy soil of outwash plains and in the vicinity of vernal pools. In California it extends through the inner south coast ranges and into the southwestern part of the Mojave Desert, at elevations of 1000 to 3500 feet. A disjunct series of populations is known in western Maricopa County, Arizona, differing morphologically in some degree from the California populations but interfertile with them (Grant & Grant, 1960).

Gilia clokeyi is found mainly in the Colorado River drainage area of northern Arizona and southern Utah, extending as far east as the San Juan River of the "four corners" region of Arizona, Utah, Colorado, and New Mexico, and west to southern Nevada and the adjacent Kingston and Clark Mountains of California. Known also farther north in the Inyo Mountains, its seems probable that *G. clokeyi* occurs in related areas between, which have been insufficiently explored. Below Grand Canyon it has been found at 1700 feet elevation, but more commonly ranges higher, from 2500 to 5300 feet.

No record is known of a sympatric occurrence of *G. clokeyi* and *G. minor* although the breaks between the ranges of the two both in Arizona and California are small and perhaps recent (see fig. 2).

Gilia aliquanta, a species related to *G. clokeyi*, replaces the latter toward the west, their ranges interdigitating in the desert mountains of the Southwest near the California-Nevada border. An ecological separation clearly exists between the species where it has been possible to observe them in contiguous areas. *Gilia clokeyi* appears to be restricted to limestone, but *G. aliquanta* is found on granite or basalt soils. The two species occur within a few miles of each other in the Kingston Mountains but remain morphologically distinct.
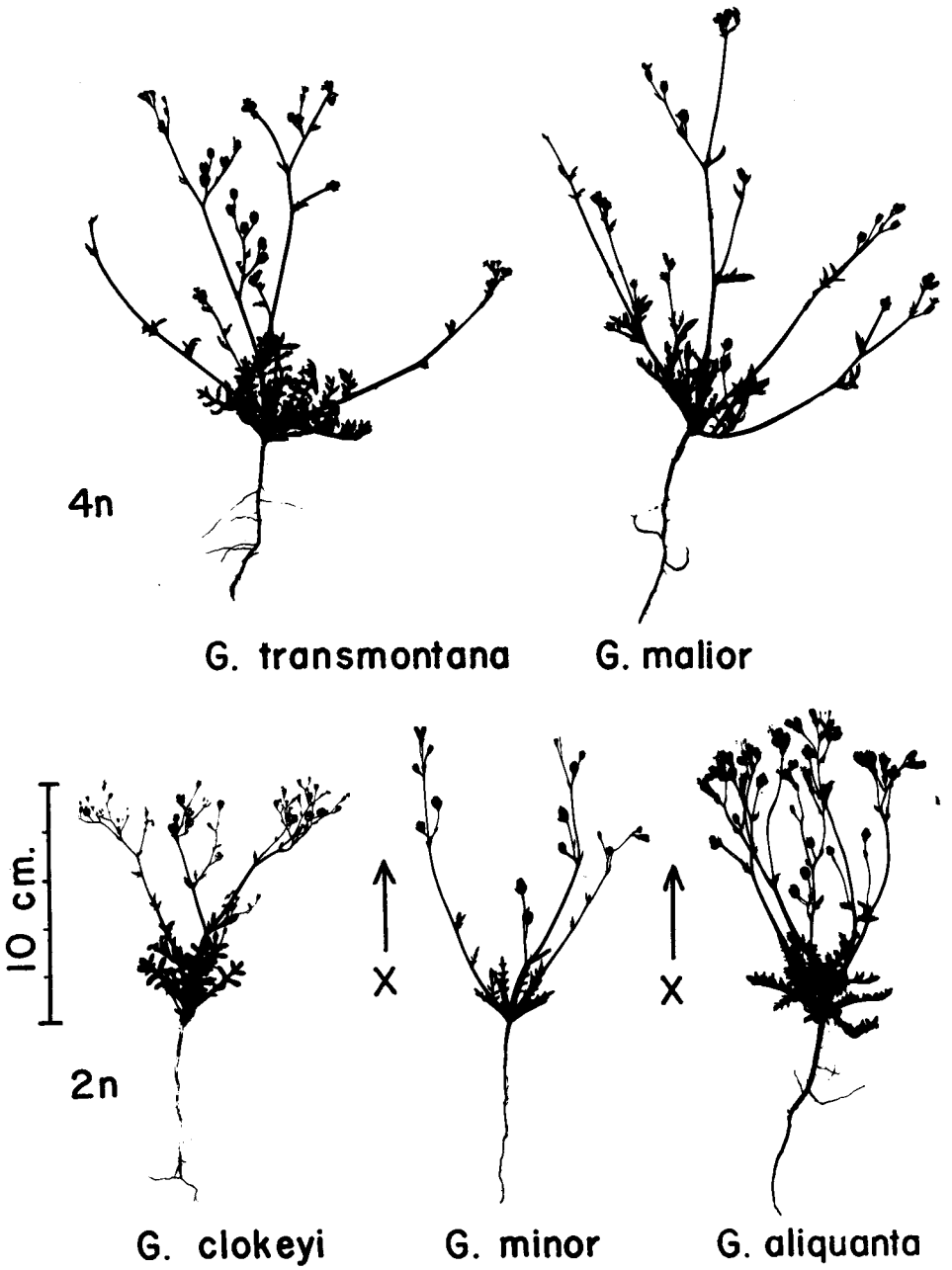
FIGURE 1

Gilia aliquanta grows at elevations of 2500 to 5000 feet. The main distributional area is in the Mojave Desert along the eastern base of the southern Sierra Nevada and the lower north sides of the San Gabriel and San Bernardino ranges. It occurs sympatrically with *G. minor* in much of this area.
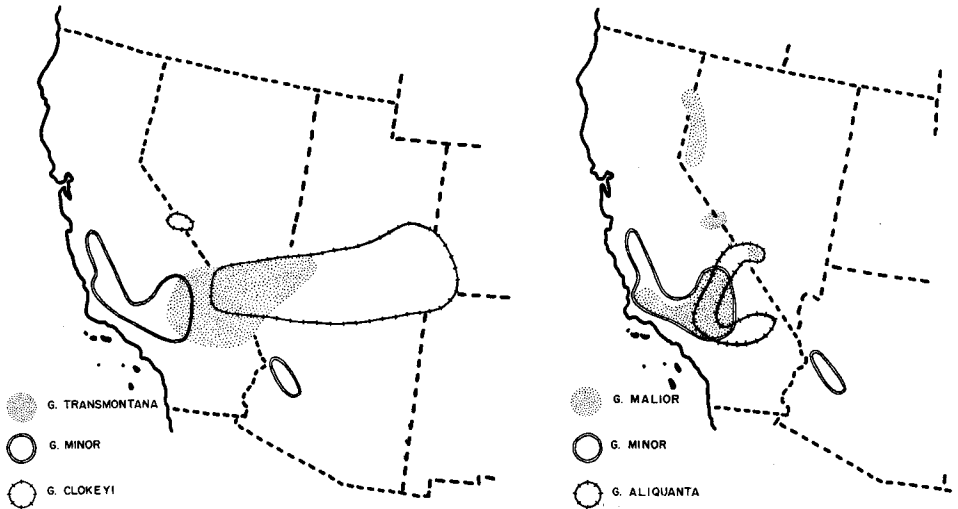

Fig. 2. Geographical distributions of the tetraploid and diploid species (southwestern United States).

The problem of the availability of water is met in similar ways by *Gilia aliquanta* and *G. clokeyi*. Both species occur in the type of habitat which may supply abundant moisture for a limited time during the spring, and both species, having succulent leaves, are able to hold the quantities of water necessary to complete a life cycle as the water supply recedes. This habitat is typically near the top and sides of rocky slopes or ridges in the path of the runoff from snow or rain, or at the base of rock outcrops where seepage occurs.

This contrasts with *Gilia minor*, as the latter species is on the lower slopes and flats in sandy soil of higher clay content. The leaves are less succulent, perhaps corresponding with a more brief life cycle, or a longer period of available water.

Gilia transmontana, as a likely product of *G. minor* × *G. clokeyi*, occurs within the ranges of these two diploid species and in the intervening areas as well (fig. 2). It is known from southwestern Utah to Mojave County, Arizona, the mountains of southwestern Nevada and of southeastern California, and the Death Valley region, as well as to the west in mountain ranges of the Mojave Desert. Altitudinally it ranges widely, from 1500 to 5300 feet.

Fig. 1. Habit silhouettes of three diploid and two tetraploid species, arranged phylogenetically.

In the eastern part of its range *Gilia transmontana* is most common. It occurs on rocky or sandy slopes and in canyons and washes, sometimes mixed in the same populations with *G. clokeyi*, but usually is more widespread than the latter species which has a more restricted habitat.

Gilia malior ranges more to the west and northwest than does *G. transmontana* and is for the most part isolated from the latter species geographically. Sympatric occurrences of the two species are known, however, in the vicinity of the Kramer Hills in western San Bernardino County, California. Where their ranges overlap in southwestern Nevada they are ecologically isolated in the manner of *G. aliquanta* and *G. clokeyi* of the same region, in that *G. malior* is upon granite and basalt formations whereas *G. transmontana* is in limestone areas.

Some distance to the north, in a disjunct area from Reno, Nevada, to Lassen County, California, *Gilia malior*, occurring independently from the other members of the complex, is found again upon basalt.

In the southern part of its range, *Gilia malior* is sympatric with both of its supposed diploid progenitors at elevations of 1800 to 5000 feet through the inner south coast ranges of California and into the Mojave Desert. Like *Gilia transmontana* it ranges more widely than its diploid relatives and may be located either in the habitat of *G. aliquanta* on rocky slopes or on outwash flats with *G. minor*, or in intermediate situations. An example of this is a population near Mojave, California, where *G. aliquanta* occurs at the base of granitic outcrop on a butte. On the lower slopes and flat below, *G. minor* is common. But the most abundant and vigorous *Gilia* in the area is *G. malior*, growing to some extent intermixed with the other species and also widespread in the less specialized situations on the hill.

In summary, the geographical range of the tetraploid, *Gilia transmontana*, overlaps the areas of the diploids, *G. clokeyi* and *G. minor*, and extends also into the intervening regions where neither diploid is known. The relatively unspecialized ecological requirements and the vigor of *G. transmontana* may have permitted its survival in regions where *G. minor* or *G. clokeyi* may not be well adapted.

Similarly, the geographical distribution of *Gilia malior* coincides with that of *G. minor* and *G. aliquanta* but includes also an area farther to the north, apart from these diploid species. Its wide tolerances as to ecological situation apparently permit it to thrive in the types of habitat preferred by the two ancestral species as well as in intermediate habitats.

IV.   THE MORPHOLOGY OF THE SPECIES

METHODS

In seeking to identify the diploid progenitors of the tetraploid species, *Gilia transmontana* and .*G. malior*, preliminary herbarium studies were conducted in 1959 which led to the observation that most of the characteristics of these species could be identified as modifications of *G. minor* in the direction of *G. clokeyi* in the first case, and toward *G. aliquanta* in the second. The suggestion of allopolyploidy as the mechanism by which the tetraploid species had arisen seemed appropriate, and the similarity between the two tetraploid

species could be identified as modifications of *G. minor* in the direction of them.

This pilot study, employing herbarium material of from 25 to 40 collections of each species, turned up many characters which seemed significant to the above hypothesis. Several of these original observations and measurements are included in the summary given in table 1. Characteristics which were especially subject to environmental modification were reexamined from a series of garden-grown specimens. Observations on corolla color were recorded in the field and in the garden from living plants.

The plants were grown under as uniform conditions as possible in a screenhouse in Claremont, California, in the spring of 1963. Two to four strains of each species (table 2) were planted as representative of the variation previously encountered in the herbarium. The plants were vigorous and appeared comparable to their counterparts in the wild.

Permanent records of the species cultures consist of a series of photographs of the leaves, flowers, and capsules of each strain and also pressed specimens which were obtained as the plants developed. The voucher specimens are deposited in the herbarium of the Rancho Santa Ana Botanic Garden.

A MORPHOLOGICAL COMPARISON OF THE SPECIES

Leaf form in the diploid species:

The following descriptions of leaf form, made from garden-grown plants, as were the illustrations of fig. 3, will apply also to wild plants which have grown in a favorable environment. Plants under poor growing conditions, which may come to maturity without having attained full development of the basal leaves, often do not adequately express the characteristics of leaf form which differentiate these species.

The basal leaf of *Gilia minor* (fig. 3 H) has lobes and rachis which are flexuous and very narrow—less than 1 mm in width and not conspicuously succulent. Secondary dissection is slight or entirely lacking although occasional populations have bipinnatifid leaves. The general outline of the leaf, which can be visualized by imagining a continous line joining the base of the leaf to the tip of each lobe and to the leaf apex, is narrowly oblanceolate, and terminally attenuate or acute. The individual lobes, somewhat similar in form to the leaf as a whole, may be described as narrowly linear and terminally acute. They are borne opposite or alternate to each other, both arrangements usually being found on the same plant. The spaces between the lobes are mostly longer that the length of the lobes themselves.

In *Gilia clokeyi* the basal leaves (fig. 3 F) are more rigid and succulent than in *G. minor*. The rachis and lobes are wider—about 1 to 2 mm in width. Secondary dissection is usually well-developed, especially near the leaf apex. The leaf outline is spatulate, being broad near the apex and terminally truncate. The lobes are also truncate and tend to be broader at the middle than at the base (see also cauline leaf, fig. 3 A). The apex of each lobe is terminated by a sharp cusp. The primary and secondary lobes are opposite to subopposite. In the upper part of the leaf the spaces between the primary lobes are shorter than the length of the lobes.

TABLE 1. *Comparison of diploid and tetraploid species in thirteen characters.*[1]

CHARACTER	G. clokeyi	G. transmontana	G. minor	G. malior	G. aliquanta
1. Leaf lobe width, mm (lateral lobe, basal lf.)	1.5 (1.1–2.2)	1.1 (0.8–1.5)	0.8 (0.6–0.9)	1.2 (0.9–1.5)	1.4 (1.3–1.5)
2. Basal leaf form (width/length of a 5-lobe segment)	0.26 (0.20–0.37)	0.21 (0.17–0.28)	0.15 (0.11–0.18)	0.18 (0.15–0.23)	0.18 (0.15–0.28)
3. Cauline leaf form (width/length)	0.53 (0.41–0.71)	0.46 (0.41–0.52)	0.34 (0.18–0.40)	0.44 (0.25–0.55)	0.52 (0.42–0.64)
4. Calyx pubescence	glabrous	lightly pubescent	lightly to densely pubescent	lightly pubescent	glabrous
5. Calyx sinus membrane	smooth	smooth	smooth	smooth to slightly puckered	slightly to very puckered
6. Corolla length, mm	5.0 (4.2–6.3)	6.2 (5.0–8.3)	6.4 (3.3–8.1)	7.5 (6.0–10.7)	11.9 (5.8–21.5)
7. Corolla lobe form	narrowly oval; somewhat attenuate	narrowly oval; acute	oval; mucronate	oval; somewhat truncate, to slightly pointed	broadly oval; truncate

TABLE 1. *Comparison of diploid and tetraploid species in thirteen characters.[1]* (continued)

CHARACTER	*G. clokeyi*	*G. transmontana*	*G. minor*	*G. malior*	*G. aliquanta*
8. Corolla color 1 (purple in tube and lower throat)	−	+ (weak)	+ (dark)	+ (reddish)	+ (reddish)
9. Corolla color 2 (purple in upper throat)	−	−	+	±	−
10. Corolla color 3 (streaks on dorsal sides of lobes)	+	+	−	−	−
11. Capsule form	ovoid; base broad, apex pointed	± ovoid; apex rounded or pointed	sub-cylindrical; narrow; apex ± rounded	ovoid; apex rounded	broadly ovoid; apex rounded
12. Capsule dehiscence	disarticulates at base	disarticulates at base	remains attached at base	disarticulates at base	disarticulates at base
13. Seed size and weight, mg	large 55 (30–85)	small to med. 27 (20–30)	small 17 (10–20)	med. to large 45 (30–60)	large 45 (30–60)

[1]The figures given are mean and range. These do not necessarily constitute a taxonomically useful analysis but represent the comparative phenotypes under the conditions indicated. Characters 1, 2, 3, and 13 refer to garden plants grown under uniform conditions. Other characters are from field notes, herbarium specimens and garden plants.

