SELECTION FOR VIGOR AND FERTILITY IN THE PROGENY OF A HIGHLY STERILE SPECIES HYBRID IN GILIA[1]

VERNE GRANT

Rancho Santa Ana Botanic Garden, Claremont, California

Received December 22, 1965

IT is known that species hybrids in plants which are highly sterile and sub-vital in the early generations may nevertheless give rise to fully fertile and viable progeny in later generations. The recovery of fertility and vigor is best documented for the general case of amphiploidy. The appearance of fertile and vigorous hybrid derivatives without change in ploidy has also been observed in a number of hybridization experiments. There are, however, no detailed studies of the generation by generation rise in fertility and viability in the progeny of highly sterile hybrids which have not undergone chromosome doubling. The purpose of the present paper is to describe such a case history in a species cross in Gilia which has been studied through the F_9 generation.

Materials: The parental species, *Gilia malior* and *G. modocensis* are desert annuals belonging to the *Gilia inconspicua* complex, Polemoniaceae (see GRANT 1964b, for review). They occur in sandy places in the Mojave Desert and bordering areas in western North America. The plants bloom in early spring, producing small inconspicuous flowers which are predominantly, but not entirely, self-pollinating. The inbred products are fully vigorous generation after generation. Both species are allotetraploids with the same chromosome number ($2n = 36$) but different genomes. In both species the chromosomes pair regularly in bivalents, and the fertility of pollen and seeds is normal.

The plants were grown from seeds collected in wild populations. The parental strain of *Gilia modocensis* came from Cajon Pass on the western edge of the Mojave Desert in southern California; that of *G. malior* from Cuyama Valley in the arid inner South Coast Range of south-central California.

These strains were crossed in 1951 in the direction *G. malior* female × *G. modocensis* male. The cross was easy to make and 53 F_1 individuals were grown in 1952. The F_1 hybrids were vigorous but meiotically irregular and highly sterile (GRANT 1964a).

Most of the 36 chromosomes in the hybrids appeared as univalents at metaphase in the pollen mother cells. The number of bivalents ranged from 1 to 10 and averaged 6.0, in a sample of 118 cells, where 18 bivalents would represent complete pairing. Chains of three or four chromosomes were found occasionally. Chromosome movement to the poles was irregular, with much lagging and unequal distributions at anaphase (GRANT 1964a).

There is considerable evidence that the reduction in chromosome pairing in this and other species hybrids in Gilia is due largely to structural differences between the parental genomes. (1) Chains and bridges are found in these hybrids (GRANT 1954b, 1964a; GRANT and GRANT 1960; DAY 1965). (2) Karyotype analysis reveals differences in chromosome morphology between certain species (DAY 1965). (3) Six different diploid hybrids with low pairing and low fertility have given rise spontaneously to tetraploid progeny with good bivalent pairing and medium or high fertility (GRANT 1954a, 1965; GRANT and GRANT 1960; DAY 1965). (4) Selection for pollen

---

[1] The work was supported in part by research grants from the National Science Foundation.

Genetics 53: 757–775 April 1966.

fertility in the early generations of one of these newly arisen allotetraploids has been effective in improving both bivalent formation and fertility (DAY 1965).

There is more specific evidence pointing to chromosome structural differentiation between *Gilia malior* and *G. modocensis*. DAY (1965) has proven that *G. malior* contains the genomes of the diploid species, *G. minor* and *G. aliquanta*. *Gilia modocensis*, on the other hand, contains the genomes of two other diploid species, *G. latiflora* (GRANT 1964a) and probably *G. brecciarum*. All four genomes are structurally differentiated; three of them are strongly differentiated and belong to different genome groups (GRANT and GRANT 1960).

The pollen fertility in F_1 was low. The percentage of well formed and well stained pollen grains ranged from 0.5 to 5% in different individuals and averaged 2%. The seed fertility of the F_1 hybrids was only 0.007% of normal seed set in the parental species. Fifty-three F_1 plants labored for ten weeks and brought forth a total of 522 F_2 seeds, 26 F_2 seedlings, and 24 more or less vigorous F_2 plants (GRANT 1964a). These F_2 plants were the starting point of the selection experiment described here.

Methods: Eleven of the most vigorous and fertile F_2 plants were chosen as parents of numerous lines grown in succeeding years. These lines were propagated from single-plant selections. A single plant in the F_2 generation produced by self-pollination of the seeds for an F_3 family; one of these F_3 individuals was then selected as the parent of an F_4 family; and so on. Meanwhile other parallel lines were propagated from sister individuals in the same families.

All the plants descended from a single parental plant are referred to as a family, while a lineal succession of families is called a line and designated by the culture number of its final family. All the lines derived from a common parental individual in F_2 are referred to as a branch.

