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Verne Grant

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GENETIC AND TAXONOMIC STUDIES IN *GILIA*V. *GILIA CLIVORUM*

VERNE GRANT\*

## INTRODUCTION

One of the commonest species of Leafy-stemmed *Gilia* in the South Coast Range of California is a somewhat sprawling plant with small blue-violet flowers decorated with purple spots. This species has not been recognized as distinct in previous treatments of the genus, some populations having been classified under *Gilia millefoliata* and others with the small-flowered forms of *G. achilleaeifolia*. The discovery that the latter species are both diploid and that the purple-spotted plant of the Coast Range hills is tetraploid suggested that the latter should be set apart as a separate species. The results obtained from artificial hybridizations confirmed this disposition. A Latin name was sought for the new species and was found in a previously published variety of Jepson, *Gilia multicaulis* var. *clivorum*, which now becomes *Gilia clivorum* (Jeps.) V. Grant. One of the purposes of this paper is to clarify the taxonomy of *Gilia clivorum* and of *G. millefoliata*.

The long-standing taxonomic confusion between *Gilia clivorum* and its relatives, *G. achilleaeifolia* and *G. millefoliata*, was revealing in another sense. The fact that the tetraploid species varies in the direction of two diploid species suggests that the former may be a segmental allotetraploid and the latter its ancestors. Cytogenetic studies provided confirmation of this hypothesis. A second purpose of the present paper, therefore, is to present the evidence leading to the conclusion that *Gilia clivorum* is an allotetraploid derived from hybridization between *G. millefoliata* and *G. achilleaeifolia*.

During the investigation of the genomic constitution of *Gilia clivorum*, an artificial allotetraploid was obtained from the cross between *G. millefoliata* and *G. achilleaeifolia*. The raw allotetraploid segregated for some of the character differences between *G. millefoliata* and *G. achilleaeifolia* and thus revealed some part of the genic basis of the differences between two intersterile species. A third purpose of this paper is to describe the raw allotetraploid, which represents a synthetic form of *Gilia clivorum*.

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NATURAL *CLIVORUM*

*Gilia clivorum* is a common annual herb on hillsides in the California Coast Range. The plant has a more or less decumbent habit of growth and small blue-

\*Rancho Santa Ana Botanic Garden. Manuscript received for publication September 1, 1952.

violet flowers with five pairs of purple spots in the corolla throat. The species exists in a series of disjunct local colonies scattered throughout the live-oak and bunch-grass communities. These colonies often consist of several diverse types of individuals.

Thus a colony on Mt. Diablo, Contra Costa County, contained three types of individuals: those with prominent purple spots in the corolla, those with faint spots, and those without spots. A colony on Grizzly Peak in the Berkeley hills consisted of some individuals with deep purple spots and others with faint spots. Parallel variations were found in a nearby colony in Strawberry Canyon. A colony in Panoche Pass, San Benito County, contained the following types of individuals: (a) floccose plants with finely dissected leaves and corollas with a clear yellow tube and deep purple spots; (b) like the former but with corollas with a salmon-yellow tube and pale purple spots; (c) plants with lightly floccose stems, slightly dissected leaves with long pinnae, and corollas with a clear yellow tube and deep purple spots; (d) like the preceding but with corollas with a salmon-yellow tube and pale purple spots.

Seeds were harvested on single plants representing the different morphological types in the Grizzly Peak, Strawberry Canyon and Panoche Pass colonies in the summer of 1948. Their progeny, numbering between 20 and 40 individuals in each family, were grown in the spring of 1949. These progeny all came true to type. The

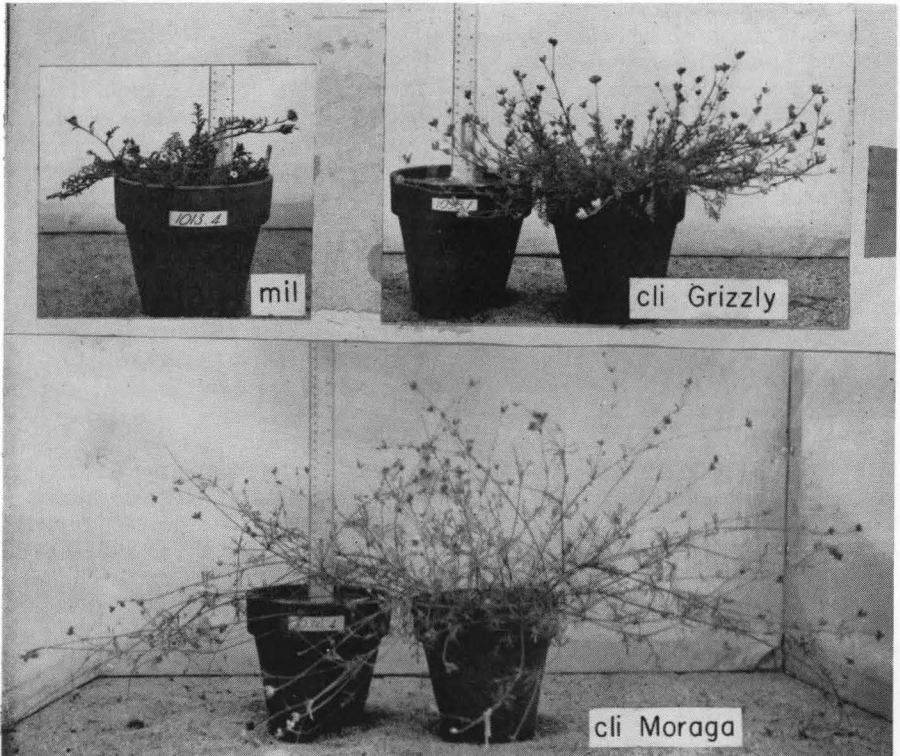


Fig. 1. Growth habit of *Gilia divorum* from Grizzly Peak and Moraga Canyon in the hills east of San Francisco Bay, and of *G. millefoliata* from Pt. Reyes Peninsula. Plants grown at Stanford.