TABLE 2. *List of strains and sources of seed.*

SPECIES	SYMBOL	LOCALITY	COLLECTION NO.[1]
Gilia aliquanta A. & V. Grant ssp. *breviloba* A. & V. Grant	MB	Butte south of Mojave, Kern Co., Calif.	60–109a
G. aliquanta A. & V. Grant ssp. *aliquanta*	Caj	Cajon Pass, San Bernardino Co., Calif.	62–17
G. aliquanta A. & V. Grant ssp. *aliquanta*	RR	Redrock Canyon, Kern Co., Calif.	9117
G. aliquanta A. & V. Grant ssp. *aliquanta*	V	Victorville, San Bernardino Co., Calif.	60–126
G. clokeyi Mason	DS	Deep Springs, Westgard Pass, Inyo Co., Calif.	9836
G. clokeyi Mason	MH	Mexican Hat, San Juan Co., Utah	10154
G. clokeyi Mason	Ky	Kyle Canyon, Charleston Mts., Clark Co., Nevada	9950
G. minor A. & V. Grant	MB	Butte south of Mojave, Kern Co., Calif.	60–101
G. minor A. & V. Grant	Kr	Kramer Junction, San Bernardino Co., Calif.	9222
G. minor A. & V. Grant	W	Wickenburg, Maricopa Co., Arizona	9298
G. malior Day & Grant	Cu	Cuyama Valley, Santa Barbara Co., Calif.	H. L. Mason, 1949
G. malior Day & Grant	MB	Butte south of Mojave, Kern Co., Calif.	60–107
G. malior Day & Grant	ShC	Short Canyon, Inyokern, Inyo Co., Calif.	9337
G. malior Day & Grant	Sp	Sparks, Washoe Co., Nevada	9795
G. transmontana (Mason & Grant) A. & V. Grant	Bd	Beaverdam Mts., Washington Co., Utah	9972
G. transmontana (Mason & Grant) A. & V. Grant	KH	Kramer Hills, San Bernardino Co., Calif.	9902
G. transmontana (Mason & Grant) A. & V. Grant	Ky	Kyle Canyon, Charleston Mts., Clark Co., Nevada	9952
G. transmontana (Mason & Grant) A. & V. Grant	MP	Mountain Pass, eastern San Bernardino Co., California	9060

[1]Collection numbers are those of the author, unless otherwise identified.

The basal leaves of *Gilia aliquanta* (fig. 3 J) are in several ways similar to those of *G. clokeyi*, being more or less rigid and succulent with rachis and lobes about 1.5 mm wide (often up to 2.5 mm in the wild). Secondary dissection is slight or in vigorous plants well-developed in the upper lobes. The whole leaf is terminally truncate in form, narrowly oblanceolate, differing from *G. clokeyi* by the loose spacing of the lobes and the relatively short length of the lobes, which together extend the proportions of the leaf longitudinally. The lobes are rounded and acute, but tend to be broader at the base than at the middle, in contrast to *G. clokeyi* (see also cauline leaf, fig. 3 E). A sharp cusp terminates each lobe. The lobes are opposite to subopposite or alternate, and as in *G. minor* this varies on different leaves of a given plant.

Leaf form in the tetraploid species:

The influence of *Gilia minor* in the tetraploid species is apparent in the nearly linear and wide-spaced leaf lobes, and the narrow, somewhat flexuous leaves. It is often difficult to distinguish with certainty from the leaves alone the tetraploids from *G. minor*. Under other conditions, due to the variability in leaf form, the tetraploid leaves are scarcely distinguishable from those of *G. clokeyi* or *G. aliquanta*. This is especially true in young and incompletely formed or depauperate specimens. The photographs in fig. 3 represent approximately the average form of leaf in each of the species. The influence of *G. clokeyi* and *G. aliquanta* may be noted in the form of the leaf outline which is more blunt or truncate rather than attenuate as in *G. minor*.

Thus far we have dealt with the two tetraploid species together, as being similarly intermediate between *Gilia minor* on the one hand and either *G. clokeyi* or *G. aliquanta* on the other. Distinguishing the leaves of *G. transmontana* from those of the sibling species, *G. malior*, requires the use of characters which correspond to the differences between *G. clokeyi* and *G. aliquanta*. As described above this has to do with the leaf proportions as determined by the length and spacing of the lobes. The tetraploid leaves shown in fig. 3 illustrate this character difference well, as the *G. transmontana* leaf is broader in relation to its length than is the case in *G. malior*.

A more accurate picture of the morphological relationships of the diploid and tetraploid species may be obtained from actual measurements and ratios between them (table 1, characters 1–3). In each of these characters *Gilia minor* has the lowest value and *G. clokeyi* the highest, with *G. aliquanta* close behind it. The two tetraploid species occupy intermediate positions, but with a tendency of *G. transmontana* toward a higher mean value in both characters having to do with leaf form. This corresponds with the visual impression of similarity of *G. transmontana* to *G. clokeyi* in that character.

The calyx in the diploid species:

The calyx in *Gilia minor* (fig. 4) is very pubescent with glandular trichomes. The sepals are acute and are joined by a narrow hyaline membrane. *Gilia clokeyi* and *G. aliquanta* both differ from *G. minor* in having a glabrous calyx, and in the calyx lobes which bear sharp cusps (fig. 4). *Gilia aliquanta* is peculiar and distinctive in the nature of the calyx sinuses, because

the membrane is expanded or puckered and forms deep folds, the outer parts of which are often tinged with intense red-violet colors.

The calyx in the tetraploid species:

In *Gilia transmontana* and *G. malior* the calyx is neither glabrous nor densely pubescent but is moderately pubescent and intermediate between the condition in *G. minor* and that in the other two diploid species. The shape of the sepals is also approximately intermediate. The sinus membrane is narrow and smooth in *G. transmontana* but is usually slightly puckered in *G. malior* and sometimes also tinged with red-violet, suggesting the influence of *G. aliquanta* in the latter species.

Corolla size, form and color in the diploid species:

The corolla is smallest in *Gilia clokeyi*, a little larger in *G. minor*, and usually comparatively large but highly variable in *G. aliquanta* (table 1). The form of the corolla lobes (figs. 4–5) is oval with a mucronate apex in *G. minor*, narrowly oval and attenuate in *G. clokeyi*, and in contrast to both of the foregoing, broadly oval and truncate in *G. aliquanta*.

Analysis of corolla color patterns seems best accomplished by comparing three critical regions of the corolla (table 1). The diagrams in fig. 5 present the total color pattern of each species, without, however, including all details and variations. In general it can be said that *Gilia minor* and *G. aliquanta* have bright corolla colors, *G. clokeyi* pale. *Gilia minor* and *G. aliquanta* both have dark purple color in the corolla tube and lower throat (region 1) but *G. minor* alone of the diploids has purple extended up into the corolla orifice (region 2). *Gilia clokeyi* has white corollas (regions 1 and 2) and it alone among the diploids has violet streaks on the dorsal sides of the corolla lobes (region 3).

Corolla size, form and color in the tetraploid species:

Corollas in *Gilia transmontana* are variable in size but are intermediate between those of the diploids *G. minor* and *G. clokeyi*. *Gilia malior* has corollas intermediate in size between *G. minor* and *G. aliquanta*. The mean size of *G. malior* corollas is larger than that of *G. transmontana* (table 1). Corolla lobe form in *G. transmontana* is much like that of *G. clokeyi*, being narrowly oval and strongly pointed or acute (figs. 4–5). *Gilia malior* corollas are much broader, somewhat truncate but slightly pointed, thus resembling both *G. minor* and *G. aliquanta* but actually intermediate and quite different from the other tetraploid species (figs. 4–5).

The tetraploids both have purple color in the corolla tube but it is light purple in *Gilia transmontana* and dark purple with a reddish cast in *G. malior* similar to *G. aliquanta* (see table 1, region 1). In *G. malior* but not in *G.*

Fig. 3. Representative leaves from garden-grown plants. Cauline leaves (above, left to right): F. *Gilia clokeyi* DS; G. *G. transmontana* MP; H. *G. minor* MB; I. *G. malior* MB; J. *G. aliquanta* MB. Basal leaves (below, left to right): A. *G. clokeyi* MH; B. *G. transmontana* Bd; C. *G. minor* Kr; D. *G. malior* MB; E. *G. aliquanta* MB.
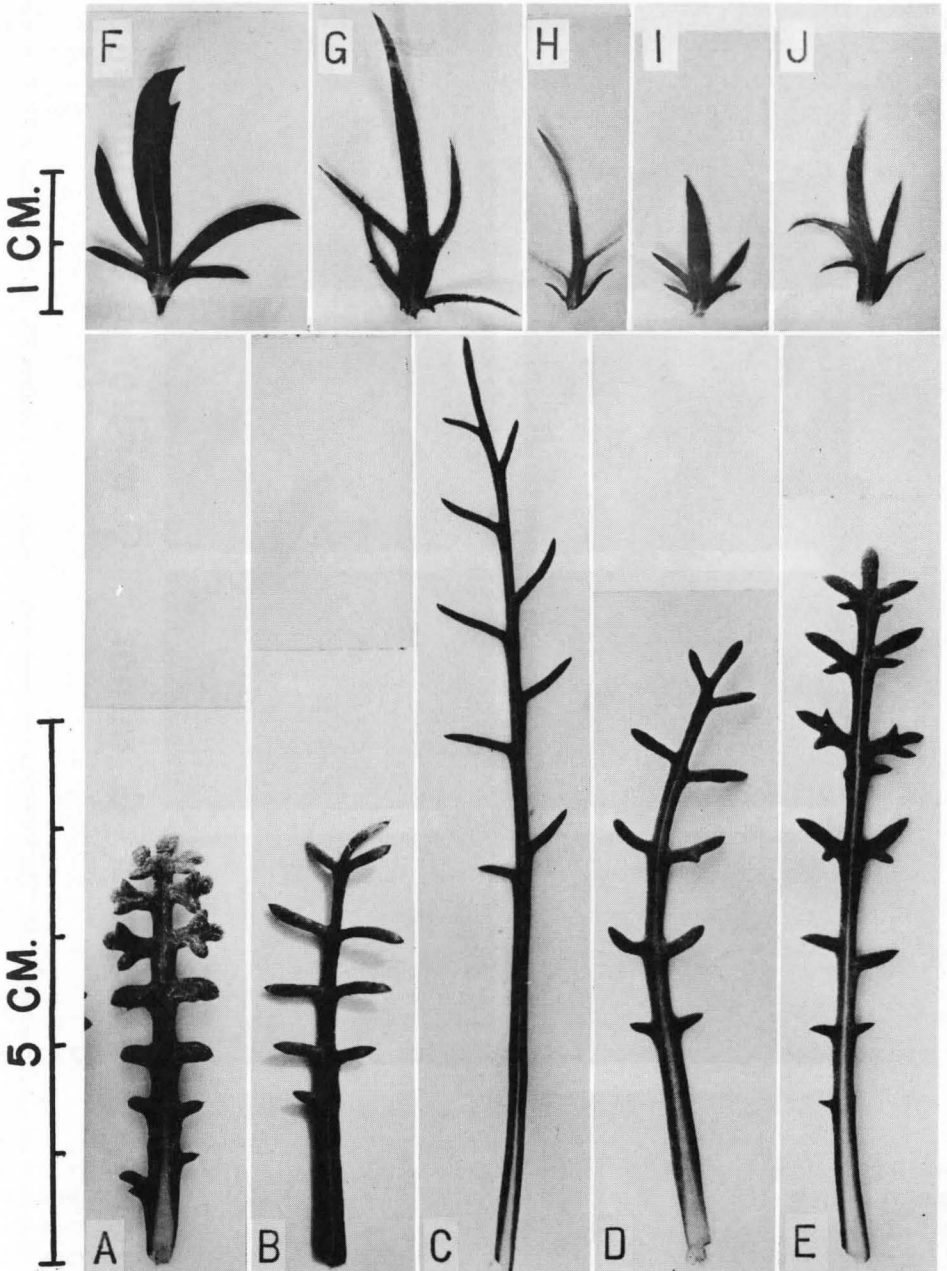
G. clok. G. trans. G. minor G. malior G. ali.

FIGURE 3

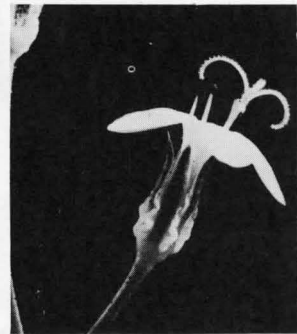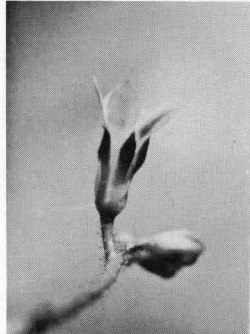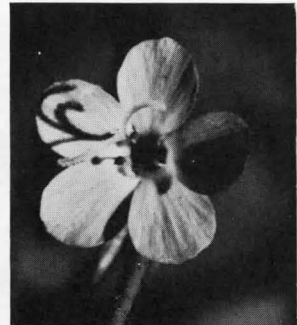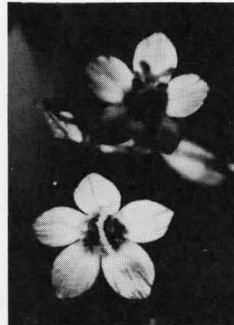Fig. 4. Flowers from garden-grown plants. Top view and side view (left to right): *Gilia clokeyi* DS, *G. transmontana* Bd, *G. minor* Kr, *G. malior* Cu, *G. aliquanta* MB.

transmontana the upper corolla throat is sometimes purple to the orifice as in *G. minor* (region 2). Violet streaks on the dorsal sides of the lobes in *G. transmontana* (not in *G. malior*) suggest relationship of the former to *G. clokeyi* (region 3).

☐ white
▒ white, violet-streaked
▥ violet
■ dark purple
▨ yellow

| Gilia | G. | G. | G. | G. |
| clokeyi | transmontana | minor | malior | aliquanta |
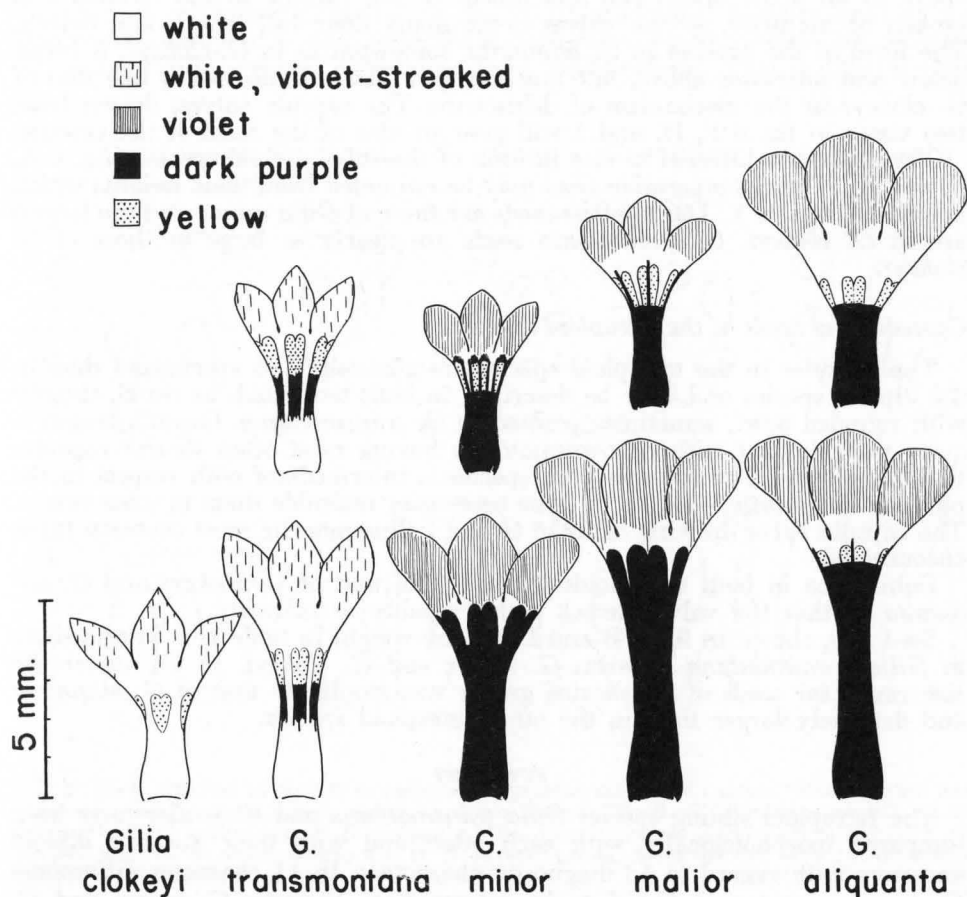
Fig. 5. Diagrammatic sketches of flowers with color patterns indicated.

In corolla region 1, then, *Gilia transmontana* is intermediate between the diploid species, and *G. malior* is like *G. minor* and *G. aliquanta* with a strong resemblance to the latter. In region 2 *G. transmontana* is like *G. clokeyi* but *G. malior* is intermediate between *G. minor* and *G. aliquanta*. In region 3 *G. transmontana* is like *G. clokeyi*, and *G. malior* is like both *G. minor* and *G. aliquanta*.

Capsules and seeds in the diploid species:

The capsule in *Gilia minor* is narrow from the base to the apex, being almost cylindrical and quite elongate. It is firmly attached to the receptacle even at maturity and dehiscence is by splitting along the sutures in the upper part of the capsule. In *G. clokeyi* the capsule is broad at the base, narrowing just above to an acute apex. The attachment of the capsule to the receptacle is broken at maturity, as the valves break apart their full length and detach. The form of the capsule in *G. aliquanta*, somewhat as in *G. clokeyi*, is broad below and narrower above, but much larger. The capsule is also like that of *G. clokeyi* in the mechanism of dehiscence. The capsule valves, drawn from two views in fig. 6 F, H, and J will give an idea of the form of the capsule.

The seeds are different in size in each of the three diploid species (fig. 6 A, C, and E). Their comparative sizes may be estimated from their weights which are given in table 1. The smallest seeds are those of *Gilia minor* and the largest are in *G. clokeyi*. *Gilia aliquanta* seeds are nearly as large as those of *G. clokeyi*.

Capsules and seeds in the tetraploid species:

The capsules in the tetraploid species are generally less specialized than in the diploid species and may be described in both tetraploids as ovoid, usually with rounded apex, sometimes pointed in *G. transmontana*. Capsule length is quite variable, but with *G. transmontana* having most often shorter capsules than *G. malior*. The form in both species is intermediate with respect to the putative ancestral species, or in some cases may resemble them to some extent. The capsule valve drawing of fig. 6 G and I illustrate the most common types encountered.