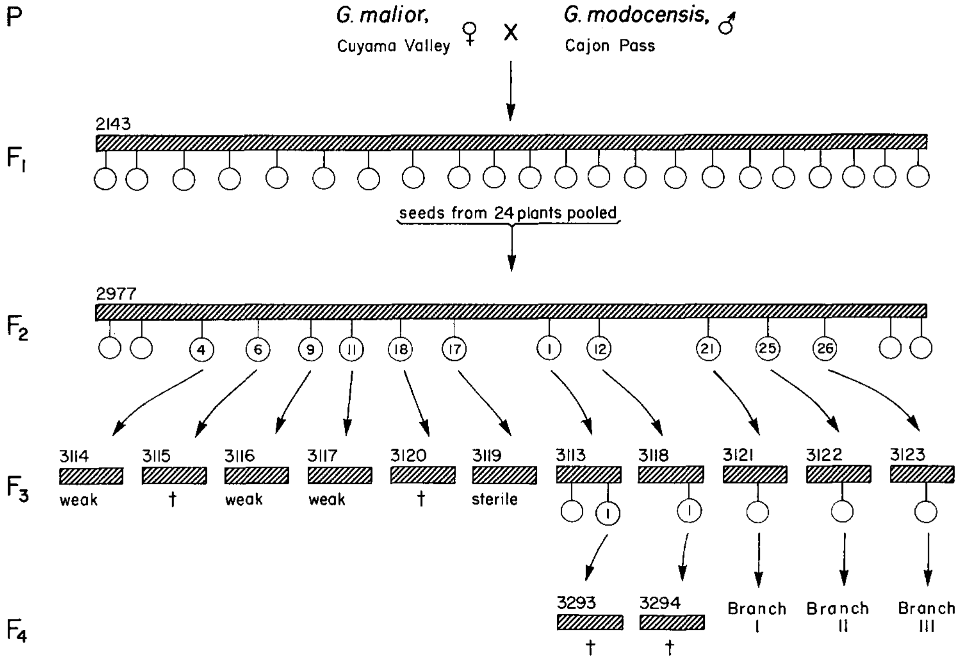


FIGURE 1.—Pedigree of the early generations derived from the cross of *Gilia malior* × *G. modocensis*. Each family is represented by a horizontal cross-hatched bar with the family number listed above and the individual plant numbers below. Only the individual plants which were selected as parents of later generations are indicated by number. See Appendix I for the complete pedigrees of Branches I, II, and III.

Twenty-eight lines were grown during the course of the experiment. Only those lines belonging to three branches got beyond the F_4. The pedigree of the early generations is shown in Figure 1; that of the later generations is presented for reference in Appendix I.

The plants were grown in a special greenhouse with glass roof and screen sides at the Rancho Santa Ana Botanic Garden in Claremont, California, from 1956 (F_2) to 1963 (F_8 or F_9), when the experiment reported here was terminated. (Some of the lines have been continued to date for other purposes, as will be described in future papers.) The exclusion of insects from the screenhouse and the autogamous reproduction ensured the uniparental descent of the families. The propagation and general environment of the plants were kept as uniform as possible.

The choice of parents for succeeding generations was determined by both natural and artificial selection. I scored the plants in each family for vigor and fertility, and bred from the one or several most vigorous and fertile individuals. This artificial selection was, however, superimposed on natural selection for the same traits, insofar as the inviable seedlings and lethal gametophytes eliminated themselves from the contest in each generation.

Pollen fertility was estimated by mounting the pollen in lactophenol and staining with aniline blue. A mount was made from all the pollen grains produced by a single flower, and all grains were counted and scored as either abortive or well formed. A single plant was often reexamined as to pollen at different times in the blooming season. The percentage of well formed and well stained grains is higher than the proportion of germinable grains by several percentage points, as other studies have shown (i.e., EAST 1921; MÜNTZING 1930), but provides a good and convenient measure of the pollen fertility.

Meiosis was examined in propiono-carmine squashes of PMCs. For critical observations a phase contrast microscope was used.

A special problem arose owing to the small size and short life span of these reduced annual plants. To obtain satisfactory meiotic preparations it is necessary to strip off most or all the flower buds from a plant. If a certain plant is to be used as a seed parent it is necessary to leave its flower buds intact. A kind of indeterminancy principle is thus involved in that a plant can usually be used for breeding or cytology but not for both. A solution to this problem which proved satisfactory in later generations, when the families had become essentially isogenic, was to obtain the cytological data and the seed stocks from sister individuals.

RESULTS

Vigor: The parental species and the F_1 hybrids were vigorous. But the internally balanced genotypes determining this vigor were broken up by hybridization, and segregation for vigor was apparent within the larger families and between families from F_3 to F_6. Weak, stunted, or semilethal individuals were found in the different families. These types appeared as rare variants in some families but were common or predominant in others (Appendix II). The frequency of inviable seedlings was 42% in all families combined from F_3 to F_6.

Much additional inviability in the seed stage is inferred from failure of germination. One obtains about 25% germination from seeds of the P, F_1, and F_7 to F_9 generations with the methods employed. Under the same conditions, the seed germination in F_2 was reduced to 5% and that in F_3–F_6 to 14%.