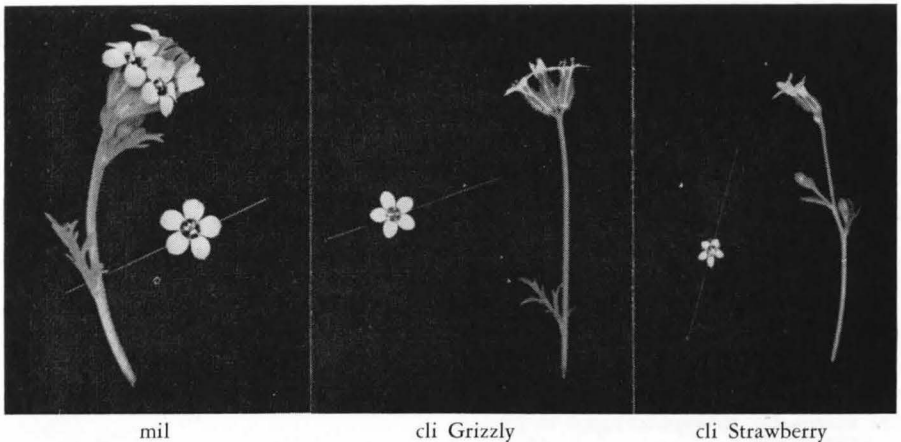


Fig. 2. Inflorescence and flowers of *Gilia clivorum* from Grizzly Peak and Strawberry Canyon in the Berkeley hills, and of *G. millefoliata* from Pt. Reyes Peninsula. Plants grown at Stanford.

garden-grown plants were quite uniform within each family, and the families differed as markedly from one another in the garden as had their parents in the wild. This shows that the variations are hereditary.

The Grizzly Peak and Strawberry Canyon strains of *Gilia clivorum* were found to be both self-compatible and automatically self-pollinating. The plants in nature are rarely visited by insects and are consequently largely inbred. This circumstance accounts for the pattern of variation encountered in the natural colonies. These colonies are composed of true-breeding biotypes between which there is only occasional gene exchange.

There are constant differences between as well as within the colonies. Colonies only a few miles apart in the Berkeley hills, the Grizzly Peak, Strawberry Canyon, and Moraga Canyon colonies, thus differ in habit of branching and size of flowers. When grown in the experimental garden, the Grizzly Peak plants are branched from the base and more or less erect, and possess large flowers, while the Strawberry Canyon and Moraga Canyon plants are spreading with horizontal stems and small flowers (figs. 1 to 3).

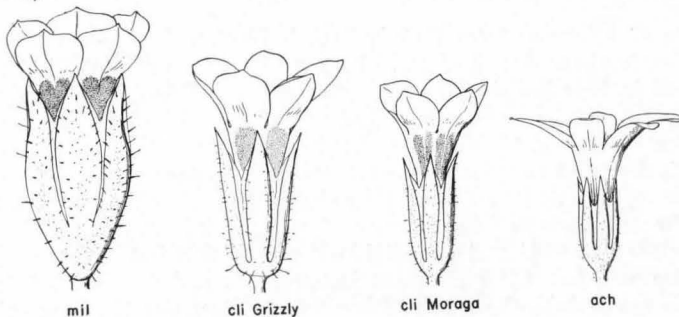


Fig. 3. Flowers of two strains of *Gilia clivorum*, of *G. millefoliata* from Pt. Reyes Peninsula, and of a small-flowered form of *G. achilleaeifolia* from Kings Mt. on the San Francisco Peninsula. Drawings made from plants growing in a uniform garden. (x 3 1/3)

The Grizzly Peak and Strawberry Canyon types were crossed in order to determine the degree of genetic relationship between them. The two strains cross freely. A sample of 30  $F_1$  hybrids was grown. These hybrids were fully fertile and produced an  $F_2$  generation of 400 individuals, all of which were vigorous. The  $F_1$  hybrid was intermediate in growth habit and flower size and the  $F_2$  generation displayed continuous variation for these characters. These facts indicate that the Grizzly Peak and Strawberry Canyon types are but variants of the same species which differ in some polygenic characters.

The  $I_1$  and  $I_2$  generations of *Gilia clivorum* and of *G. millefoliata*, which is also autogamous, are quite free of inviable or subnormal individuals. The interstrain hybrids of *G. clivorum*, furthermore, show no trace of hybrid vigor; that is, the  $F_1$  hybrids do not attain a larger size or longer blooming period, etc., than the mean of their parents. In *Gilia capitata*, a predominantly outcrossing species, by contrast, the first and second inbred generations usually contain some dwarfish or malformed or weak individuals; while hybrid vigor is manifested in the  $F_1$  hybrids between different strains, but disappears again in  $F_2$ .

These facts are consistent with the view that populations of a cross-breeding organism like *Gilia capitata* contain numerous deleterious recessive genes. Such genes are pretty well sheltered by their dominant alleles in the natural populations; they are even more completely covered up in  $F_1$  hybrids derived from crosses between different populations; but they are exposed by inbreeding. The mutation process probably produces such deleterious recessives also in *Gilia clivorum* and *G. millefoliata*, but here the self-pollinating habit soon exposes them to elimination by natural selection. Inasmuch as deleterious recessive genes cannot accumulate in the populations under these conditions, there is no basis for hybrid vigor in the interstrain hybrids (see Dobzhansky, 1950).

As previously mentioned, *Gilia clivorum* is tetraploid and *G. millefoliata* is diploid. The following counts were obtained from carmine smears of pollen mother cells:

<i>Gilia clivorum</i> Grizzly Peak, Alameda Co.	<i>V. Grant</i> 8536	n=18
<i>Gilia clivorum</i> Strawberry Canyon, Alameda Co.	<i>V. Grant</i> 8535	n=18
<i>Gilia clivorum</i> Moraga Canyon, Contra Costa Co.,	<i>V. Grant</i> 8647	n=18
<i>Gilia clivorum</i> Panchoe Pass, San Benito Co.,	<i>V. Grant</i> 8435	n=18
<i>G. millefoliata</i> Pt. Reyes Peninsula, Marin Co.,	<i>V. Grant</i> 8419	n=9

Horticultural strains identified as *Gilia millefoliata*, but in reality probably belonging to *G. clivorum*, have been reported as tetraploid by Langlet (1936) and Flory (1937).

Meiosis in *G. clivorum* from Grizzly Peak is illustrated in figure 4; meiosis in *G. millefoliata* has been described and illustrated elsewhere (Grant, 1952). Meiosis was examined in four individuals of *G. clivorum*, and found to be fairly regular. Among 29 pollen mother cells seen at first metaphase, 27 had the full complement of 18 bivalents, one had 17 bivalents plus 2 univalents, and one contained 16 bivalents, a trivalent and an unpaired chromosome. Separation of the chromosomes at anaphase was regular, no laggards or bridges being found in either the first or second division. The tetrads were likewise normal without micronuclei. Pollen fertility ranged from 66% to 95% and all individuals were highly fertile as to seeds.