Dehiscence in both tetraploids is about like that of *G. clokeyi* and *G. aliquanta* in that the valves detach rather readily at maturity.

Seed size, shown in fig. 6 B and D and by weight in table 1, is intermediate in *Gilia transmontana* between *G. minor* and *G. clokeyi*. In *G. malior* the size range for seeds of the strains grown was similar to that of *G. aliquanta* and definitely larger than in the other tetraploid species.

SUMMARY

The tetraploid sibling species *Gilia transmontana* and *G. malior* have been compared morphologically with each other and with their putative diploid ancestors with regard to 13 diagnostic characters. In 11 character differences *G. transmontana* was found to be intermediate between *G. minor* and *G. clokeyi*. In ten characters *G. malior* was found to be intermediate between *G. minor* and *G. aliquanta*. *Gilia transmontana* and *G. malior* differed from each other in seven of these characters which also represented their resemblances to *G. clokeyi* and *G. aliquanta*, respectively. The influence of *G. minor*, the diploid ancestor which the sibling tetraploids share, is responsible for the strong resemblance between the two tetraploids. The other diploid ancestors, *G. clokeyi* and *G. aliquanta* are held to be responsible for the differences between the sibling tetraploids.

The emphasis in the foregoing morphological analysis has been the inter-
mediate nature of the tetraploids, as species, between the parental diploid
species. Yet the range of variation for a number of these characters is wide and
actually encompasses recognizable racial differences. Growth habit of the plant,
form and size of leaf and calyx, color and size of corolla, form and size of the
capsule, all are variable within the limits described. These variations in a
number of combinations make up the racial attributes. A refinement of the
descriptive analysis to deal with these deviations would involve the problem
of distinguishing genetic differences from modifications. This is difficult even
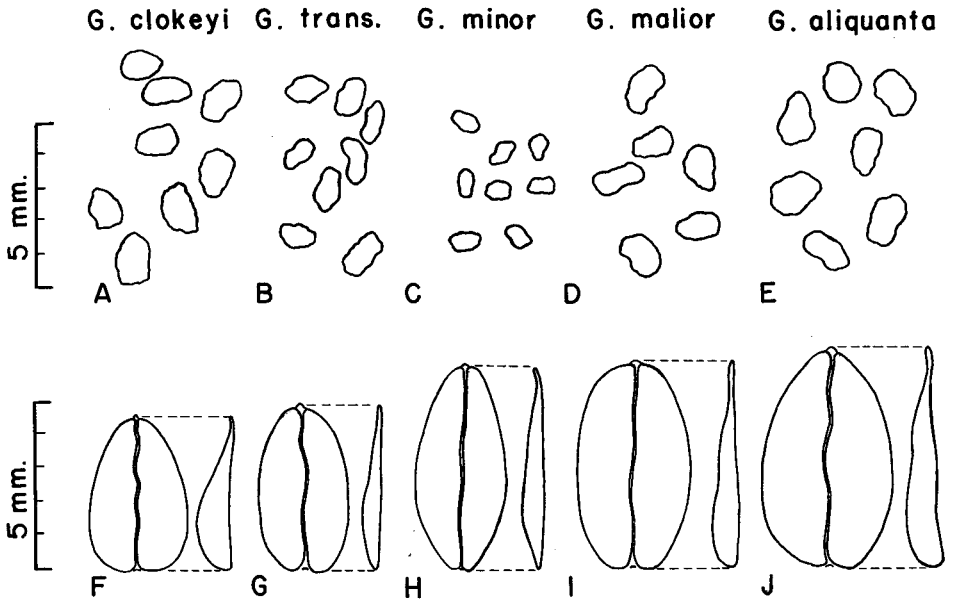
Fig. 6. Seeds sketched in outline to indicate comparative sizes; and capsule valves, ventral and
side views. Seeds (above, left to right): A. *Gilia clokeyi* DS; B. *G. transmontana* BD; C. *G.
minor* W; D. *G. malior* MB; E. *G. aliquanta* MB.—Capsule valves (below, left to right): F. *G.
clokeyi* MH; G. *G. transmontana* Ky; H. *G. minor* Aguila, Maricopa Co., Ariz.; I. *G. malior*
MB; J. *G. aliquanta*, Rosamund, Kern Co., Calif.

when the plants are grown in a relatively uniform environment due to the
small size of the plants, the brief annual cycle and the variation which occurs
on a single plant as it approaches maturity. But some degree of genetic basis
for the observed racial differences cannot be denied.

If the tetraploid races have had separate geographical origins by means of
hybridization between races of the ancestral diploids then interracial differ-
ences in the diploids may be directly responsible for the differences in their
derivatives. Another means for the evolution of the diversity in form may have
been genetic segregation in the allotetraploid plants. Experimental evidence,

described in sections VI and VII, will show that this phenomenon is likely to have been involved in the case of *Gilia transmontana.*

V.   CHROMOSOME NUMBER AND MORPHOLOGY

METHODS

Chromosome number determinations were made from pollen mother cells. Slides were prepared by the propiono-carmine squash method and were made permanent by the use of Hoyer's medium (Beeks, 1955).

For karyotype studies of mitotic chromosomes, root tips and shoot apices, along with the cotyledons, were taken from newly germinated seedlings or from very young embryo-cultured plants. The latter material was useful in the case of species which germinated poorly, and provided actively growing plants within a week after the embryos were excised.

The fresh material was pretreated in 0.2% colchicine and fixed in 3:1 absolute alcohol to propionic acid. After hydrolysis for about 30 minutes in a warm mixture of 1:1 1 N HCl to propiono-carmine stain, the latter diluted half with water, the material was rinsed in propiono-carmine. It was then teased apart on a slide in a drop of fresh stain. Slides were made permanent by mixing in a small drop of Hoyer's medium before applying the cover slip.

CHROMOSOME NUMBER

A list of chromosome counts is given in table 3. The diploid chromosome number of $2n = 18$ and the tetraploid number of $2n = 36$ have so far proven to be invariable for Section *Arachnion* with only rare exceptions. *Gilia malior* from just south of Mojave, California, is reported here to have a chromosome number of $n = 19$. The members of the extra pair are smaller than the other chromosomes of the set and have been observed to segregate regularly from a quadrivalent configuration involving a larger pair of chromosomes. At other times one or two univalents are produced.

In table 3, new chromosome counts are indicated by an asterisk. The majority of these have been done by myself, others by Verne Grant. The remaining chromosome numbers which are listed have been published by Grant, V., Beeks, and Latimer (1956); Grant, V. (1959); and Grant, V., and A. Grant (1960). The taxonomic disposition of some of the plants in the earlier published lists has since been changed.

CHROMOSOME MORPHOLOGY

Analysis of the chromosome morphology of the three diploid and two tetraploid species should be useful for the present study if the diploid species are found to have particular differences which can also be distinguished in their respective tetraploid derivatives. Similarity in a general way between the karyotypes of *Gilia minor*, *G. clokeyi*, and *G. aliquanta*, and the small size of the chromosomes exclude the possibility of differentiating their karyotypes, chromosome for chromosome, without employing more refined techniques than those which have been used in this study. Even less may the tetraploids, with the large number of chromosomes involved, easily be analyzed in great detail.

A few clearly discernible differences among the chromosome sets have been found, however, which may provide some helpful evidence. These concern the form of the satellited chromosomes, the length of the chromosomes, and their relative lengths. Tentative idiograms of the karyotypes are presented in order to compare these specifically emphasized features.

The following strains (see table 1) of the diploid and tetraploid species were employed in the chromosome studies: *Gilia minor* MB; *G. clokeyi* MH, DS; *G. aliquanta* MB, V and Caj.; *G. transmontana* Ky; *G. malior* MB, Sp.

Chromosome form:

The karyotypes of five of the six strains of the three diploid species included: (1) one metacentric, and (2) one subtelocentric pair of satellited chromosomes as in fig. 7 A–B, D–G. The satellite was borne on the short arm of the subtelocentric. The Deep Springs strain of *Gilia clokeyi* differed in having two pairs of subtelocentric, satellited chromosomes and in lacking the metacentric pair (fig. 7 C). All three strains of *G. aliquanta* had, in addition to the standard two pairs described above, a subtelocentric pair with the satellite at the end of the long arm (fig. 7 F–G).

The two tetraploid species differed from each other in several ways as to the nature of their satellited chromosomes. The *Gilia transmontana* strain studied had, in every case seen, only two pairs of satellited chromosomes and both of these were subtelocentric with the satellite on the short arm. In fig. 8 A the two members of a medium-length pair and two members of a longer pair of such chromosomes are in view. The highly contracted chromosome set of fig. 8 B reveals only two satellited chromosomes, these representing again one medium-length and one longer subtelocentric chromosome pair, but with a greater size contrast between the two than is seen in the other cell.

The two strains of *Gilia malior* which were analyzed exhibited at least four pairs of satellited chromosomes, but with no one cell displaying all of these at once. Four morphological types were identified as follows: two pairs of metacentric chromosomes, one pair being large and the other small; one pair of subtelocentric chromosomes with the satellite on the short arm; and one pair of subtelocentric chromosomes with the satellite on the long arm (fig. 8 C–E and fig. 9).

Chromosome length:

The length of the chromosomes in this group of species varied from 2.0 to 8.4 microns (table 4). *Gilia minor* had the smallest chromosomes (mean length $=3.7\ \mu$); *G. aliquanta* had the largest (mean length$=5.8\ \mu$); and *G. clokeyi* had chromosomes of an intermediate size (mean length$=4.5\ \mu$). The chromosomes in the tetraploid species were also about intermediate in their average lengths (table 4).

The chromosomes of *Gilia minor* differed from those of the other diploid species in another aspect, that of the relative sizes of the longest and shortest members of the set. The longest chromosomes in *G. minor* were just over two times as long as the shortest (fig. 7 E). The average length ratio for this species was $1/s=2.2$. Figures 7 A, F illustrate the more nearly uniform lengths of

TABLE 3. *List of chromosome numbers.*

Gilia aliquanta $n = 9$

> Nevada:
>> East of Leadville, Nye Co. (10100)

> California:
>> Red Rock Canyon, Kern Co. (9117)
>> Butte south of Mojave, Kern Co. (Day 60–109a; Day 60–102)*
>> Pear Blossom, Los Angeles Co. (9324)
>> Desert Springs, San Bernardino Co.-Los Angeles Co. (Keck 6261)
>> Victorville, San Bernardino Co. (Day 60–126)*
>> Cajon Pass, San Bernardino Co. (Day 62–17)*

Gilia clokeyi $n = 9$

> California:
>> Deep Springs, Westgard Pass, Inyo Co., (9836)

> Nevada:
>> Kyle Canyon, Charleston Mts., Clark Co. (9950)

> Arizona:
>> Kayenta, Navajo Co. (10157)*

> Utah:
>> Mexican Hat, San Juan Co. (10154)
>> Moab, San Juan Co. (10150)*

> New Mexico:
>> Between Shiprock and Farmington, San Juan Co. (10140)*

Gilia minor $n = 9$

> California:
>> Homewood Canyon, Argus Mts., Inyo Co. (9350)
>> Butte south of Mojave, Kern Co. (Day 60–101a; Day 60–116)*
>> Oak Creek, west of Mojave, Kern Co. (Day 62–14)*
>> Kramer Junction, San Bernardino Co. (8851; 9222)
>> Gorman, Los Angeles Co. (9092)
>> Ballinger Canyon, Cuyama Valley, Santa Barbara Co. (2677; 2684; 9098)
>> Cuyama Valley, Santa Barbara Co. (9099)

> Arizona:
>> Wickenburg, Maricopa Co. (9298)
>> Aguila, Maricopa Co. (10061a,b)

Gilia malior $n = 18$ (with one exception, see below)

> Nevada:
>> Sparks, Washoe Co. (9795)
>> Virginia City, Ormsby-Storey Co. (Mason 13988)
>> South of Carson City, Douglas Co. (9785)

> California:
>> Short Canyon, near Inyokern, Kern-Inyo Co. (9337)

TABLE 3. *List of chromosome numbers. (Continued)*

Gilia malior $n = 18$ (with one exception, see below)

California:
        Cuyama Valley, Santa Barbara Co. (8696)
        Butte south of Mojave, Kern Co. $n = 19$ (Day 60–101b, Day 60–107)*
        Boron, Kern Co. (9907)*
        South of Kramer Hills, San Bernardino Co. (Day 60–106)*

Gilia transmontana $n = 18$

California:
        Kramer Hills, San Bernardino Co. (9902)*
        South of Kramer Hills, San Bernardino Co. (10046)*
        Mountain Pass, east of Baker, San Bernardino Co. (9060)

Nevada:
        Kyle Canyon, Charleston Mts., Clark Co. (9952; 9953*; 9959*)
        Searchlight, Clark Co. (10082)*
        Mormon Mesa, Clark Co. (9960)*
        Mesquite, Clark Co. (9963)

Arizona:
        Littlefield, Mojave Co. (9964)*
        Beaverdam, near Beaverlodge, Mojave Co. (9966)*

Utah:
        Beaverdam Mountains, Washington Co. (9968; 9970; 9972)
        Eight miles east of St. George, Washington Co. (9975)*
        St. George, Washington Co. (9977)*

---

both *G. clokeyi* and *G. aliquanta* in which the longest chromosomes were nearer to one and one-half times as long as the shortest (mean $1/s=1.7$).

The length ratios in the tetraploids were about as high as, or higher than, the *Gilia minor* ratio. *Gilia malior* had the most extreme length differential, the longest chromosome averaging nearly two and one-half times as long as the shortest (mean $1/s=2.4$). The size contrast between the longest and shortest *G. malior* chromosomes in fig. 8 C–D, on close inspection, will be seen as slightly greater than that found in *G. transmontana* (fig. 8 A–B), which has a mean ratio of $1/s=2.1$. This is also to be noted in the idiograms of these species (fig. 9).

DISCUSSION AND SUMMARY

The foregoing observations on the chromosome morphology of the diploid and tetraploid species offer some useful evidence in the problem of the origin of the tetraploids. An unusual satellited chromosome, a subtelocentric chromosome with the satellite on the end of the long arm, was seen in all strains of *Gilia aliquanta*, and in no other diploid species. A chromosome of this same description was also seen in the two strains of *G. malior* which were studied, but not in *G. transmontana*. This marker was among the larger chromosomes
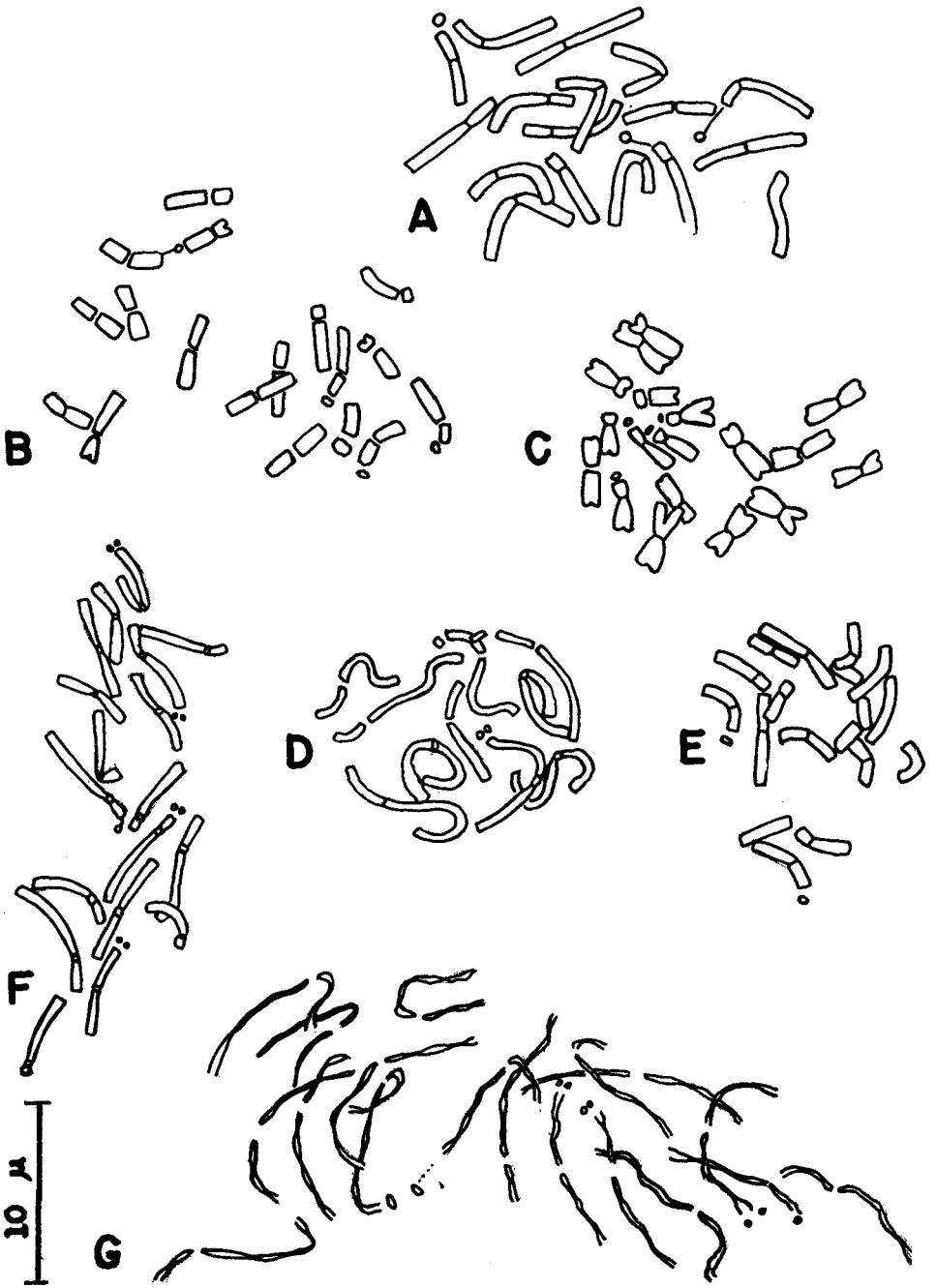
FIGURE 7

in the *G. malior* complement (fig. 9). The identification of the *G. aliquanta* genome at the large end of the *G. malior* karyotype is thus suggested.

The second chromosome set making up the *Gilia malior* karyotype would need to include numerous chromosomes of a smaller size than those of *G. aliquanta* (see *G. malior* idiogram in fig. 9). This size requirement is met by

TABLE 4. *Absolute and comparative lengths of chromosomes at mitiotic metaphase.*

SPECIES (Strain)	CHROMOSOME LENGTH (Microns)	LENGTH RATIO (Longest/shortest chromosome)
G. clokeyi Mexican Hat Deep Springs	4.5 (2.0–7.8)	1.7 (1.6–1.8)
G. minor Mojave Butte	3.7 (2.0–6.2)	2.2 (2.0–2.6)
G. aliquanta Mojave Butte Cajon Pass	5.8 (4.4–8.4)	1.7 (1.5–2.0)
G. transmontana Kyle Canyon	4.4 (3.0–6.4)	2.1 (2.0–2.1)
G. malior Sparks	4.5 (3.0–8.0)	2.4 (2.1–2.9)

G. minor which has a smaller average chromosome size than *G. aliquanta*. In another respect, however, *G. minor* appears to fail to match with the *G. malior* karyotype. This is the lack of correlation as to satellited chromosomes in their respective karyotypes (fig. 9).

There is some evidence from karyotype analysis as to the genomes contained in *Gilia transmontana* (Kyle Canyon strain). As noted above, the marker chromosome which was found in *G. malior* is missing in *G. transmontana*. The lack of this particular marker in *G. transmontana* would tend to exclude *G. aliquanta* from its ancestry. A second type of satellited chromosome, a metacentric, which is found in *G. malior* and in the diploid strains except *G. clokeyi* from Deep Springs, does not occur in *G. transmontana*. The two satellited chromosomes which do occur in *G. transmontana* are both subtelo-

Fig. 7. Mitosis in the diploid species. Divisions are from root-tips and shoot apices of young seedlings, except D, which is from a tapetal cell. Camera lucida drawings. A and D are from broken cells and lack the complete complement.—A–B. *Gilia clokeyi* MH.—C. *G. clokeyi* DS.—D–E. *G. minor* MB.—F. *G. aliquanta* MB.—G. *G. aliquanta* V.

10 μ
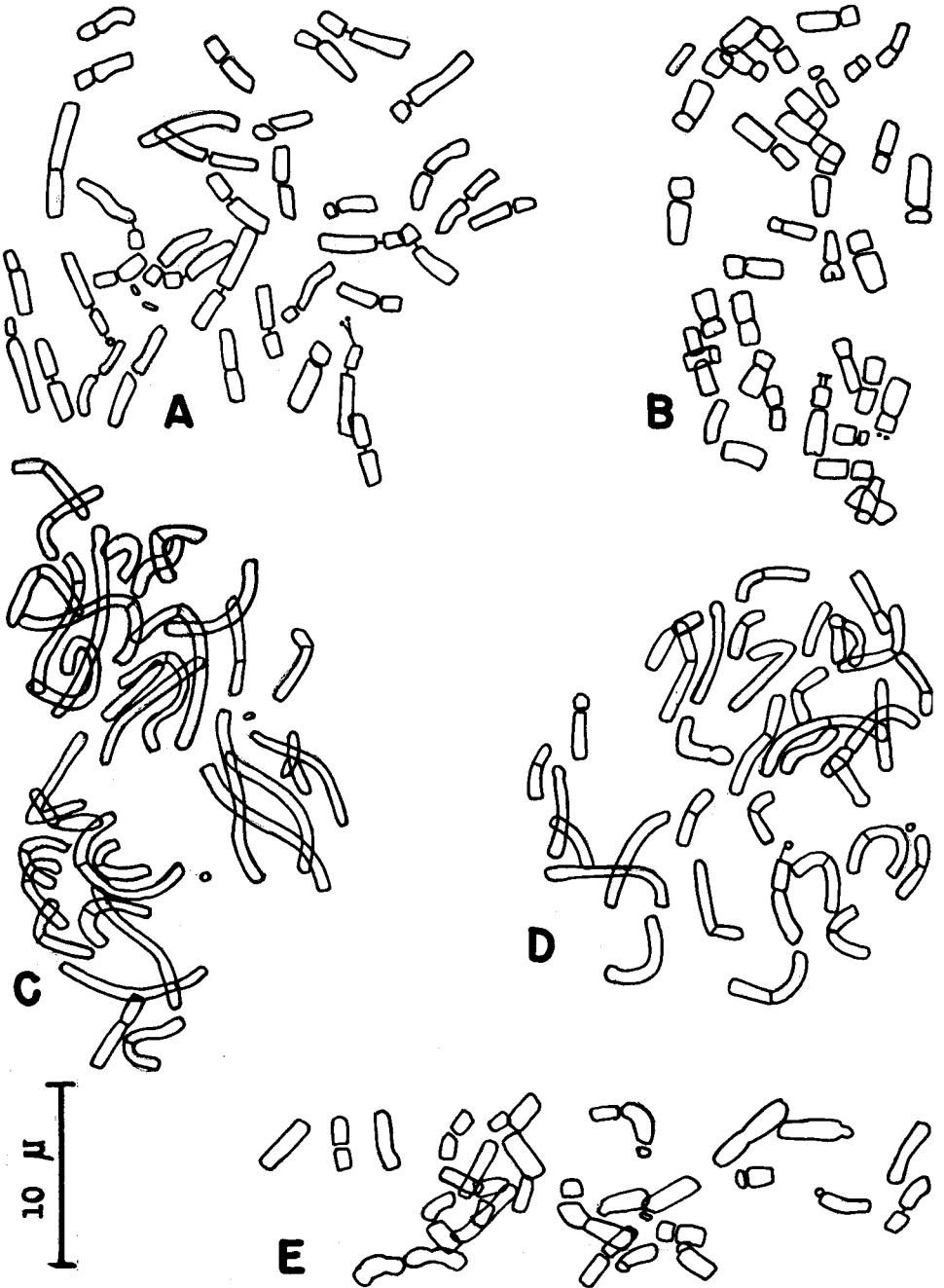
FIGURE 8
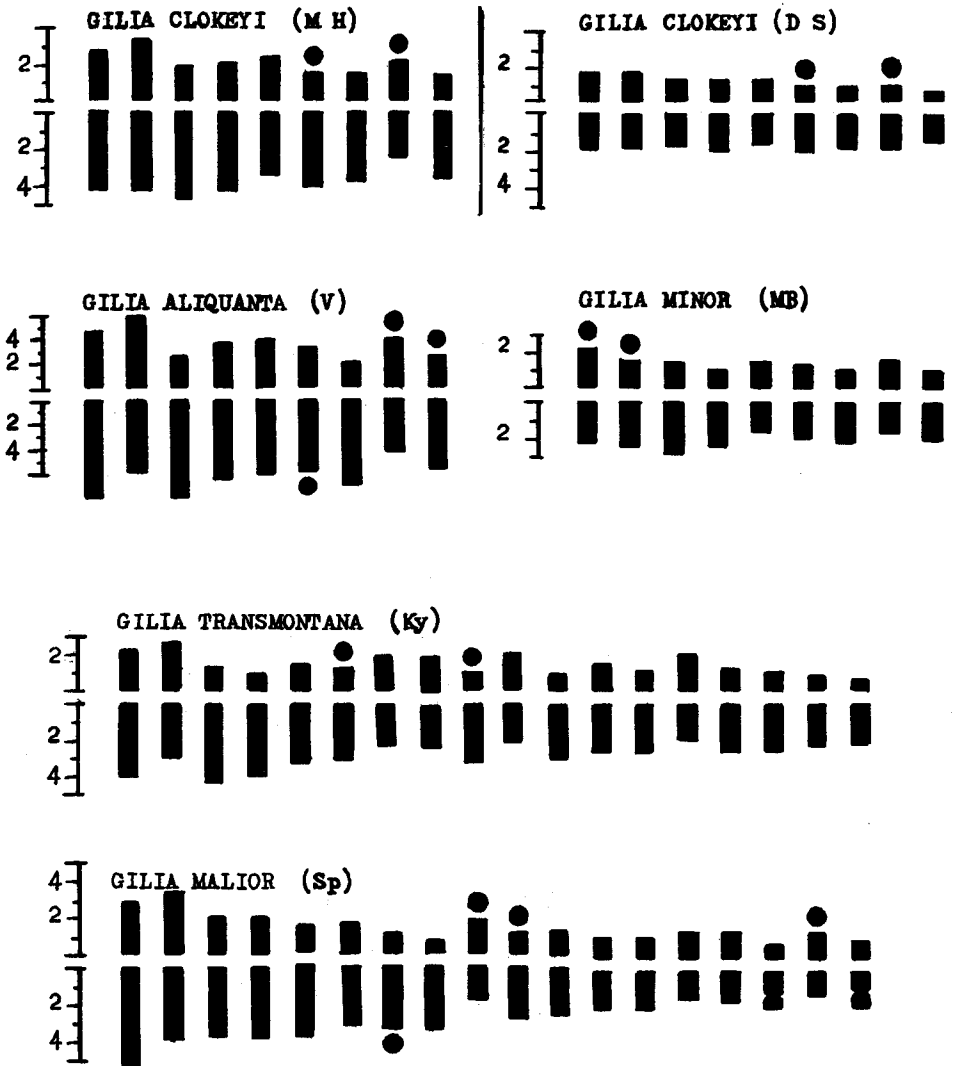
Fig. 9. Idiograms of the chromosomes of the diploid and tetraploid species, from somatic cell divisions. The scale is in microns, and all are to same scale except *Gilia aliquanta* which is reduced because measurements are from relaxed, prophase chromosomes. The strains are indicated with the species names.

Fig. 8. Mitosis in the tetraploid species. Divisions are from root-tips and shoot-apices of young seedlings. Camera lucida drawings. E is from a broken cell and lacks the complete complement.—A–B. *Gilia transmontana* Ky.—C–D. *G. malior* Sp.—E. *G. malior* MB.

centric and have the satellite on the short arm (fig. 9). Two satellited chromosomes of this type are characteristic of the Deep Springs strain of *G. clokeyi*. It is not possible, however, to identify both of the satellited chromosomes in *G. transmontana* with a *G. clokeyi* genome just from the coincidence of these observations, because the second satellited chromosome could as well have been derived from the second genome in the tetraploid karyotype.

Another problem in the interpretation of *Gilia transmontana* chromosomes is that only two pairs of satellited chromosomes have been seen. Since the diploid species being considered are known to have two to three pairs of satellited chromosomes, *G. transmontana* would be expected to have at least four pairs. Thus it must be that satellite suppression is taking place, in which case evidence based upon the satellited chromosomes could be misleading. However, it can be said without question that *G. transmontana* has a different karyotype structure from that of *G. malior*.

The ratio of longest to shortest chromosome in *Gilia transmontana* was lower than in *G. malior*. From this we can conclude that the two genomes in *G. transmontana* consist of chromosomes more nearly alike in length than are those in *G. malior*. The chromosomes of *G. clokeyi* and *G. minor*, the putative ancestors of *G. transmontana* are, in fact, more alike in length than are those of *G. aliquanta* and *G. minor*, the putative ancestors of *G. malior*. The size relationships then are roughly in harmony with the hypothesis that *G. minor* is the contributor of one genome to both tetraploid species.

VI. CYTOLOGY OF THE INTERSPECIFIC HYBRIDS AND OF THE SYNTHETIC ALLOTETRAPLOID PLANTS

The relationships of the genomes of the diploid and tetraploid species have been examined from the point of view of the morphology of the chromosomes and of the chromosome sets (Section V). Further understanding of these relationships may be obtained from studies of hybrids between the species, at both the diploid and the tetraploid levels.

The classical technique for determining the ancestry of allotetraploids would include crossing the tetraploid species to the putative diploid ancestors so that from the chromosomal pairing in the triploid hybrids, some indication as to genomic relationships should result. But in the present study attempts to obtain triploid hybrids failed. *Gilia transmontana* and *G. malior* were crossed to each of the three diploid species using, in all, nine combinations of species and strains. In none of these attempts was any seed produced. It was possible, however, to obtain interspecific hybrids at both the tetraploid and the diploid levels. An additional source of information was from the synthetic allotetraploid progeny of diploid species hybrids which presumably corresponded to the natural tetraploid species, *G. transmontana* and *G. mailor*.

METHODS

The hybridizations were performed in an insect-proof screenhouse. Flowers were emasculated in bud with a dissecting needle bent at the tip. Pollen was applied to the reflexed stigma lobes with an anther held by the attached filament. Bud fixation for cytological studies was in 3:1 absolute ethanol to pro-

pionic acid. After four to eight hours the buds were transferred to 70% alcohol and stored in the refrigerator. The pollen mother cell squash preparations were stained with propiono-carmine. Iron was introduced from the dissecting needles during preparation. Slides were made permanent with Hoyer's medium by the method described by Beeks (1955).

Metaphase pairing in the parental species, with only rare exceptions, is regular and the chromosomes are associated as nine bivalents. However, stickiness among the chromosomes, reduced chiasma frequency, and lowered pollen fertility have been found in some of the experimental plants late in the growing season when the plants are apt to become male sterile. Buds were collected from the hybrid plants when they were in vigorous condition.

A chronological list of the crosses made between *Gilia minor* and *G. clokeyi* and between *G. minor* and *G. aliquanta*, identification of the strains employed, and other hybridization data are given in table 5.

Colchicine treatment for inducing somatic doubling as performed on plants in flowering stage was as follows: the small branches were removed and larger branches cut back, leaving numerous young buds; then the plant in its pot was inverted over a container of 0.2% colchicine solution where the shoot portion of the plant remained immersed for a period of two hours. After a water rinse the plant was returned to the greenhouse. Numerous tetraploid branches developed after several weeks. They differed from the normal branches by their greater size and vigor, and in the presence of abundant, fertile pollen.

THE TETRAPLOID HYBRIDS

In 1953 *Gilia malior* Cu was crossed with *G. transmontana* MP (V. Grant, 1964). Many hybrid seeds were produced but the F_1 plants had a pollen fertility of only 1–5% and chromosomal pairing varied from 7 to 15, with an average of 9.1 bivalents. Four years later the cross was repeated with *G. transmontana* MP and a different strain of *G. malior* from Sparks, Nevada (V. Grant, 1964). Again many hybrid seeds were obtained but the F_1 pollen fertility was low (2–5%) and chromosomal pairing reduced [9.2_{II} (7–11_{II})]. The low fertility and low pairing permitted the conclusion that *G. transmontana* and *G. malior* are biological species. For our present purposes the point of significance is that the number of bivalents in the F_1 hybrids of these species, being approximately nine, corresponds with the number of homologous chromosomes that would be expected if they share a single diploid genome. This is in line with the hypothesis that *G. transmontana* and *G. malior* arose from different hybrids involving *G. minor*.

THE DIPLOID HYBRIDS

F_1 GILIA MINOR × CLOKEYI:

The cytology of the F_1 generation of *Gilia minor* × *clokeyi* was described and illustrated by Grant and Grant (1960). The range of bivalent pairing in the pollen mother cells was 2–7 with a mean of 4.6 (table 6). Translocation chains were observed in 9% of the PMC's, and a chain of three which was illustrated appeared to include metacentric (or submetacentric) and subtelo-

TABLE 5. *The diploid species crosse:.*

CROSS[1]	YEAR	NO. FLS. POLLINATED	NO. SEEDS FOR F_1	NO. F_1 PLANTS	F_1 POLLEN FERTILITY[2]	NO. SEEDS FOR F_2	NO. F_2 PLANTS	F_2 POLLEN FERTILITY[2]
Gilia minor $\times$ *clokeyi*								
clok ♀ DS $\times$ min Kr	1958	6	44	2	1%	3	1 (inv.)[3]	
min ♀ Kr $\times$ clok Ds	1958	7	70	2	1–3%	12	3 (2 inv.)	91%
Gilia minor $\times$ *aliquanta*								
min ♀ W $\times$ ali RR	1959	21	68	2	3%	1	0	
min ♀ MB $\times$ ali V	1960	18	14	3	1%	0		
min ♀ MB $\times$ ali MB	1960	11	0	0				
ali ♀ MB $\times$ min MB	1960	21	91	17	1%	0		
min ♀ MB $\times$ ali MB	1961	37	70	2 (weak)		0		
ali ♀ MB $\times$ min MB	1961	11	25	7	8%	0		
					47–65%[4]	150	61	96% (91–99%)

[1]abbreviations refer to strains listed in table 1
[2]percentage of stainable pollen
[3]inv. = inviable
[4]chimeral branches

TABLE 6. *Meiotic behavior of F_1 diploid hybrids.*

CULTURE	PAIRING FREQUENCIES (II's+ multivalents)	MULTIVALENT FREQUENCIES
F_1 *Gilia clokeyi* DS × *minor* Kr	4.6 (2 – 7)	9%
F_1 *Gilia aliquanta* MB × *minor* MB	4.5 (1 – 8)	36%

centric chromosomes (fig. 10 A). Heteromorphic bivalents as in fig. 10 C (arrows) and strong attenuation of a number of the bivalents were noted. Micronuclei were contained in 88% of the sporads. A very low percentage of the pollen grains was stainable (1–3%), and sterility was almost complete.