Having learned that *Gilia clivorum* is tetraploid and *G. millefoliata* and *G. achilleaefolia* are diploid, the next problem was to determine the geographical distribution of the tetraploid and diploid species. Efforts to correlate differences in the number of chromosome sets with differences in diameter of pollen grains or stomata have met with no success whatever in *Gilia*, perhaps owing to a tendency for the size of the chromosomes to decrease as their number increases so that the cell size

remains about constant. It was necessary, therefore, to discover taxonomic characters other than pollen grain or stomatal size for distinguishing the tetraploid from the diploid populations. There are some fairly reliable morphological distinctions between *Gilia clivorum* and *G. millefoliata* and *G. achilleaefolia*. These key characters will be set forth in the taxonomic section of this paper.

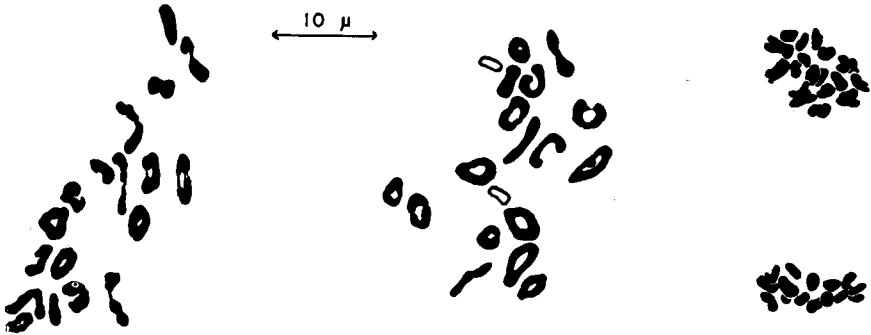


Fig. 4. Dividing pollen mother cells of *Gilia clivorum* from Grizzly Peak showing a metaphase I with 18 bivalents, a metaphase I with 17 bivalents plus 2 univalents, and an anaphase I. (x 1400)

The distribution map presented in figure 5 shows that *Gilia clivorum* occurs through the Coast Ranges from Lake County in the north to Ventura County in the south, and from the dry hills bordering the San Joaquin Valley to the coast line and onto the islands in Santa Barbara Channel. *Gilia achilleaefolia* occurs in the South Coast Range back of the coast line (Grant, 1954), while *G. millefoliata* occupies sand dunes along the coast of northern California and southern Oregon (fig. 5). It seemed logical to include *Gilia nevinii* on the present map, because it is a close relative of *G. millefoliata*, but this island endemic does not enter into the present problem and will not be considered further here.

Ecologically, *Gilia clivorum* possesses a somewhat broader tolerance range than either of the diploid species to which it is related. Whereas *G. millefoliata* is a strict maritime plant and *G. achilleaefolia* is largely restricted to inland hills, *G. clivorum* ranges from the arid interior hills to the very coast and onto the offshore islands. It is equally at home in open sunny places and in the semi-shade of oak woodlands. Its range extends farther north than *Gilia achilleaefolia* and farther south than *G. millefoliata*.

*Gilia clivorum* also spans the gap between *G. millefoliata* and *G. achilleaefolia* in a number of morphological characters. Many strains of *G. clivorum* resemble *G. millefoliata* in having a spreading growth form with more or less horizontal stems (fig. 1); other strains approach *G. achilleaefolia* in being more erect. Some strains of *G. clivorum* resemble *G. millefoliata* in possessing relatively large flowers with prominent purple spots, oval corolla lobes, and a predominantly herbaceous calyx; other strains approach the small-flowered types of *G. achilleaefolia* in having small flowers, or spotless corollas, or pointed corolla lobes, or more membranous calyces. Most populations of *G. clivorum* have blue pollen like *G. achilleaefolia*, but some forms found in Lake County have white pollen like *G. millefoliata*. On morphological, as well as ecological, ground, therefore, there are reasons for suspecting that *Gilia clivorum* is a product of hybridization between *G. millefoliata* and *G. achilleaefolia*.

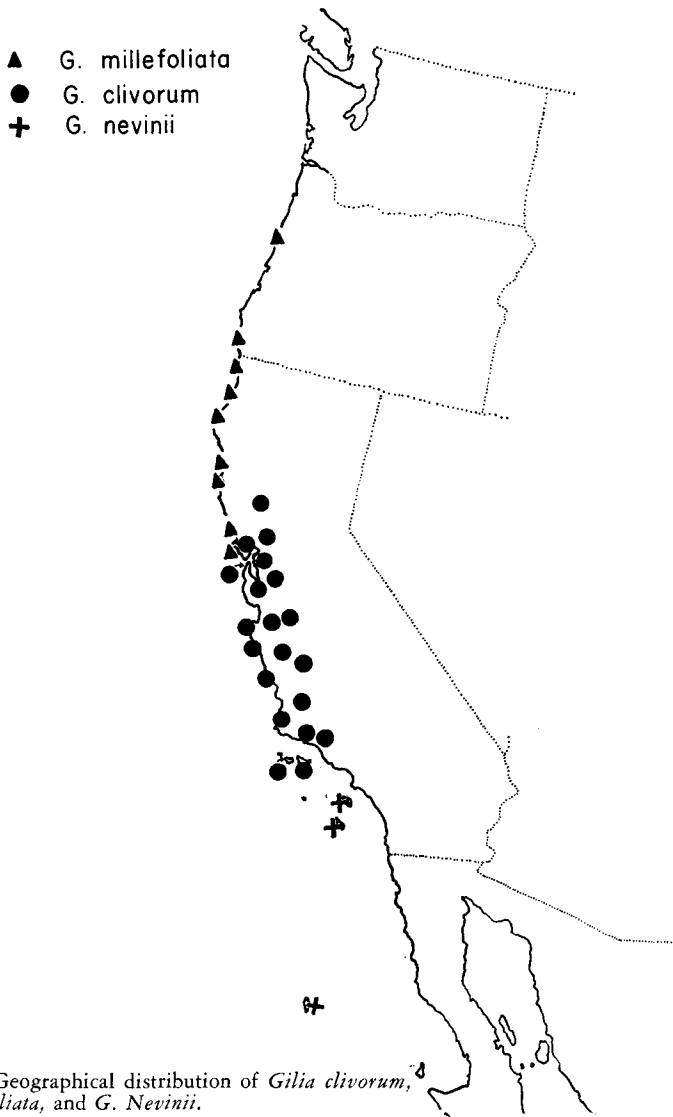


Fig. 5. Geographical distribution of *Gilia clivorum*, *G. millefoliata*, and *G. nevinii*.

So far as is known, *G. millefoliata* and *G. achilleaefolia* do not come into contact with one another today. The two species could easily have met in past centuries on San Francisco Peninsula or at some point on the Marin coast north of San Francisco Bay where wooded areas suitable for the small-flowered types of *G. achilleaefolia* approach the Pacific strand. It is also possible that in a cooler geological age *G. millefoliata* may have ranged many miles farther south along the California coast than it does today and so might have had innumerable opportunities to meet and hybridize with *G. achilleaefolia*.

Efforts were made to confirm the foregoing hypothesis concerning the origin of

*G. clivorum* by means of hybridization experiments. It was soon learned that *G. millefoliata* and *G. achilleaefolia* will cross in the experimental garden if the proper strains are employed. These two species, moreover, possess essentially different genomes between which there is only partial pairing in the  $F_1$  hybrids (Grant, 1952).

Repeated attempts to cross two strains of *G. clivorum* reciprocally with *G. millefoliata* were unsuccessful. In all, 51 flowers were cross-pollinated and 51 capsules with 145 apparently sound seeds were harvested, but these seeds did not germinate and no  $F_1$  individuals were obtained. The cross between *G. clivorum* and *G. achilleaefolia* was almost equally difficult to make. Two strains of *clivorum* were intercrossed with four strains of *achilleaefolia*. 45 flowers were cross-pollinated and set 18 capsules which contained two apparently sound seeds. One of these seeds germinated and developed into an  $F_1$  hybrid.

This hybrid was a product of the cross between the Kings Mt. strain of *G. achilleaefolia* and the Grizzly Peak strain of *G. clivorum*. In growth habit the hybrid was intermediate with a central stem more erect than the *clivorum* parent but not as straight and tall as the *achilleaefolia* parent, and with lateral branches longer than the latter but shorter than the former parent. Its stigma lobes were intermediate between the very long stigmas of *clivorum* and the short ones of the *achilleaefolia* parent. The glandular hairs on the calyx were shorter than those of *clivorum* but longer than those of *achilleaefolia*. The spots in the corolla throat were very faint. In corolla size, the hybrid approached the *clivorum* parent more than *achilleaefolia*.

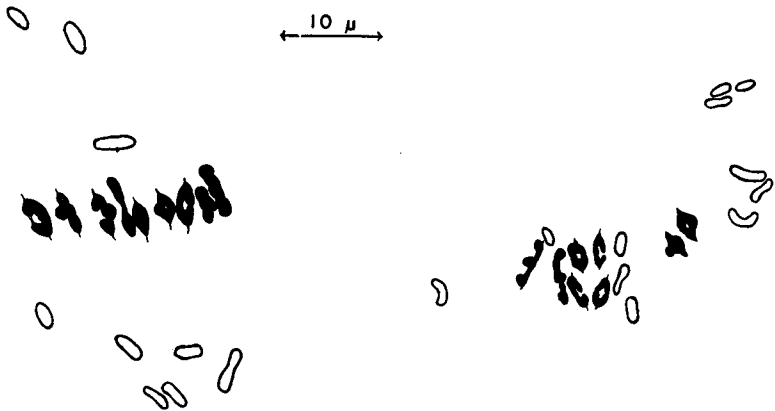


Fig. 6. First metaphase in a PMC of an  $F_1$  hybrid between *G. achilleaefolia* and *G. clivorum*. 9 bivalents plus 9 unpaired chromosomes in the left-hand cell, 8 bivalents plus 11 univalents on the right. (x 1400)

The hybrid was triploid with 27 chromosomes. At meiosis the chromosomes paired according to the *Drosera* scheme with 9 bivalents and 9 univalents. Among 33 cells examined at first metaphase, 32 showed pairing of this type and one cell contained 8 bivalents and 11 univalents (fig. 6). Lagging chromosomes were found in about half of the first anaphase figures, in almost all of the second anaphase cells, and micronuclei occurred in nearly all the sporads. No chromatid bridges were found at anaphase or multivalents at metaphase. The hybrid was completely sterile. The type of meiosis exhibited by this hybrid indicates that one of the genomes of *Gilia clivorum* is homologous with the chromosome set present in *Gilia achilleaefolia*.



## ARTIFICIAL CLIVORUM

The sterile hybrid between *Gilia millefoliata* and the San Luis Obispo strain of *G. achilleaefolia* spontaneously gave rise on two occasions to a few vigorous and fertile allotetraploids, as a result of the union of unreduced gametes (Grant, 1952). According to our hypothesis, this allotetraploid should be a synthetic form of *Gilia clivorum*. It resembled natural occurring *clivorum* in the general form of the plant and cut of the leaves and in several characteristics of the flowers, particularly the distribution of herbaceous and membranous tissue in the calyx, the light purple spots in the corolla throat, and the blue pollen (see fig. 7). It was autogamous like natural *clivorum*. It differed from natural *clivorum* in its broader leaf segments, stiffer and more erect stems and larger flowers. The difference in flower size is to be expected since the *achilleaefolia* parent of the artificial allotetraploid was a particularly large-flowered type, whereas a small-flowered form of this species is believed to have entered into the parentage of natural *clivorum*.



Fig. 7. Artificial *Gilia clivorum* as obtained in  $F_2$  from the cross *G. millefoliata*  $\times$  *G. achilleaefolia*. Plant grown at Stanford. Reproduction is to the same scale as in figs. 1 and 2.

Attempts were made to cross natural *Gilia clivorum* with the allotetraploid *G. millefoliata-achilleaefolia* during two successive hybridization seasons. The female parent was in all cases *G. clivorum* from Grizzly Peak, which, owing to its larger flowers, is more easily emasculated than the other strains of this species. The male parents in 1950 were two highly fertile individuals in the  $F_2$  generation of *G. millefoliata*  $\times$  *achilleaefolia*. A total of 34 flowers were pollinated and nearly as many capsules were set. These capsules contained numerous abortive seeds and 4 apparently sound seeds. The latter did not germinate. In 1951 various fertile and vigorous individuals in  $F_3$  family 1 were used as male parents. Twenty flowers pollinated by several of these individuals led to a full set of capsules containing masses of abortive seeds and a few sound seeds which developed into selfs.