After abundant flowering through the season the plants matured 15 seeds. Three seeds of *Gilia clokeyi* × *minor*, and 12 from the reciprocal cross were obtained. One seed of the first cross germinated but the plant died at an early stage, whereas three seeds of *G. minor* × *clokeyi* germinated and one plant survived to maturity. This F_2 plant was vigorous, tetraploid in chromosome number ($2n = 36$) and was fertile, both as to pollen (table 7) and seed, and despite the variable presence of univalents and multivalents (figs. 10 D–F, 11 A–H). Meiotic behavior of this hybrid will be described in some detail in a later section (pp. 58–62).

F_1 GILIA ALIQUANTA × MINOR:

The cross of *Gilia minor* × *aliquanta* was made in three successive years using three different combinations of strains (table 5). In each case the diploid F_1 hybrid plants were almost or entirely seed sterile. The percentage of stainable pollen varied from less than 1% to 8%.

The hybrid combination studied in greatest detail was the series of crosses between the Mojave Butte strains of *Gilia aliquanta* and *G. minor*. This cross was made reciprocally in two successive years, producing viable diploid F_1 progeny from each attempt, but with the greater success when *G. aliquanta* was the maternal parent. Meiosis was studied in two F_1 individuals.

At metaphase I in PMC's of F_1 *Gilia aliquanta* × *minor*, chromosome pairing was sometimes high (fig. 12 I–J), and in all cases bivalent separation was clear cut. Attenuated bivalents such as were common in F_1 *G. minor* × *clokeyi* were lacking. But most of the cells had numerous unpaired or loosely-paired chromosomes (fig. 12 D–H), or pseudobivalents with matrix connections rather than chiasmata, in addition to normal bivalents.

Distinguishing between the pseudobivalents and normal bivalents was in some cases difficult. The appearance and behavior of the pseudobivalents were somewhat like those of *Bromus* hybrids described by Walters (1954) in that they often had an orientation with respect to the equator similar to that of true bivalents. Since they were joined only by matrix, the *Bromus* pseudobivalents, like the *Gilia* bivalents, lacked the stretched or thin areas between centromere region and terminal chiasma.
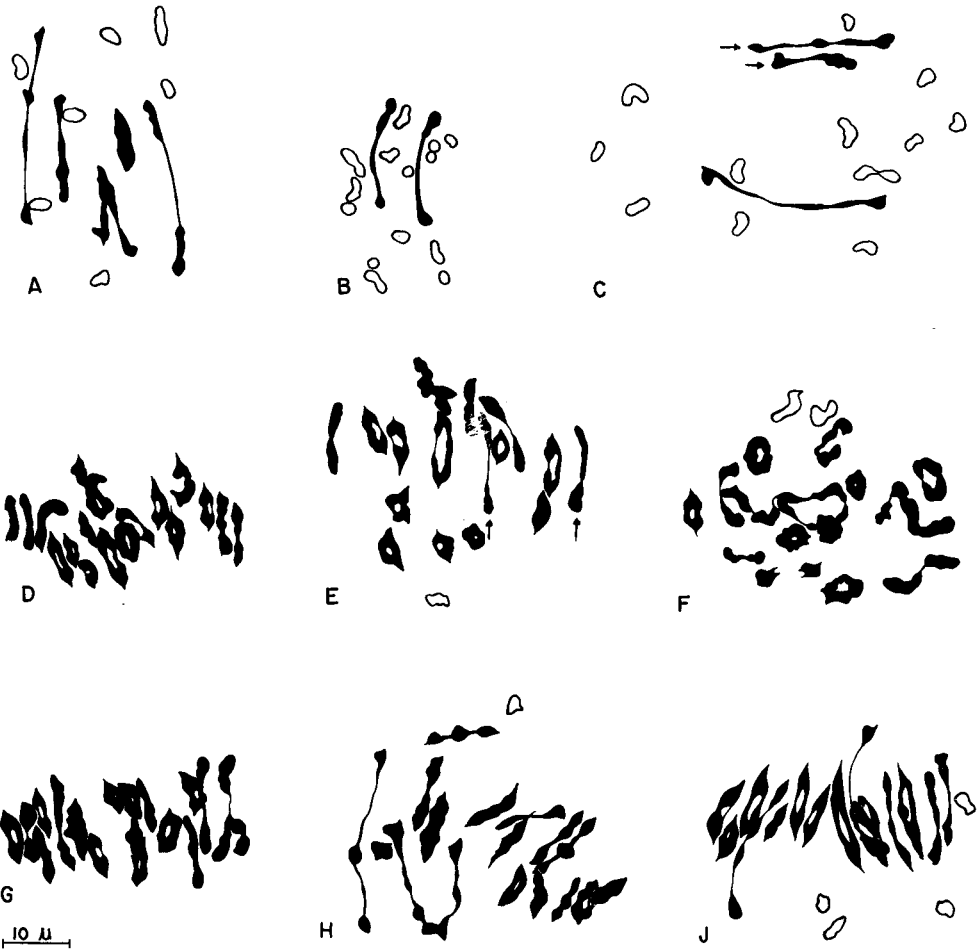
Fig. 10. Meiotic metaphase in F_1 *Gilia minor* $\times$ *clokeyi* and in the allotetraploid progeny.—
A–C. F_1 hybrid ($2n = 18$).—D–J. Allotetraploid progeny ($2n = 36$).—D–F. F_2 generation.
—G. F_5 generation, line 1.—H. F_4 generation, line 6.—J. F_5 generation, line 8.—Arrows
indicate heteromorphic bivalents. Bivalents and multivalents are black, univalents white.

Figure 12 E shows a cell with four bivalents, at least one of which is a pseudo-
bivalent with a single strand of matrix between the univalent-like chromo-
somes. Other pairs of chromosomes which appear as univalents, situated oppo-
site to each other in this cell, probably are pseudobivalents which have dropped
their matrix connections. Paired orientation of univalents at diplotene, dia-
kinesis and prometaphase stages in a *Lilium* hybrid has been termed position

correlation (Ribbands, 1937). This term would seem to describe the meta-phase situation of many of the univalents in F_1 *Gilia aliquanta* × *minor*.

At a later stage one or more univalent-like chromosomes were observed at or near each of the poles of the cell, while the true bivalents remained term-inalized at the equator. Often the numbers of univalents which had segregated precociously in this way were equal or nearly equal at the two poles (fig. 12 E, F, I), indicating that they may have been joined as pseudobivalents, and had become oriented normally with respect to the spindle.
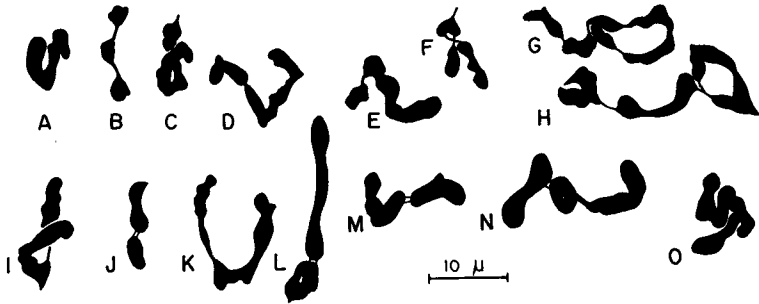

Fig. 11. Multivalent configurations at M_I from PMC's of synthetic allotetraploid plants, *Gilia minor-clokeyi* and *G. aliquanta-minor*.—A–H. F_2 *G. minor-clokeyi*.—A–B. Trivalent chains.—C–F. Quadrivalent chains.—G–H. Configurations of 6 chromosomes.—I–O. F_2 *G. aliquanta-minor*.—I–J. Trivalent chains.—K–L. Quadrivalent chains.—N–O. Configurations of 6 chromosomes.

Failure of such chromosome pairs to orient normally with the spindle, as inferred from the anaphase position of univalents joined by matrix (fig. 12 G), would result in unequal segregation. Among twenty cells which could be scored at this stage of meiosis, when the ture bivalents were still terminalized at the equator, 50% had equal segregation of the univalents, 30% had an excess of one chromosome at one pole, and 20% had more strongly unbalanced distri-butions.

Both normal chains and pseudoassociations of three or four chromosomes were seen at diakinesis and at metaphase. The frequency of these was higher at diakinesis than at metaphase since the loosely-paired chromosome associa-tions tended to break up into smaller units.

In fig. 12 A, on the lower right, a heteromorphic configuration is seen which resembles a translocation ring of four with alternate disjunction, al-though chiasmata are lacking. A further stage in the breaking up of such a configuration is suggested by the bivalent and two univalents at the bottom of fig. 12 B. In both of these cases two of the chromosomes would appear to be submetacentric and the other two subtelocentric.

Heteromorphic bivalents involving submetacentric-subtelocentric chromo-some combinations were frequently seen at metaphase (fig. 12 H), and may
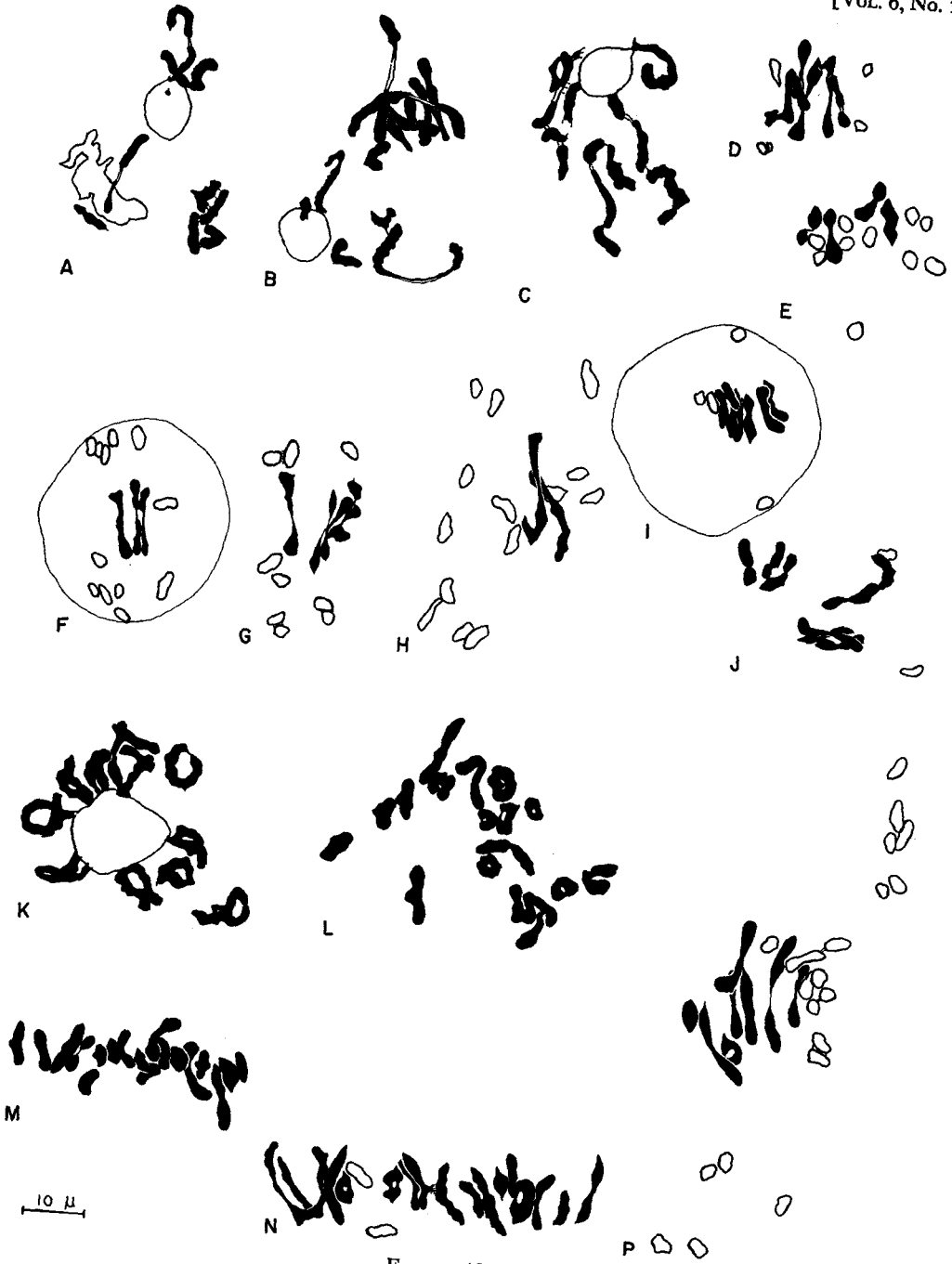
10 µ

FIGURE 12

represent associations of the interchange group of chromosomes. It will be recalled that similar chains and heteromorphic bivalents were observed in F_1 *Gilia minor* × *clokeyi*.

Scoring of the pairing in this hybrid was restricted to mid-metaphase. Chromosomes which were paired and joined by chiasmata or pseudochiasmata, if oriented normally, were counted as pairs. A chain of three or four was counted as 1.5 and 2.0 pairs, respectively. The pairing range was 1.0–8.5 per cell, with a mean frequency of 4.5 for 28 cells in two F_1 plants, very nearly the same degree of pairing as was recorded for F_1 *Gilia minor* × *clokeyi* (table 6).

At metaphase II more than two nuclei were usually found in a PMC, suggesting that in division I the precociously segregating chromosomes were not combining with those which moved to the poles later in anaphase. At telophase II numerous micronuclei were present. Out of 27 sporads examined, none had less than two micronuclei, and one sporad contained 10 small nuclei, none as large as a normal microspore. The mean number of micronuclei was 3.8 per sporad. The percentage of stainable pollen was less than 1%.

Failure to produce functional haploid gametes seems then to be due at least in part to the poor timing brought about by precocious segregation of the weakly-paired chromosomes, which fail to be included in the same nucleus with those segregating later. There was not observed any case of restitution nuclei or of dyads of microspores, such as were noted in F_1 *Gilia minor* × *clokeyi*.

Although these plants bloomed profusely through the season no seeds were produced. A repeat of this cross, F_1 *Gilia aliquanta* × *minor*, with both strains again being from Mojave, produced similar plants which also flowered profusely and were sterile. One plant which was treated with colchicine produced several fertile branches. From the abundant seeds set on these branches, numerous tetraploid F_2 plants were grown the following year (table 5).

Discussion of meiosis in the F_1 diploid hybrids:

It could be said of the diploid hybrids, *Gilia minor* × *clokeyi* and *G. aliquanta* × *minor*, that the occasional high pairing at metaphase I is indicative of some homology between nearly every chromosome of *G. minor* and the corresponding chromosomes in both *G. clokeyi* and *G. aliquanta*. On the other hand, the frequent low pairing in these hybrids is evidence that the homologies may be incomplete and that at least slight structural differentiation has occurred between most of the chromosomes of the parental species.

In addition there have been some larger structural changes which involved the chromosomes composing translocation chains in both F_1 hybrids, *Gilia minor* × *clokeyi* and *G. aliquanta* × *minor*.

Fig. 12. Meiosis in diploid hybrids and tetraploid progeny of *Gilia aliquanta* × *minor.*—A–J. F_1 hybrid ($2n = 18$).—A–C. Diakinesis stage.—D–J. Metaphase I.—K–P. F_2 progeny ($2n = 36$).—K. Diakinesis stage (broken cell lacking complete complement).—L–P. Metaphase I. Bivalents and multivalents are black, univalents white, in D–J and N–P.

Although several similarities in meiotic behavior have been indicated between these two hybrid combinations, the outcome of the first hybrid generation was different, in that *Gilia minor* × *clokeyi* made a few tetraploid seeds spontaneously, and *G. aliquanta* × *minor* made none.

A somewhat parallel case in another section of *Gilia* was described by V. Grant (1952). Sterile diploid hybrids between *G. millefoliata* and *G. achilleaefolia* in the section of leafy-stemmed Gilias were produced in two different years, the first attempt resulting in a much greater relative rate of tetraploid production than the second attempt. The higher rate of tetraploid production was associated with lower bivalent pairing and a more frequent occurrence of attenuated bivalents. These features were associated with a "scattered distribution" of chromosomes "throughout the cytoplasm," the formation of restitution nuclei, and dyads of diploid microspores. The formation of tetraploid seeds therefore appeared to be by the fertilization of diploid ovules by diploid male gametes.

The two sets of plants differed in environmental conditions, and probably differed in genetic factors affecting pairing. Grant (1952) concluded the poor nutritional conditions and genetic factors were effectively responsible for the poor chromosome pairing and attenuation of bivalents in hybrid set 1, and that the high polyploidy rate was a consequence of that low pairing.