The failure of the cross between natural *clivorum* and the allotetraploid *millefoliata-achilleaefolia* is not surprising in view of the fact that strong incompatibility barriers exist within *achilleaefolia* itself (Grant, 1953). The artificial allotetraploid contained a genome of *achilleaefolia* from San Luis Obispo. Natural *clivorum*, on the other hand, is most probably derived from a hybrid involving a strain of *G.*

*achilleaefolia* occurring in the same geographical areas as *G. millefoliata*. The only contact between the two diploid species in recent times would lie in the area of central California. It is known that strains of *achilleaefolia* from central California do not cross freely with strains from San Luis Obispo. The genetic factors responsible for this incompatibility between different strains of *achilleaefolia* may also prevent crossing between their respective allotetraploid derivatives.

There were 16 tetraploid plants in the  $F_2$  generation of *G. millefoliata*  $\times$  *achilleaefolia* grown in 1950 and five in the culture grown in 1952. These plants were uniform among themselves with respect to some of the interspecific differences. They segregated for some other characters differentiating the parental species. The segregating and non-segregating characters are listed in table 1. The fact that some interspecific differences segregate in  $F_2$  shows that the tetraploids arose by failure of meiosis rather than by somatic doubling. There is cytological evidence that pollen mother cells with a low degree of pairing in  $F_1$  can sometimes produce restitution nuclei (Grant, 1952). Wherever pairing and crossing-over occurred between *millefoliata* and *achilleaefolia* chromosomes prior to the formation of such restitution nuclei in the diploid  $F_1$ s diploid gametes will have arisen which differ among themselves with respect to the genes located on the cross-over segments. Fusion of these diploid gametes will then lead to the partial segregation observed in  $F_2$ .

This segregation was studied further in three  $F_3$  families comprising respectively 176, 114 and 95 individuals. These families were derived from single plant selections in  $F_2$ . Segregation in  $F_3$  can, of course, be caused by pairing and crossing over between *millefoliata* and *achilleaefolia* chromosomes in the tetraploid  $F_2$  as well as in the diploid  $F_1$ . That such interspecific chromosome pairing did occur in the tetraploid  $F_2$  is indicated by the occasional presence of heteromorphic bivalents and multivalents in pollen mother cells (Grant, 1952). This factor cannot have been a major source of variation in the later generation progeny of the hybrids, however, inasmuch as no new segregating characters were discovered subsequent to the  $F_2$  generation.

As shown in table 1, the raw allotetraploid segregates with respect to at least ten different characters. Detailed measurements were made on several of these char-

TABLE 1. SEGREGATION IN ALLOTETRAPLOID *GILIA MILLEFOLIATA*-*ACHILLEAEFOLIA* ( $F_2$  TO  $F_1$ )

Plant part	Segregating characters	Non-segregating characters	
		like <i>achilleaefolia</i>	like <i>millefoliata</i>
Shoot	color of herbage	growth habit	
Leaf	width of segments	dissection	
Pubescence	on calyx	on leaf	on leaf
	on stem	(mixture of <i>acb</i> and <i>mil</i> types of hairs in all individuals)	
Calyx	shape of lobes	accrescence	
Corolla	presence of scale		spots on throat
	size		
Stamens	ratio throat to tube		
	length of filaments	color of pollen	
	pollen fertility		
Pistil	chromosome pairing in pollen mother cells		
	seed fertility		stigma exertion

acters, namely length of corolla, ratio between length of corolla throat and corolla tube (which we can refer to simply as "throat ratio"), width of leaf segments, color of herbage, presence of scale-like appendages on outer part of corolla ("corolla scale"), and fertility.

The 16  $F_2$  plants of the 1950 culture varied in fertility. Eleven plants were fully fertile as to seeds, while five were sterile or semisterile. In one individual pollen fertility was as high as in the parental species, in 13 others it ranged from 85% down to 28%, and two individuals produced no pollen at all. Three of the seed sterile plants produced a fairly large proportion (30 to 40%) of good pollen grains, whereas the two other seed sterile plants were also male sterile. The strong but imperfect correlation between seed and pollen sterility suggests that the hereditary factors governing the two types of sterility are independent but fairly closely associated.

Twelve individuals in the  $F_2$  generation of 1950 had a high degree of chromosome pairing with an average of 17 bivalents per pollen mother cell, the total possible number of bivalents being 18, while two other plants were asynaptic (Grant, 1952). One of the asynaptic individuals was seed fertile, the other was seed sterile; one of them had 75% good pollen, the other 35% good pollen. The  $F_2$  is thus probably segregating for a gene controlling chromosome pairing which is independent of the genes causing pollen and seed sterility.

Two of the  $F_3$  families, namely families nos. 1 and 14, descended from fertile  $F_2$  individuals, while the third family, no. 4, came from a moderately sterile parent with 29% good pollen. The ratio of sterile to fertile individuals in  $F_3$  family 1 was 9 to 165; the corresponding ratio in family 14 was 3 to 90; while family 4 included a much higher proportion of sterile or semisterile plants in the ratio of 11 to 96.

Lethality and various inhibitions of growth were also more frequent in  $F_3$  family 4. The seedling mortality in this family was 6%, as compared with 1% and 2% in families 1 and 14, and among the plants which grew to maturity many had deformed corollas and leaves. Abnormal corollas with 4, 7, 8 or 9 lobes, instead of the usual 5, were fairly common. An  $F_4$  generation derived from a single individual in family 4 had similar characteristics in that 25% of the individuals died as seedlings and another 25% developed into dwarfs.

The data on segregation for corolla throat ratio (table 2) are typical of those obtained for other quantitative characters. The  $F_1$  tends to be more or less intermediate between the parental species. The mean of the  $F_2$  is greater than that of the  $F_1$ , due, perhaps, to the increase in the number of chromosome sets. The three  $F_3$  families frequently differ rather widely among themselves in the mean values for the quantitative character. They also differ conspicuously from their respective parents in  $F_2$ , being skewed in the direction of the *millefoliata* types. This is perhaps a consequence of the fact that the *millefoliata* parent is self-pollinating and highly homozygous, whereas the *achilleaefolia* parent is heterozygous and carries various deleterious recessive genes (Grant, 1954). There may be a selective elimination in the hybrid progeny of the *achilleaefolia*-like segregates which contain such deleterious genes.

Regarded from the standpoint of variance, a population of self-pollinating *millefoliata* is much more uniform than a population of cross-fertilized *achilleaefolia*. The  $F_1$  is more variable than the former but less variable than the latter. The  $F_2$  exhibits a great increase in variation, which fully spans the gaps between the parental types. This variability falls off again in the  $F_3$  and  $F_4$  generations.

The continuous nature of the variation in throat ratio is clearly apparent in the table. Continuous variation was observed also in corolla length, leaf segment width,

TABLE 2. SEGREGATION FOR RATIO OF COROLLA THROAT LENGTH TO TUBE LENGTH IN ALLOTETRAPLOID  
*GILIA MILLEFOLIATA-ACHILLEAEFOLIA*.

(P and F<sub>1</sub> diploid, F<sub>2</sub> to F<sub>4</sub> tetraploid. Letter P indicates character of parent of each F<sub>3</sub> and F<sub>4</sub> family.)

Generation	Length of throat expressed as per cent of length of tube																					Statistics							
	35	40	45	50	55	60	65	70	75	80	85	90	95	100	105	110	115	120	125	130	135	140	145	150	155	No. individ.	Mean	Std. dev.	
P <sub>m11</sub>			1	4	2	2		2																		11	55.91±2.50	8.31	
P <sub>ach</sub>											2	3		5	1	2			2					2	1	2	20	113.25±5.46	24.4
F <sub>1</sub>					2		4	1	2	3	1		1													14	72.14±3.05	11.4	
F <sub>2</sub>					2	3	2	2		1	1	2	2		1		3					1				20	84.00±5.32	23.8	
F <sub>3</sub> family 1			3	2	4	11	15	11	28	18	9	22	15	17	7	3	4	1	3	1						174	82.87±1.30	17.1	
F <sub>3</sub> family 14																										93	57.80±.80	7.7	
F <sub>3</sub> family 4																										107	59.06±1.12	11.6	
F <sub>4</sub>																										20	54.00±1.23	5.5	

calyx lobe shape, and density of stem glands. There was a semi-continuous series of subtle differences in herbage color. The pattern of variation just described can readily be explained as the result of crossing individuals differing in multiple factors.

There is evidence that the tetraploid individuals which arose in the  $F_2$  generation were derived from gametes containing a small number of crossover chromosomes. The average number of chromosome pairs in pollen mother cells of the parental  $F_1$  hybrid was  $2.6 \pm 0.19$  out of a possible number of nine (Grant, 1952). Restitution nuclei, from which diploid gametes could be derived, were probably never formed in cells with more than three or four bivalents. Yet the occurrence of segregation in the tetraploid  $F_2$  generation shows that one or more chromosome pairs were usually present in these same spore mother cells. Most of the gametes involved in the origin of the artificial tetraploids must therefore have developed from meiotic cells with at least one, but not more than four, bivalents.

It is not known whether pairing took place between the same identical chromosomes in each cell. It is, however, quite clear, from the absence of segregation in a considerable number of characters in  $F_2$ ,  $F_3$  and  $F_4$ , that some *millefoliata* and *achilleaefolia* chromosomes never paired in cells ancestral to these hybrid individuals. In our study of segregation, therefore, we are dealing with genes borne on between one and four, or perhaps slightly more than four, but well less than nine, chromosome pairs. Considering the fact that several of the characters are evidently polygenic, we might expect to find that some of the segregating genes are linked.

TABLE 3. CORRELATIONS BETWEEN CHARACTERS IN ALLOTETRAPLOID  
*GILIA MILLEFOLIATA-ACHILLEAEFOLIA*

Character 1	Character 2	$F_3$ family	Size sample	Correlation coefficient	Probability*	Linkage
Throat ratio	Corolla length	1	174	.327	< .01	yes
		14	93	.142	> .05	
Throat ratio	Corolla scale	1	174	.019	> .05	
		14	93	.183	> .05	
Throat ratio	Leaf width	1	174	.185	.01-.02	yes
		14	88	.297	< .01	yes
Throat ratio	Herbage color	1	174	.129	> .05	
		14	93	.243	< .01	
Throat ratio	Seed fertility	1	174	.243	< .01	yes
		14	93	.107	> .05	
Corolla length	Corolla scale	1	174	.060	> .05	
		14	93	.013	> .05	
Corolla length	Leaf width	1	174	.257	< .01	yes
		14	93	.169	> .05	
Corolla length	Seed fertility	1	174	.285	< .01	yes
		14	93	.379	< .01	yes
Corolla scale	Leaf width	1	174	.039	> .05	
		14	93	.248	.01-.05	
Corolla scale	Herbage color	1	166	.210	< .01	yes
Corolla scale	Seed fertility	1	168	.218	< .01	yes
		14	93	.110	> .05	
Leaf width	Herbage color	1	174	.079	> .05	
Leaf width	Seed fertility	1	174	.151	ca. .05	yes?
		14	93	.131	> .05	
Herbage color	Seed fertility	1	174	.219	< .01	yes

\*i.e., probability that a given correlation coefficient is the result of chance alone.

Linkage would be difficult to detect in the small F<sub>2</sub> generation, but might become apparent in the larger F<sub>3</sub> progenies. The characters of corolla length, throat ratio, width of leaf segments, color of herbage, presence of corolla scale, and seed fertility were accordingly paired in all possible combinations and studied for correlation. The correlation studies were carried out in F<sub>3</sub> families 1 and 14; family 4 was omitted from the study because the presence of sterility and lethality phenomena in this family would alter the genetic segregations. Correlation between herbage color and other characters could not be studied in family 14, inasmuch as this family did not segregate for herbage color.

The characters chosen are known to be only slightly affected by ordinary environmental variables. Furthermore, the F<sub>3</sub> progeny were all grown concurrently in the same experimental plot. Developmental correlations are not anticipated between such dissimilar characters as corolla throat ratio, leaf segment width, herbage color, and seed fertility. Any statistically significant correlations found between such characters may therefore be attributed with a fair degree of safety to genetic linkage.