In comparing F_1 *Gilia minor* × *clokeyi*, which spontaneously produced tetraploid seed, with F_1 *G. aliquanta* × *minor*, which did not, there were no conspicuous environmental differences, and it is not apparent from table 6 that there was any important difference in the rate of chromosomal pairing. But like the high tetraploid-producer in *G. millefoliata* × *achilleaefolia*, there were in *G. minor* × *clokeyi* numerous attenuated bivalents and a scattered distribution of the chromosomes. Restitution nuclei and dyads were observed. The metaphase I to anaphase behavior of *G. aliquanta* × *minor*, on the contrary, usually exhibited clear separation of chromosome pairs, and a well-developed spindle mechanism which brought about a precocious segregation of univalents. Instead of dyads, numerous small nuclei were found in the sporads.

The principal cause for failure to produce tetraploid seed spontaneously in F_1 *Gilia aliquanta* × *minor* may then be an internal genetic control which maintains a strong polarity during meiosis, regardless of poor chromosomal pairing, and which effectively prevents the formation of restitution nuclei.

THE CYTOGENETICS OF THE SYNTHETIC ALLOTETRAPLOIDS

GILIA MINOR × CLOKEYI *in the F_2 and later generations:*

The meiotic chromosome behavior of the raw allotetraploid, F_2 *Gilia minor* × *clokeyi*, contrasted strikingly with that of the diploid F_1 parental generation. As can be seen in fig. 10 D–F, the chromosomes at metaphase I were normal in form, not attenuated, and were mostly paired as bivalents (mean frequency = 12_{II} per cell), many of these as ring bivalents. Considering chromosomes in configurations of all types, the pairing frequency was 17.3 (15–18) (table 7), or to make a comparison, the pairing had increased from 51% in the F_1 diploid to 95% in the F_2 tetraploid. Pollen fertility was also high, with 91% stainable grains.

The extent to which this plant failed to attain perfect preferential pairing, however, may be assessed from the frequencies of univalents (mean frequency$=1.4$ per cell) and multivalents (mean frequency$=2.4$ per cell), as shown in table 7, and from the presence of heteromorphic bivalents (fig. 10 E). As would be expected segregation of the univalents was irregular and micropollen was present in many of the sporads.

TABLE 7. *Meiotic behavior at metaphase* I *of allotetraploid progeny of* Gilia minor $\times$ clokeyi.

PLANTS	POLLEN FERTILITY[1]	NO. PMC'S EXAMINED	M_1 FREQUENCIES OF:			TOTAL PAIRING[2]	% OF FULL PAIRING
			I	II	multiv.		
First tetraploid generation F_2	91%	10	1.4(0–6)	12.0 (6–16)	2.4(1–5)	17.3	96%
Line 1 ....F_5							
#H2–2b	93%	19	0.4(0–2)	17.6(16–18)	0.2(–)	17.5	97%
#H2–17a	95%	11	0.4(0–2)	17.6(14–18)	0.2(–)	17.8	99%
Line 1 ....F_6							
#KI–t	99%	40	0.1(0–2)	17.5(15–18)	0.3(–)	17.9	99%
Line 8 ....F_5							
#H4–7	48%	7	3.8(1–5)	10.4 (7–16)	2.7(0–5)	16.1	89%
#H4–15	85%	25	1.4(0–4)	——	——	17.3	96%
Line 8 ....F_6							
#L1–j	98%	16	1.2(0–4)	16.0(12–18)	0.8(0–2)	17.3	96%
#L1–k	98%	13	1.7(0–5)	14.4(10–18)	1.5(0–3)	17.0	94%

[1]percentage of stainable pollen
[2]frequencies of bivalent + multivalent pairing

Since translocation chains of three and four were observed in the parental diploid hybrid, configurations of as many as eight chromosomes are theoretically possible in the tetraploid offspring. Representative multivalents consisting of three, four, and six chromosomes are pictured in fig. 11 A–H. Multivalents of more than four chromosomes occurred in 50% of the PMC's. Included in this category were configurations in which some of the chromosomes were joined by matrix strands rather than chiasmata. In fig. 11 H, for example, three bivalents may be considered a translocation multivalent of six. Such an interpretation would be on the basis that the matrix connections represented earlier, more intimate associations between homologous regions. The lack of chiasmata at the triple intersections of the chromosomes is understandable, considering that the parental species characteristically have a low chiasma frequency.

Multivalents of the type pictured in fig. 11 G, H involve intergenomic pairing to some extent. Also suggesting this are heteromorphic bivalents such

as exhibited in fig. 10 E. As a result, segmental or whole-chromosome recombinations may be expected occasionally in the progeny.

This expectation appeared to be verified among the offspring. In the F_3 and F_4 generations pollen fertility was variable (fig. 13), and among 133 F_4 plants several inviable individuals were segregated. The remaining plants included some quite normal-appearing and fertile types, but others were malformed, slower growing, and with variously depressed fertilities. The majority of the plants of which meiotic chromosome counts were made had the normal tetraploid number of $2n = 36$. Other plants, some of high fertility, had $2n = 35$ and 37. The plant with the lowest recorded fertility, 26%, had $2n = 40$.

One F_4 family was exceptional in having uniformly normal morphology and also high pollen fertility (line 1, fig. 13). This family, carried into the F_6 generation, continued to be highly fertile. Meiotic behavior in the F_5 and F_6 generations of line 1 was markedly more regular than in the F_2 ancestor (table 7). Univalent frequency had dropped from 1.4 per cell in the F_2 to 0.4 in the F_5 and as low as 0.1 in a highly fertile F_6 plant. PMC's with 18 regular-appearing bivalents at metaphase I (fig. 10 G) were frequent. Multivalent frequency had decreased from 2.4 per cell to 0.2 and 0.3. Translocation configurations, present in 50% of the F_2 cells, were found in only 8% of the F_5 cells. The mean frequency of bivalents increased from 12 in the F_2 to more than 17 in the later generations.

The mechanism by which this quite stable line was established so rapidly after the raw allotetraploid is not known. It seems likely, however, that the initial step would have occurred in the F_2 generation by a chance combination of like gametes with complete and unmodified sets of the parental genomes, resulting from strictly homogenetic pairing. The continued operation of mainly preferential pairing would be favored, perhaps, in subsequent generations by the balanced genetic constitution and its advantageous physiological effects.

To explore the evolutionary possibilities of a less regular family, line 8 was also grown through the F_6 generation. In the F_5 progeny of a semisterile individual (pollen stainability = 48%), the range of fertilities was similar to that of the F_4 generation (fig. 13), and also as before, a number of inviable plants were segregated. The low fertility was associated with numerous univalents and multivalents, exceeding the frequencies of the F_2 ancestor (table 7). In fig. 10 J a representative metaphase I is illustrated in which there are four univalents, a chain of four, and a complex configuration of six chromosomes.

If the cause of the increased irregularities was heterogenetic pairing and segmental interchanges in the preceding tetraploid generations, which seems likely, the segmental alterations must have been few and nonextensive, for there were several plants of quite high fertility, as evidence of successful gametic recombinations.

The F_6 progeny of one of these fertile individuals showed an improvement over the F_5, since all but two plants scored above 90% in pollen stainability. Meiotic behavior in the best of these plants differed from that of line 1, however, in that a greater proportion of the PMC's formed multivalents, and produced them in frequencies $2\frac{1}{2}$ to 5 times as great as in line 1 (table 7). High univalent frequency apparently was a consequence of variability in the expression of the multivalent configurations, or to their instability.

Gilia minor   Gilia clokeyi   Pollen fertility

P_1 Fully fertile

9_{II} 9_{II}

F_1 1 – 3%

$4.6\ (2-7)_{II}$

F_2 91%

$12\ (15-18)_{II}$

F_3 45% ⊢——— 64% ———⊣ 91%

F_4 26% ⊢——— 77% ———⊣ 96%

line 8

F_5 48% ⊢——— 70% ———⊣ 94%

$10.4\ (7-18)_{II}$

line 1

F_5 91% ⊢ 96% ⊣ 99%

$17(14-18)_{II}$

F_6 65% ⊢—— 91% ——⊣ 98%

$12-18_{II}$

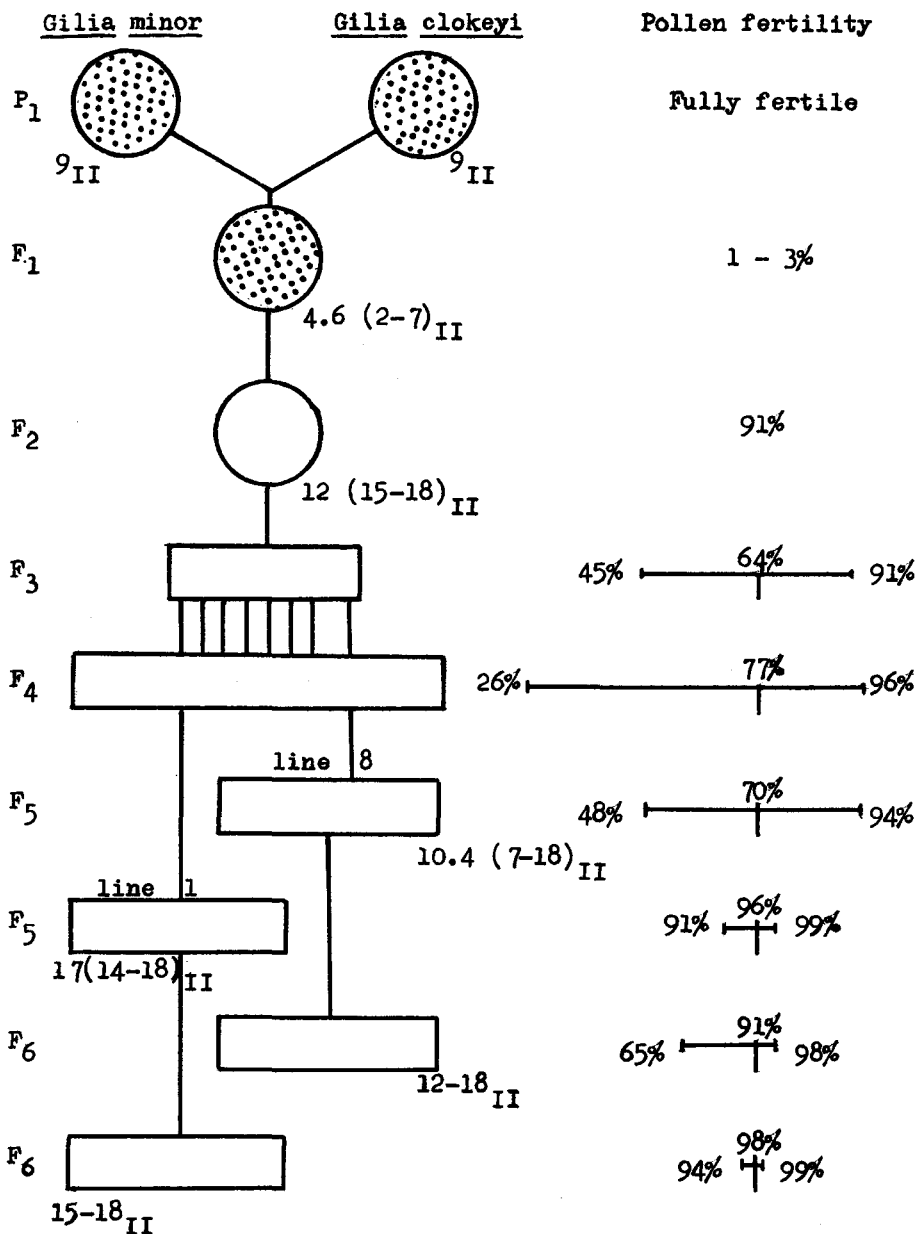F_6 94% ⊢ 98% ⊣ 99%

$15-18_{II}$

Fig. 13. Pedigree of *Gilia minor* × *clokeyi*. Pollen fertilities, as percentages of stainable pollen, are shown at the right, bivalent pairing, below. Diploid plants are indicated by stippling, the tetraploid by white.

A high frequency of multivalents in an allotetraploid is a circumstance which may lead to the segregation of characters by which the parental diploids differ. It was important then to observe carefully any evidences for such secondary segregation in the synthetic tetraploid plants. As will be described in Section VIII, several morphological variants did arise in line 8, as well as in another, line 6, which also was known to have numerous multivalents at metaphase I.

The meiotic regularity and high fertility of line 1 would favor its success were it to arise in nature. Line 8, in competition with line 1, would have the selective disadvantage of a less stable cytological behavior and the consequent potential for segregating plants of reduced fertility. However, there would perhaps be a compensating advantage in the possession of the mechanism of secondary segregation by which means new and possibly adaptively useful recombination genotypes might be derived.

F$_2$ Gilia aliquanta × minor:

A sample of 27 plants of F$_2$ *Gilia aliquanta* × *minor* was grown to maturity. There were no inviables; the morphology of leaves, flowers, and plant habit was very uniform, and all plants were fertile [pollen stainability = 96% (91%–99%)], comparing well with the most fertile line of F$_5$ *G. minor* × *clokeyi* which was grown simultaneously (see the pedigree, fig. 14, and table 7). The chromosome number did not vary from $2n = 36$ among the five plants counted.

Pairing at metaphase I was usually close, the chromosomes forming 12–18 normal-appearing bivalents (mean frequency of bivalents = 15.6). Heteromorphic bivalents were rare. Some of the cells had no univalents and others had as many as two, (mean frequency of univalents = 0.5). In Figure 12 M is shown a typical PMC at metaphase I with 18 bivalents.

At diakinesis the pairing relations of many of the chromosomes were obscure because of the concentration of many chromosomes about the nucleolus. A high number of nucleolar chromosomes is expected, since, as shown in Section V, *Gilia aliquanta* has three satellite chromosome pairs and *G. minor* has two. Figure 12 K is drawn from a broken diakinesis cell in which the paired nucleolar chromosomes were well spread. One trivalent and a quadrivalent, in addition to the bivalents, illustrate high pairing at diakinesis as compared with the incomplete and loose pairing at the same stage in the F$_1$ hybrid (fig. 12 A–C).

Multivalents of from three to six chromosomes at metaphase I (mean frequency = 1.2) were usually oriented as a series of bivalents with terminal connections, such as the chain of six in fig. 11 N and the chain of four in fig. 11 K. The chain of six in fig. 11 O was the only case observed of a multivalent of more than four chromosomes in which the members appeared to be joined by chiasmata, rather than partly by matrix strands or bands. Stickiness between series of bivalents was fairly common (figs. 11 L, 12 N), and it is not known if this was a consequence of weak homology and loose heterogenetic pairing at earlier stages of meiosis, as seemed to be true of the pseudobivalents in the F$_1$ generation. Observations at diakinesis were difficult due to the tendency described above for a large number of chromosomes to be massed

about the nucleolus. Resolution of the complexities of meiotic prophase in metaphase I seemed the most common pattern of behavior, however, and this was reflected in the high pollen fertility of the plants.

An aberrant cell having very low pairing is mentioned because of the behavior of the univalents (fig. 12 P) which appear to have segregated pre-
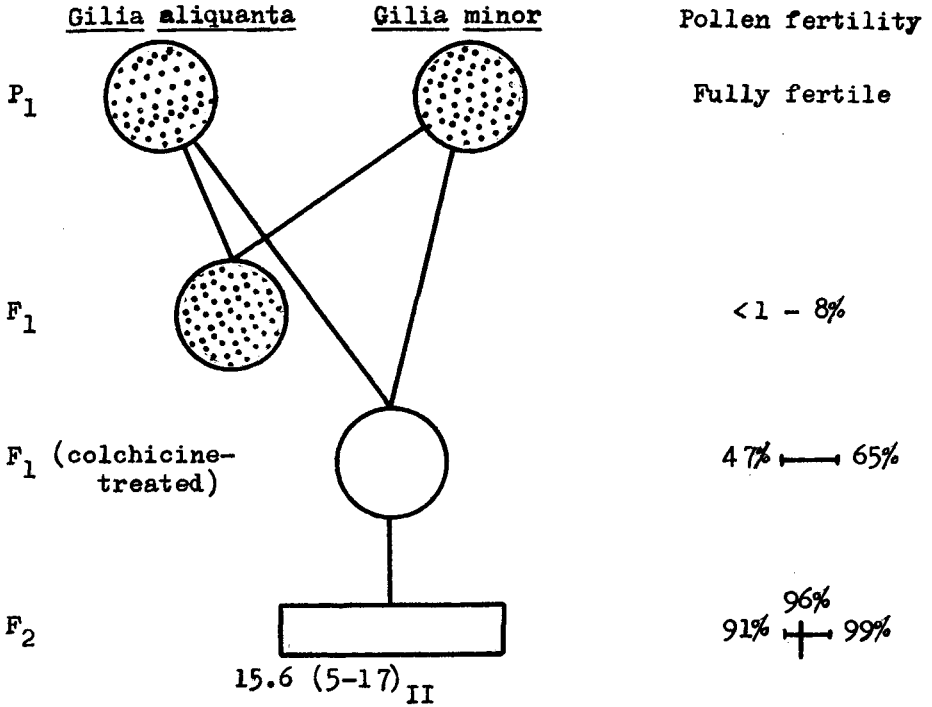

Fig. 14. Pedigree of *Gilia aliquanta* × *minor*. Pollen fertilities, as percentages of stainable pollen, are shown at the right, bivalent pairing, below. Diploid plants are indicated by stippling, the tetraploid by white.

cociously, such as commonly occurred in the diploid F_1 generation. Since this cell obviously deviated strongly from the norm of regular behavior at metaphase I, it was not included among the eleven PMC's scored. It is of interest, nevertheless, to note that precocious univalent segregation is an inherited trait in this hybrid, and would perhaps serve as a mechanism to prevent the development of chromosomally unbalanced gametes. This mechanism would involve the formation of separate telophase I nuclei for the early-segregating and later-segregating chromosomes, each of which would be deficient and would consequently give rise to deficient and probably abortive microspores.

The uniformly high fertility (91% to 99%) of the first tetraploid generation to be produced from a tetraploid parent in *Gilia aliquanta* × *minor* was

in contrast to the varied pollen fertilities (45% to 91%) in the comparable F_3 generation of *G. minor* × *clokeyi*. The difference may be attributed perhaps to a more effective operation of preferential pairing in *G. aliquanta* × *minor*, and probably in addition a stronger elimination of unbalanced gametes in the parental, tetraploid flowers by the mechanism described above. The relatively low pollen fertility of those flowers (47% to 65%), as compared to the fertility of the plant which gave rise to F_3 *G. minor* × *clokeyi* (91%), may be evidence of such a mechanism. The meaning of the low pollen fertility is in some doubt, however, since the extent of the distribution of tetraploid tissue within the chimaeral branches and flowers is unknown.

VII. THE COMPARISON BETWEEN NATURAL AND SYNTHETIC ALLOTETRAPLOID PLANTS

The production of fertile allotetraploid progeny from hybrids between the diploid species believed to represent the ancestral parents of *Gilia transmontana* and *G. malior* was described in Section VI. These synthetic allotetraploid plants provide a means of checking the morphological and genomic identity of the natural tetraploid sibling species. Morphological comparisons were made between garden cultures of the natural and artificial tetraploid plants grown the same season under uniform conditions as described in Section IV. During the previous season, however, notes on variability of the F_4 progeny of *G. minor* × *clokeyi* had been made. These will be reported below as of significance to the question of genetic segregation as a source of variation in the natural tetraploid species.

Finally the genomic constitution of *Gilia transmontana* will be considered through a study of its hybrids with allotetraploid *G. minor-clokeyi*.

THE MORPHOLOGICAL COMPARISON

If we are to identify the synthetic tetraploid plants with one or other of the natural tetraploids we must also understand well the chracteristics which differentiate the natural tetraploids. This was done in Section IV but will be reviewed at this time along with the comparisons of the synthetic tetraploids. A summary of the character analysis to follow is given in table 8.

The two tetraploid species have basal leaf shapes which are intermediate between the diploid species, but the *Gilia transmontana* leaf, with a resemblance to *G. clokeyi*, is broader on the average than that of *G. malior* (see fig. 3). Additional leaf samples which illustrate this difference well are shown in fig. 15. They represent three strains of each tetraploid species. The two leaves in the center are from the artificial tetraploids. It will be seen that *G. minor-clokeyi* (fig. 15 D) corresponds with the broad form of *G. transmontana* (fig. 15 A–C) and that *G. aliquanta-minor* (fig. 15 E) corresponds with the narrower form of *G. malior* (fig. 15 F–H).

The leaf form characteristics may also be compared mathematically using the ratio of width to length as employed in Section IV. (The "width" of the leaf was the length of the longest lobe, and the "length" was the distance from the leaf apex to the position of the lobe pair fifth below the apex. When only four lobe pairs were present an additional segment was supplied which

was equivalent to the distance between two pairs of lobes. The ratio was termed the "basal leaf form.")

Gilia malior, as compared with *G. transmontana*, tends to have lobes which are shorter, if considered in relation to the distances between them. Consequently the basal leaf ratio is generally lower for *G. malior* than for *G. transmontana* (table 1). The ratios which were calculated for each of the leaves in fig. 15 are given at the top of the figure. Allotetraploid *G. minor-clokeyi* with a ratio of 0.26 is at the upper end of the range of *G. transmontana* (0.17–0.28), and contrasts with allotetraploid *G. aliquanta-minor* which has a ratio

TABLE 8. *Morphological comparison of natural and synthetic* Gilia transmontana *with natural and synthetic* G. malior.

| *Gilia transmontana* and *G. minor-clokeyi*	*G. malior* and *G. aliquanta-minor*
1. Leaf in outline oblanceolate to subspatulate. Basal leaf ratio = 0.20 (0.17–0.28)	Leaf in outline oblanceolate. Basal leaf ratio = 0.18 (0.15–0.23).
2. Calyx sinus membrane smooth.	Calyx sinus membrane usually slightly puckered.
3. Corolla throat pale in color.	Corolla throat dark purple at least in lower part.
4. Corolla lobes streaked with violet on dorsal sides.	Corolla lobes not streaked.
5. Corolla lobes narrow.	Corolla lobes broad.
6. Corolla lobes acute at apex.	Corolla lobes rounded or slightly pointed at apex.

of 0.16 and is in the lower part of the range of *G. malior* (0.15–0.23). Thus in basal leaf form the synthetic tetraploid plants presented the same character contrast which differentiates the corresponding tetraploid plants.

The calyx in the synthetic tetraploids (fig. 16 I–J) is pubescent as in both *Gilia transmontana* (fig. 16 G–H) and *G. malior* (fig. 16 K–L). Variations in degree of pubescence of the calyx are known in the natural tetraploids but no case has yet been found of a completely glabrous calyx as in the diploid species *G. clokeyi* and *G. aliquanta* (fig. 4).

Gilia transmontana has a smooth membrane in the sinuses of the calyx lobes (fig. 16 G–H). Allotetraploid *G. minor-clokeyi* also has a smooth membrane (fig. 16 I). The membrane in the calyx sinuses is slightly puckered in most strains of *G. malior* (fig. 16 K) or sometimes smooth (fig. 16 L) but never extremely puckered as in *G. aliquanta* (fig. 4 J). Allotetraploid *G. aliquanta-minor* had a slightly puckered sinus membrane (fig. 16 J) like that usually seen in *G. malior*.

Corolla colors in *Gilia tranmontana* are pale, the throat weak violet in the lower part, and with violet streaks on a white background on the dorsal sides of the corolla lobes. Synthetic *G. minor-clokeyi* had corolla colors fitting this

W/L = 0.23 0.20 0.20 0.26 0.16 0.17 0.16 0.18
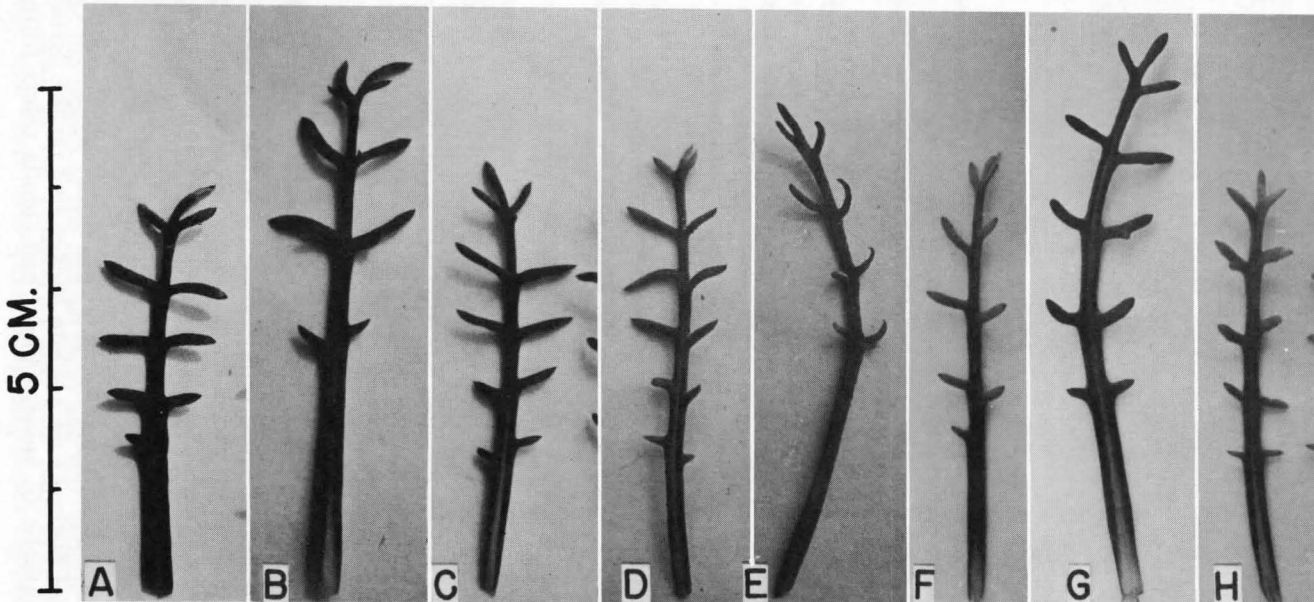
Fig. 15. Basal leaves from garden-grown plants of natural and synthetic allotetraploids. Basal leaf form (width/length) is indicated above each leaf (see text).—A–C. *Gilia transmontana* Bd, KH, and MP, respectively.—D. *G. minor-clokeyi.*—E. *G. aliquanta-minor.*—F–H. *G. malior* ShC, MB, and Sp, respectively.

description except that the background color of the corolla lobes was pale violet. *Gilia malior* has brighter corolla colors than *G. transmontana,* with a deep purple area in the throat, and violet lobes. Allotetraploid *G. aliquanta-minor* resembled *G. malior* closely in these characters. The dark purple color in the throat can be seen as black in the photographs (fig. 16 J–L).

    Gilia transmontana and *G. malior* are well distinguished by the form of the corolla lobes which are narrow and acute in the former species (fig. 16 A–B), and broad with a slightly pointed apex in *G. malior* (fig. 16 E–F). *Gilia minor-clokeyi* corolla lobes (fig. 16 C and 17 A–C) had a form much like that of *G. transmontana.* Corolla lobes in *G. aliquanta-minor* (fig. 16 D) were broader and had only a slightly pointed apex, being very similar to those of *G. malior.*

VARIABILITY IN THE SYNTHETIC ALLOTETRAPLOIDS

    Among the 133 F_4 progeny of *Gilia minor* $\times$ *clokeyi* there was noted some variation in morphology. There were difficulties in analyzing this variation, however, as some of the plants were abnormal in form, in at least some cases, due to genic or chromosomal unbalance. The most abnormal plants were sterile or semisterile, grew slowly and reached flowering stage late. These plants were alike in being unusually succulent, with dark green, nearly glabrous herbage. The leaves were often entire or of peculiar form with a few irregularly disposed lobes. Flowers were also poorly formed and set few, if any, seeds.

    Variation among the more normal plants involved herbage color, pubescense, leaf form, and calyx and corolla size and form, but due to the tendency for irregularities in these plants too, analysis of segregation seemed hardly feasible. One conspicuous deviant, however, will be especially considered, as evidence of morphological segregation among the progeny. This plant, a member of F_4 family 6, was highly pollen fertile and had the normal tetraploid chromosome number of $2n = 36$, but due to the peculiar relationship of the flower parts it was unable to set many seeds. Both corolla and calyx were abnormally long (fig. 17 B), and the adnate stamens were carried up beyond stigma level so that pollination was only occasionally completed. The leaves, like the corolla and calyx, were of unusually long, narrow proportions.

    Three flowers of F_4 *Gilia minor* $\times$ *clokeyi,* shown in fig. 17, include two from family 6 which show the extremes of corolla and calyx proportions (fig. 17 A–B) and a third (fig. 17 C) of more intermediate form typical of family 1. The latter family was morphologically very uniform as were its F_5 and F_6 progeny. The similarity between the flowers of family 1 and *G. transmontana* (Kyle strain) is apparent from fig. 17 C–D.

    An unusually plump type of capsule was first noted in the F_6 generation of *Gilia minor* $\times$ *clokeyi* line 8. The majority of the 17 plants in the family had plump capsules, but three were most extreme in this regard. The scatter graph in fig. 19 and the capsule drawings of fig. 18 permit a comparison of capsule form in line 8 with that of line 1, as well as with those of the natural species. The form of the capsule, expressed as a ratio between width and length (W/L) is plotted against calyx length, which was also variable. Plump capsules, with a high W/L appear to be correlated with short calyces. The
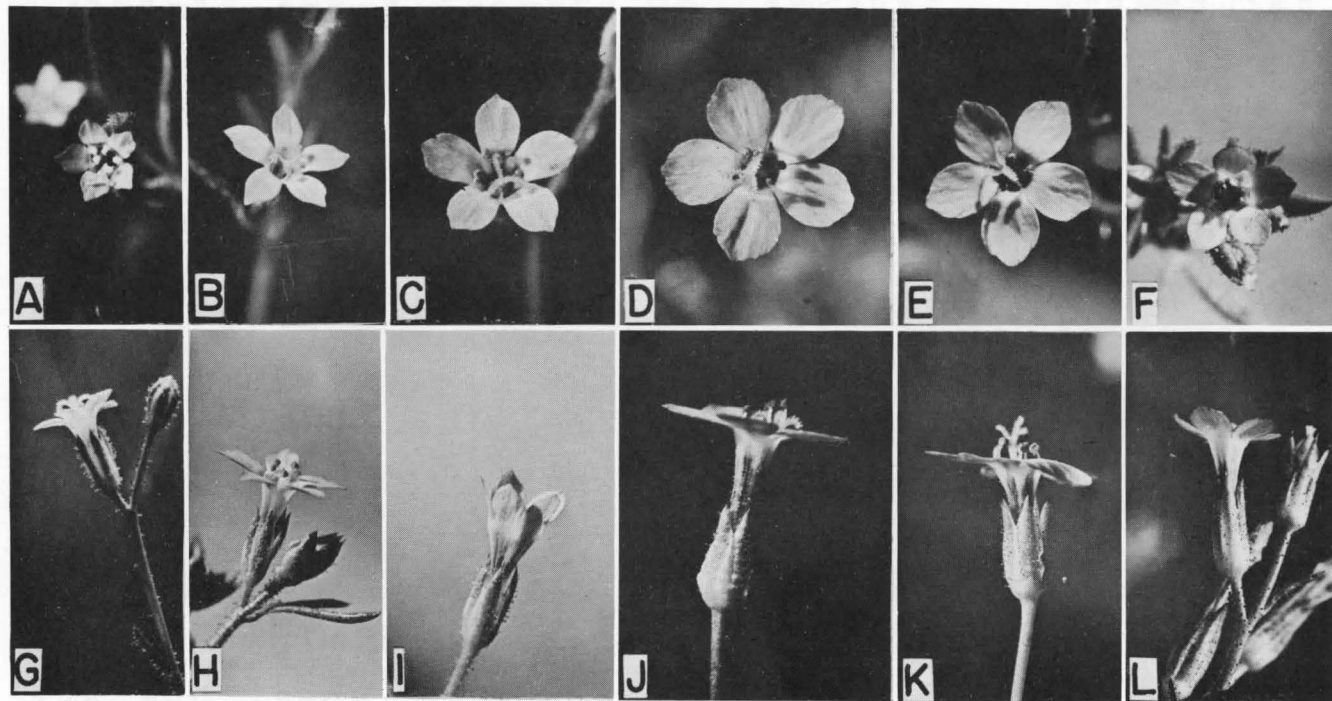
Fig. 16. Flowers from garden-grown plants of natural and synthetic allotetraploids.—Top view (left to right): A–B. *Gilia transmontana* KH and Bd, respectively.—C. *G. minor-clokeyi*.—D. *G. aliquanta-minor*.—E–F. *G. malior* Sp and MB, respectively.— Side view (left to right): G–H. *G. transmontana* Ky and Bd, respectively.—I. *G. minor-clokeyi*.—J. *G. aliquanta-minor*.—K–L. *G. malior* Sp and MB, respectively.

three plants of line 8 with the plumpest capsules and shortest calyces are found situated adjacent to those of *G. clokeyi* on the scatter graph.

Gilia minor occupies the opposite extreme, due to its proportionately narrower capsules and moderately long calyces. In the drawing (fig. 18) the capsules are arranged from left to right in order of their increasing W/L. The natural species in the upper half may be compared with the synthetic tetraploids immediately below. Capsule D, from line 1, has the lowest W/L among the two lines and is the closest approach to the form of *G. minor*, although less extreme, as the respective ratios indicate.
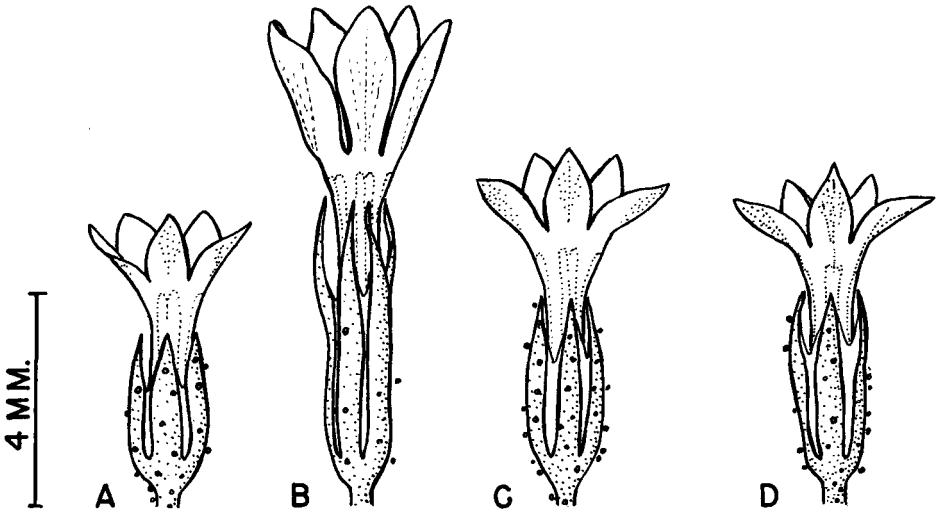

Fig. 17. Flowers drawn from garden-grown *Gilia transmontana* and 4n *G. minor-clokeyi*.—A–B. F_4 *G. minor* × *clokeyi*, line 6.—C. F_4 *G. minor* × *clokeyi*, line 1.—D. *G. transmontana* Ky.