The results of the computations are given in table 3. This table shows that positive correlation exists between the parental characteristics in at least one of the F<sub>3</sub> families for 10 of the 14 character pairs. The values of the statistically significant correlation coefficients, ranging from .38 down to .15, are in all cases small. The low magnitude of these values may be due to the circumstance that linkage between the systems of polygenes responsible for different characters is only partial. Random non-genetic variations in the phenotypes may be another contributing factor. It seems fair to conclude, in any case, that there is some weak linkage between most of the six characters studied. Furthermore, a seventh character, density of glands on the stem, is perceptibly associated with color of herbage.

TAXONOMY

The following key provides a means of distinguishing most populations of *Gilia clivorum* from *G. millefoliata* or the small-flowered types of *G. achilleaefolia*. Taxonomic descriptions are given here for *G. clivorum* and *G. millefoliata*, and in the preceding paper of the series (Grant, 1954) for *G. achilleaefolia*.

- Corolla throat usually with purple spots; herbaceous portion of calyx lobes 1 mm. wide; withered corolla persistent for a time on the ripening capsule; capsule containing 24-50 seeds.
  - Stems densely glandular; fruiting calyx 8-11 mm. long; pollen white; spots in corolla throat very dark and conspicuous..... *G. millefoliata*
  - Stems floccose below, never densely glandular; fruiting calyx 5-6 mm. long; pollen blue (rarely white); spots in corolla throat moderately dark to faint or absent..... *G. clivorum*
- Corolla throat never with purple spots; herbaceous portion of calyx lobes 0.8 mm. wide or less; corolla deciduous shortly after anthesis; capsule containing 10-18 seeds..... *G. achilleaefolia*

*GILIA clivorum* (Jepson) comb. nov.

- Gilia multicaulis* var. *clivorum* Jepson, Fl. Calif. 3 (2): 187. 1943 (type seen.)
- Gilia millefoliata*, of authors.
- Gilia multicaulis*, of authors.

Annual herbs; plants low, 6-30 cm. high, stems abundantly branched from base and erect, or divaricately branching and spreading, or simple in depauperate individuals, sparsely to densely floccose below and glandular above; leaves uni- or bipinnate, 1-6 cm. long, the ultimate segments frequently elliptical, or sometimes filiform, 0.5-2.0 mm. wide, the upper cauline leaves similar to the lower; flowers

grouped in 2-5-flowered glomerules, each borne on a peduncle 0.5-3 cm. above uppermost leaf, the peduncle elongating as capsules mature, pedicels 1-10 mm. long, or the flowers sometimes solitary, and the pedicels then 1-4 cm. long; calyx floccose or glandular, 4-5 mm. long in flower, accrescent, 5-6 mm. long in fruit, the lobes acute with a central band 1 mm. wide, without hyaline wings, but joined below by a very narrow hyaline sinus about 0.1 mm. wide, this frequently purple, or sometimes colorless; corolla funnellform, 6-8 mm. long, frequently less than twice as long as the calyx, the limb 3-5 mm. wide when pressed flat, corolla persistent for a time upon the ripening capsule, tricolored with a clear yellow tube, blue-violet limb and 5 pairs of purple spots in the throat, or the spots sometimes faint, the lobes oval, rotately spreading, 1-2 mm. wide; stamens inserted in the sinuses of the corolla, included, the filaments 0.2-0.4 mm. long, the pollen blue-violet, rarely white; style included, stigmas at the level of the anthers, 1.0-1.5 mm. long; capsule ovoid, dehiscent, 24-36-seeded; seeds ovoid, angular, 1.0-1.5 mm. long, brown;  $n=18$ .

*Type*.—Paso Robles, San Luis Obispo County, California. J. H. Barber (1899). Jepson Herbarium, Univ. of Calif., Berkeley.

*Range*.—Open fields and grassy hillslopes, often near the coast, Central and South Coast Ranges from Lake and Solano counties to w. Ventura Co., California; 30-3500 ft. Occurs with *Gilia tricolor*, *G. achilleaefolia*, *G. angelensis*, *G. gilioides*. April-May, or sometimes in March.

*Variation*.—Considerable variation frequently exists within populations of this entity. Thus on Grizzly Peak near Berkeley, Mt. Diablo, Contra Costa County, and undoubtedly elsewhere, individuals possessing deep purple corolla spots coexist with individuals having faint spots or none at all. A population in the Panoche Pass region of San Benito County, again, is composed of one biotype having finely dissected leaves and heavily floccose stems, and another possessing larger leaves with long entire segments and lightly floccose stems. Visible differences between populations also exist. Two populations less than one mile apart in the Berkeley hills, for example, differ in the relative size of the corolla and habit of branching. In general the morphological differences to be found within the whole area of the species are not of a greater order than those existing within the confines of one population. This pattern of variation is undoubtedly due to the self-pollinating method of reproduction of the species.

*Specimens cited*: CALIFORNIA. Santa Barbara Co.: Santa Barbara, A. D. E. Elmer 3913; Lompoc, A. Eastwood 18922, P. A. Munz 10249. San Luis Obispo Co.: Cholame, I. L. Wiggins 5778; San Luis Mt., R. W. Summers (1886); Spanish Ranch, P. L. Johannsen 1017; Atascadero, A. Eastwood 14388. Monterey Co.: Big Sur, M. Stason (1926); Jolon, H. L. Mason 11763; King City, A. Eastwood 4066; Pajaro, H. L. Mason 7040. San Benito Co.: Pinnacles, J. T. Howell 4609; Panoche Pass, V. & A. Grant 8435. Santa Clara Co.: Pacheco Pass, V. & A. Grant 8427. San Mateo Co.: Crystal Springs Lake, C. F. Baker 467; San Bruno, C. F. Baker 1899. San Francisco Co.: San Francisco, H. L. Mason 4375, E. Zeile (1924), A. A. Heller 6594. Alameda Co.: Arroyo Mocho, J. T. Howell 829; Sunol, H. L. Mason 1308; Tesla, H. L. Mason (1935), A. Eastwood 1945; Oakland, L. Constance 503, H. L. Mason 4358; Berkeley, H. L. Mason 902, V. & A. Grant 8535, 8536. Contra Costa Co.: Mt. Diablo, H. L. Mason 5128, M. L. Bowerman 604, 2840, 2857, 2858, 2920; 2932. Solano Co.: Putah Canyon, H. Cohen (1940). Marin Co.: Mill Valley, A. Eastwood (1909); Fairfax, V. & A. Grant 8417; Mt. Tamalpais, A. Eastwood 1898, J. T. Howell 21601; Sausalito, J. T. Howell 23146. Lake Co.: e. side Clear Lake, M. Baker 3977, 12158, 12180.