Capsule E of line 8 occupies an intermediate position with a similar W/L to that of *Gilia transmontana*. Referring again to the scatter graph, most of the individuals of line 1 and several of line 8 are in an intermediate position between the two extremes, but the majority of line 8 plants are out of the variation range of line 1, in the direction of greater W/L.

Although conclusions based upon such small progenies are perhaps premature it appears that a divergence in form toward the *Gilia clokeyi* parent has occurred in line 8. This observation, along with the fact that line 8 had a high multivalent frequency (Section VI) leads one to suspect secondary segregation in this allotetraploid line. Evidence that the mechanism of secondary segregation could be involved also in the evolution of diversity in *G. transmontana*, the natural counterpart of *G. minor-clokeyi*, is in the observation of the nature of interracial differences. It seems significant that some of

the variation from race to race (Summary, Section IV) concerns the same characters—corolla length and capsule form—which segregated in the synthetic allotetraploid plants.
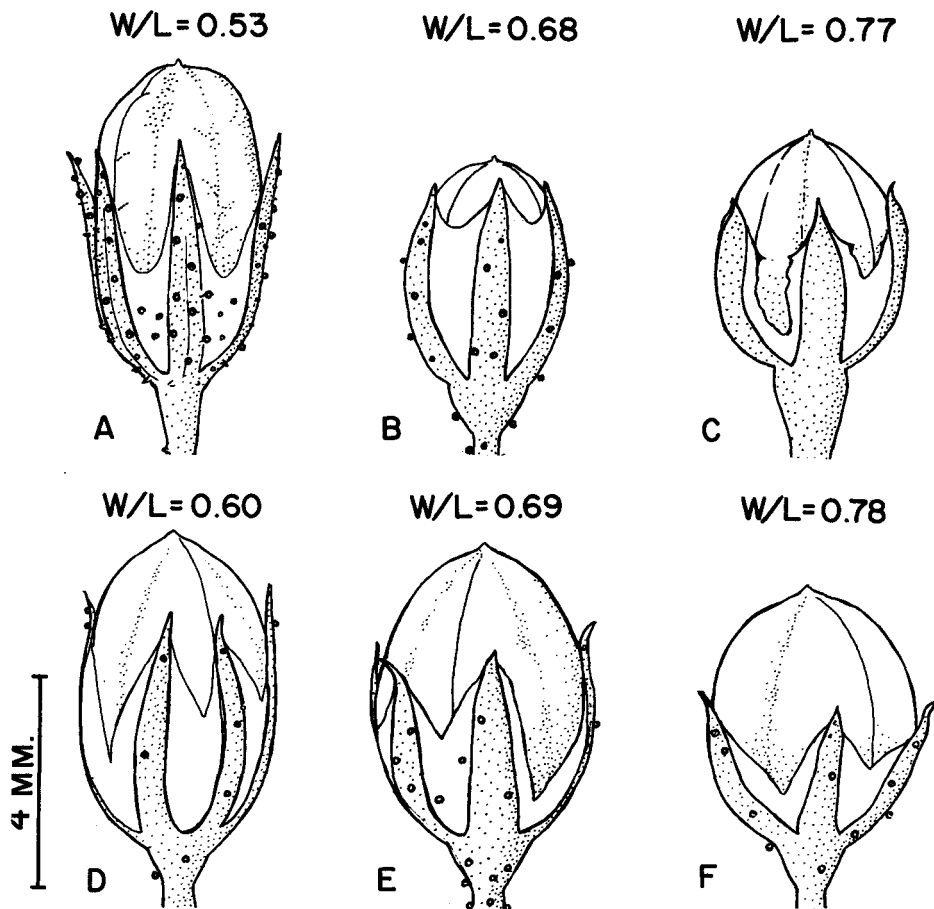

Fig. 18. Mature capsules and calyces drawn from garden-grown plants.—A. *Gilia minor* MB. —B. *G. transmontana* Ky.—C. *G. clokeyi* MH.—D. F_6 *G minor* × *clokeyi*, line 1.—E–F. F_6 *G. minor-clokeyi*, line 8.—The width to length ratio of each capsule is indicated above.

The allotetraploid F_2 progeny of *Gilia aliquanta* × *minor*, consisting of 27 plants which were grown to maturity, provided no example of morphological segregation since they were quite uniform in all characters examined.

FERTLITY AND GENOMIC RELATIONSHIPS BETWEEN
NATURAL AND SYNTHETIC ALLOTETRAPLOIDS

Hybrids between the tetraploid species and the synthetic allotetraploids were produced in order to test the genomic constitution of the natural tetraploids. This series of crosses included two to three strains of the species, in view of the possibility that genomic differences may occur between geograph-
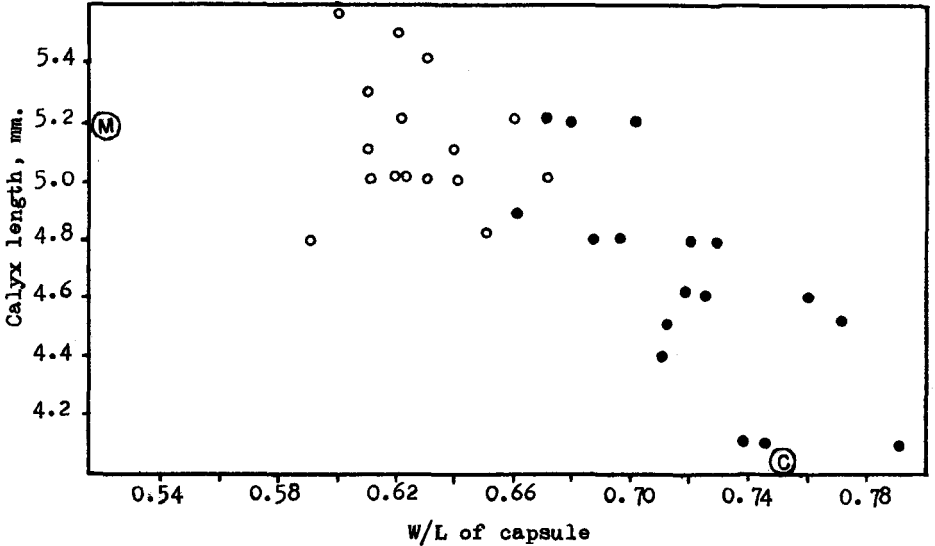

Fig. 19. Diagram of variation in F_6 *Gilia minor* $\times$ *clokeyi* with respect to capsule shape (width/length) and calyx length. Line 1 is represented by white dots, line 8 by black. Typical capsules of *G. minor* (M) and of *G. clokeyi* (C) are plotted for comparison.

ical races. Both *Gilia transmontana* and *G. malior* were crossed to 4n *G. minor-clokeyi* and to 4n *G. aliquanta-minor*. The results of these and some other crosses will be reported in some detail at a later date, but a brief summary and some of the data which are pertinent (table 9) will be presented here.

All of the hybrids involving the synthetic tetraploids produced seeds, but in some more than in others. The seed set was normal in the F_1 hybrids of *Gilia transmontana* $\times$ 4n *G. minor-clokeyi*. Seed set was also normal in F_1 *G. malior* $\times$ 4n *G. aliquanta-minor*. Correspondingly, in both of these hybrid combinations chromosomal pairing in the offspring was nearly complete—87%–94% and 89%–97% of full pairing, respectively. This was slightly reduced pairing, as compared with natural wild *G. transmontana* and *G. malior* and with the most fertile lines of synthetic tetraploids. It was, however, very similar to the meiotic pairing of 4n *G. minor-clokeyi*, line 8, with its score of

89%–96% of full pairing, and in which the reduction is believed due to heterozygosity for a few small structural rearrangements.

These results then support the hypothesis that *Gilia transmontana* is an allotetraploid combining two genomes essentially like those of diploids *G. minor* and *G. clokeyi*, and that *G. malior* is an allotetraploid combining genomes like those of *G. aliquanta* and *G. minor*.

Seed set was considerably reduced in hybrids between the tetraploid species and synthetic allotetraploids with which they presumably shared only one genome. Chromosomal pairing in the F_1 hybrids, 56%–61% of full pairing (table 9), exceeded slightly the amount expected if the chromosomes of only the two *Gilia minor* genomes were to pair, or—10.1–10.9 chromosome pairs rather than only 9 pairs.

It is appropriate to compare these results with those of the cross between the two synthetic allotetraploids, in which were combined two complete genomes of *G. minor* with one genome each of *G. aliquanta* and *G. clokeyi*. The degree of chromosomal pairing in this F_1 hybrid was in the same general range as the above outcrosses—62% of full pairing, or 11.1 pairs. These results present additional evidence in support of the hypothesis as to the genomic constitution of the tetraploid species, and in addition, demonstrate a certain degree of reliability in the sensitivity of the hybridization test in this species complex.

VIII. SUMMARY

The group of plants under investigation comprises a 5-species subgroup of the *Gilia inconspicua* complex, a network of diploid and tetraploid species. These are small-flowered, self-pollinating plants, members of Section *Arachnion*, or the "Cobwebby Gilias." The hypothesis was advanced that two of the species, *G. transmontana* and *G. malior*, which are closely similar sibling species, and tetraploid in chromosome number $(2n = 36)$, are derived by allopolyploidy from two different combinations of three diploid species $(2n = 18)$ as follows: *G. minor* $(2n)$ $\times$ *G. clokeyi* $(2n)$ = *G. transmontana* $(4n)$; *G. minor* $(2n)$ $\times$ *G. aliquanta* $(2n)$ = *G. malior* $(4n)$.

Evidence pertinent to this hypothesis has been assembled from the following lines of investigation: (1) geographical distribution and ecology of the species; (2) morphological comparisons of the species; (3) karyotype analysis; (4) the cytology of interspecific tetraploid and diploid hybrids and of synthetic allotetraploid plants; (5) morphological comparisons between the tetraploid species and the corresponding synthetic allotetraploids; and (6) the cytology of hybrids between the tetraploid species and the synthetic allotetraploids.

The diploid species which represent the putative ancestors of the tetraploid species occur in adjacent or overlapping geographical regions. Where they occur sympatrically their distinctive ecological preferences prevent extensive contact. The distributional areas of the tetraploid species coincide with the zone of contact or near contact of the diploid species. The tetraploids also occur beyond this immediate area to some extent. Locally, the tetraploids, having less specific ecological requirements than the diploids, are more widespread and abundant.

TABLE 9. *Meiotic behavior and fertility of* F_1 *hybrids between natural and synthetic allotetraploid plants.*

F_1 HYBRID	CULTURE	POLLEN FERTILITY[1]	NO. PMC'S EXAMINED	TOTAL PAIRING[2]	PERCENT OF FULL PAIRING
G. transmontana Bd × G. minor-clokeyi	#G1–1c	83%	10	15.7	87%
	#G1–1d	78%	33	16.9	94%
G. transmontana Ky × G. minor-clokeyi	#C1, C7a	64%	26	16.7	93%
G. transmontana MP × G. minor-clokeyi	#G2–1e	88%	10	17.0	94%
	#G2–1a	91%	13	16.2	90%
G. transmontana Bd × G. aliquanta-minor	#I1–2a	9%	24	10.9	60%
G. transmontana Ky × G. aliquanta-minor	#I2–a	6%	30	10.2	57%
G. malior MB × G. aliquanta-minor	#F1–5b	80%	22	17.4	97%
	#F1–5f	69%	14	16.8	93%
G. malior Sp × G. aliquanta-minor	#F2–1a	62%	8	16.7	93%
	#F2–1d	65%	12	16.0	89%
G. malior Sp × G. minor-clokeyi	#H1–3c	18%	34	10.1	56%
G. minor-clokeyi × G. aliquanta-minor	#E1–2a	46%	28	11.1	62%

[1]percentage of stainable pollen
[2]frequencies of bivalent + multivalent pairing

Morphological comparisons were made between the tetraploid and diploid species from herbarium specimens, wild plants, and also from numerous strains grown in a uniform environment. In most of the characters examined the tetraploids were intermediate between their putative ancestral diploids. It may be said that the two tetraploid species usually resemble each other more closely than they resemble their diploid ancestors. This may be explained by the premise that each combines one and the same genome—that of *Gilia minor*—with a second genome which in each case belongs to the same morphological species group. This is the *G. ochroleuca* group to which *G. aliquanta* and *G. clokeyi* belong. Differences between the tetraploids consist of a moderated expression of the features which differentiate *G. clokeyi* from *G. aliquanta*.

From karyotype studies it was noted that the chromosome sets of the three diploid species were similar to each other in many ways but were also different with respect to the size and proportions of satellited chromosomes. A marker chromosome found only in *Gilia aliquanta*, among the diploids, has a satellite on the long arm. This chromosome was identified in *G. malior* but not in *G. transmontana*, indicating the probability that *G. aliquanta* has contributed a genome to *G. malior*. Satellite suppression, which appears to occur in *G. transmontana*, prohibits use of evidence as to the constituent genomes involving the types of satellited chromosomes present. It seems clear at least that the chromosomes making up the karyotypes of the two tetraploid species are not entirely alike in form. It was also shown that the size relationships of the chromosomes differ in the two tetraploids and that these size relationships correspond with those of the diploid species which are the supposed sources of the component genomes.

Attempts to obtain triploid hybrids between the diploid and the tetraploid species failed, but interspecific crosses at both the diploid and tetraploid levels yielded information on genomic relationships. Hybrids between the tetraploid species, *Gilia transmontana* and *G. malior* had very low pollen fertility but formed an average of about nine bivalents, suggesting that the two species have one genome in common.

Chromosomal pairing in the F_1 diploid hybrids between *Gilia minor* and *G. clokeyi* and between *G. aliquanta* and *G. minor* varied from very low to very high, but with a mean bivalent frequency of 4.6 and 4.5, respectively. The low pairing in the hybrids, together with the karyotype differences between the species shows that structural changes differentiate the chromosomes of *G. minor* from the semihomologous ones of *G. clokeyi* and *G. aliquanta*. The existence of some larger structural differences between the species was surmised from the presence of occasional translocation chains of three and four chromosomes in both of the F_1 hybrids. The occasional high pairing is evidence that there is some homology between the chromosomes of the two genomes.

Allotetraploid progeny of F_1 *Gilia minor* $\times$ *clokeyi*, which appeared spontaneously in the F_2 generation, were variable in fertility but one line was highly fertile and morphologically uniform with chromosomal pairing mostly as bivalents. The first generation, synthetic, colchicine-induced allotetraploids of *G. aliquanta* $\times$ *minor* proved to be as uniform and fertile as the most

fertile tetraploid line of *G. minor* × *clokeyi*. Chromosomal pairing was largely as bivalents.

Morphological segregation was observed in the tetraploid offspring of *Gilia minor* × *clokeyi*, involving proportions of the leaf, flower, and capsule. Since variation in the proportions of the leaf, flower, and capsule are known to occur between races of *G. transmontana* it is appropriate to consider secondary segregation in a segmental allotetraploid as a mechanism by which such variation may have arisen.

A series of crosses between the synthetic allotetraploids and several strains of the tetraploid species were made to test their genomic relationships. Hybrids of *Gilia transmontana* and 4n *G. minor-clokeyi* and of *G. malior* × 4n *G. aliquanta-minor* had nearly full chromosomal pairing, which was in the range characteristic for some of the selfed plants of 4n *G. minor-clokeyi*. This indicated a high degree of homology between the artificial tetraploids and the natural species and strains which correspond with them. Therefore it may be said that the two genomes of *G. transmontana* are essentially like those of *G. minor* and *G. clokeyi*, and that the two genomes of *G. malior* are like those of *G. minor* and *G. aliquanta*.

Thus from six lines of evidence the hypothesis of allopolyploidy in the origin of the sibling tetraploid species is upheld.

LITERATURE CITED

Beeks, R. M. 1955. Improvements in the squash technique for plant chromosomes. El Aliso 3: 131–134.

Grant, A., & V. Grant. 1956. Genetic and taxonomic studies in *Gilia*. VIII. The Cobwebby Gilias. El Aliso 3: 203–287.

Grant, V. 1952. Cytogenetics of the hybrid *Gilia millefoliata* × *achilleaefolia*. I. Variations in meiosis and polyploidy rate as affected by nutritional and genetic conditions. Chromosoma 5: 372–390.

———. 1956. The influence of breeding habit on the outcome of natural hybridization in plants. Am. Nat. 90: 319–322.

———. 1959. Natural History of the Phlox Family. Nijhoff. The Hague.

———. 1964. Genetic and taxonomic studies in *Gilia*. XII. Fertility relationships of the polyploid Cobwebby Gilias. Aliso 5: 479–507.

———, R. M. Beeks & H. L. Latimer. 1956. Genetic and taxonomic studies in *Gilia*. IX. Chromosome numbers in the Cobwebby Gilias. El Aliso 3: 289–296.

———, & A. Grant. 1960. Genetic and taxonomic studies in *Gilia*. XI. Fertility relationships of the diploid Cobwebby Gilias. Aliso 4: 435–481.

Ribbands, C. R. 1937. The consequences of structural hybridity at meiosis in *Lilium* × *testaceum*. J. Gen. 35: 1–24.

Walters, M. S. 1954. A study of pseudobivalents in meiosis of two interspecific hybrids of *Bromus*. Am. J. Botan. 41: 160–171.