## GILIA MILLEFOLIATA Fisch. &amp; Mey.

*Gilia millefoliata* Fisch. & Mey., Ind. Sem. Hort. Petrop. 5: 35. 1838. (transcribed in *Erythea* 2: 164. 1894.)

*Gilia millefoliata* var. *maritima* Brand. Pflzr. IV (250): 100. 1907.

*Gilia multicaulis* var. *millefoliata* Jeps., Man. Fl. Pl. Calif. 796. 1925.

*Gilia multicaulis* subsp. *millefoliata*, Mason & Alva Grant, Madroño 9: 209. 1948.

Annual herbs with strongly scented herbage; plants low, 2-20 cm. high, branching from base, the central leader often reduced and the lateral stems then spreading and decumbent, a rosette of leaves developed at the base of the plant; stems and leaf rachis very glandular and somewhat succulent; lower cauline leaves pinnate, 2-4 cm. long, the rachis straight, the lateral pinnae short, 2-5 mm. long, the upper cauline leaves reduced, often apparently palmate; flowers grouped in 2-6-flowered glomerules, each borne on a peduncle 2-5 mm. above uppermost leaf but peduncle elongating as capsules mature, the pedicels 2-5 mm. long; calyx 4-6 mm. long in flower, very accrescent, 8-11 mm. long in fruit, glandular, the lobes acute, with a central green band 1 mm. wide flanked by very narrow hyaline wings joining below into a hyaline sinus about 0.2 mm. wide, this purple or colorless; corolla funnel-form, 8-11 mm. long, about twice as long as the calyx, the limb 4-5 mm. wide when pressed flat, corolla persistent for a time upon the ripening capsule, limb blue-violet, shading off into white near the throat, with 5 pairs of purple spots in the throat, and lemon yellow tube, the lobes oval, acute, 1.0-1.5 mm. wide, rotately spreading; stamens inserted in the sinuses of the corolla, included, the filaments 0.4-0.6 mm. long, the pollen very scanty, white; style included, stigmas at the level of the anthers, 1.0-1.5 mm. long; capsule ovoid, dehiscent, 25-50-seeded; seeds ovoid, angular, 1.0-1.5 mm. long, brown;  $n=9$ .

*Type*.—Plant grown in St. Petersburg Imperial Botanic Garden from seeds gathered by an unknown collector near the Russian colony at Bodega Bay, Sonoma County, California.

*Range*.—Along stabilized sand dunes, Pacific strand from Marin County, California, to Lincoln County, Oregon; 10-300 ft. Sympatric with *Gilia capitata* ssp. *chamissonis* and *G.c.pacifica*. Flowers April-May.

*Variation*.—Generally quite uniform. There is a form of *G. capitata pacifica* on Cape Mendocino, with spreading habit, densely glandular stems, loose flowering heads and accrescent calyces, which strongly suggests introgression from *G. millefoliata* (J. P. Tracy 15962, C. Wolf 5519).

*Specimens cited*: CALIFORNIA. Marin Co.: Pt. Reyes, V. & A. Grant 7909, 8419; Tomales Pt., B. O. Scriber 2177; Abbott's Lagoon, J. T. Howell 21997. Sonoma Co.: Bodega Pt., A. Eastwood (1901). Mendocino Co.: Cleone, A. Eastwood 2631; Fort Bragg, H. L. Mason 12775; Pudding Creek, H. L. Mason 12776, 12784. Humboldt Co.: Humboldt Bay, H. P. Chandler 1135, J. P. Tracy 2189, 2190; Samoa, J. P. Tracy 179, 14833; Little River Beach, J. P. Tracy 4794. Del Norte Co.: Crescent City, L. Constance 2620.

OREGON. Curry Co.: Pistol River, Leach 2482, M. E. Peck 23944. Lincoln Co.: Seal Rocks, M. E. Peck 10506.

## CONCLUSIONS

*Gilia clivorum* ( $n=18$ ) is a tetraploid species of Leafy-stemmed *Gilia* occurring in the Coast Ranges of California and along the coastline south of San Francisco Bay. The species is both variable and self-pollinating, with the result that its colonies consist of assemblages of homozygous and true-breeding biotypes. Some of the variants of *G. clivorum* have been referred in the manuals to *G. multicaulis* (now a synonym of *G. achilleaeifolia*), and others to *G. millefoliata*.



*Gilia clivorum* is an allotetraploid derived from hybridization between two diploid species, *G. millefoliata* ( $n=9$ ) and *G. achilleaefolia* ( $n=9$ ), which possess different genomes. (1) Morphologically and ecologically, *G. clivorum* spans the gap between the two diploid species. (2) The triploid hybrid between *G. clivorum* and *G. achilleaefolia* has *Drosera* type chromosome pairing with 9 bivalents plus 9 unpaired chromosomes. (3) The artificial allotetraploid *G. millefoliata-achilleaefolia* ( $n=18$ ) resembles *G. clivorum* in breeding behavior and in several morphological characters.

The artificial allotetraploid segregates for some of the morphological differences between *G. millefoliata* and *G. achilleaefolia*. The intermediacy of the  $F_1$  hybrids and the continuous variation in their allotetraploid progeny show that the phenotypic differences between the species are determined by allelic differences at multiple loci. Linkage might be expected between the multiple factors controlling the different segregating characters, inasmuch as these factors must be borne by a small number of chromosomes which paired in the  $F_1$  hybrid. There is evidence of weak linkage between corolla throat ratio, corolla length, corolla scale, leaf segment width, herbage color, density of stem glands, and seed fertility.

*Gilia millefoliata* and *G. achilleaefolia* are reproductively isolated by incompatibility, chromosomal sterility, genic sterility, and general disharmonies of the genotypes of the two species which lead to the formation of some inviable types in the hybrid derivatives. These barriers can be circumvented, the first by combining the right strains, the second by polyploidy, and the third and fourth by segregation in the raw allotetraploid followed by selection for the viable and fertile recombinations. The original plants of *Gilia clivorum* must have passed through some such series of bottlenecks as this before the successful establishment of the new species in nature.

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