The known number of inviable seedlings and the estimated number of inviable embryos combined amount to about 75% of all germinable seeds in the F_2 to F_6 generations. The vigorous plants were an estimated 18% of the germinable seeds in F_2, 24% in F_3, 25% in F_4, 22% in F_5, and 37% in F_6.

The effects of five generations of selection for vigor are seen in a marked decline in the frequency of inviable segregates from F_7 onwards. In the F_7, 80%

of the seedlings developed into vigorous plants, in F_8 97%, and in F_9 100%. Seed germination was also normal in these generations suggesting that the pregermination inviability had been largely eliminated.

In many of the families vigorous segregates either did not appear, or did not breed true, so that selection was powerless to establish normal vigor. The dashed selection curves in Figure 2 show how three lineages ran downhill to extinction from F_2 to F_6. In all, 18 of the 28 lines in the experiment eventually became extinct owing to poor viability in spite of efforts to keep them alive by selecting for vigor.

Constitutional weakness was evident from F_3 to F_8 in the whole Branch III. Three of the four lines belonging to this branch died out from F_5 to F_7. The fourth, Line 3803, was weak and difficult to maintain. The individuals were small and weak and produced few flowers. This line was outcrossed to a vigorous line in Branch II. The plants in the first outcross generation were vigorous, but this condition broke down again in the next (O_2) generation.

In other lines (belonging to Branches I and II) selection was successful in raising the relative frequency and absolute numbers of vigorous individuals to a level comparable to that found in the parental species. The selection curves in Figure 2 for Lines 3800 and 3854 show such upward trends in vigor. It may be

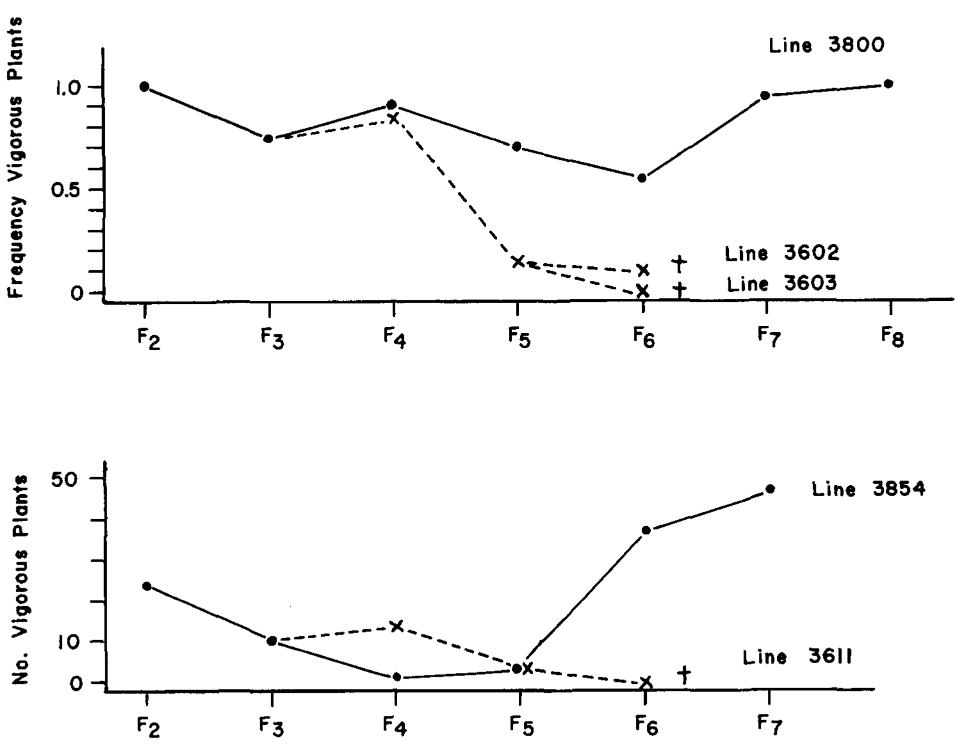


FIGURE 2.—Changes during successive generations in the relative frequency or in the absolute numbers of vigorous plants in several lines.

noted that while Line 3800 was vigorous, the parallel Line 3605 derived from a sister individual in F_5 was not and became extinct.

Pollen fertility: The percentage of well formed and well stained pollen in the parental species is usually above 90%. In the F_1 generation 11 individuals fell in the range of 0.5 to 5.0% good pollen and averaged 2%. In F_2 three individuals had 10 to 13% good pollen. There was considerable segregation for pollen fertility in the following generations from F_3 to F_6 or F_7. In F_3, for example, 20 individuals were spread out fairly evenly from 5 to 67% good pollen.

The percentage of the *vigorous* individuals in each generation that had 80% or more good pollen was as follows:

F_1	0%	F_4	5%	F_7	35%
F_2	0%	F_5	4%	F_8	88%
F_3	0%	F_6	32%	F_9	100%

In several independent lines subjected to selection for pollen fertility there was a rapid rise in the level of this trait up to the F_6 or F_7. In F_6 some segregates appeared with normal pollen fertility in the range 90 to 97%. Their descendant

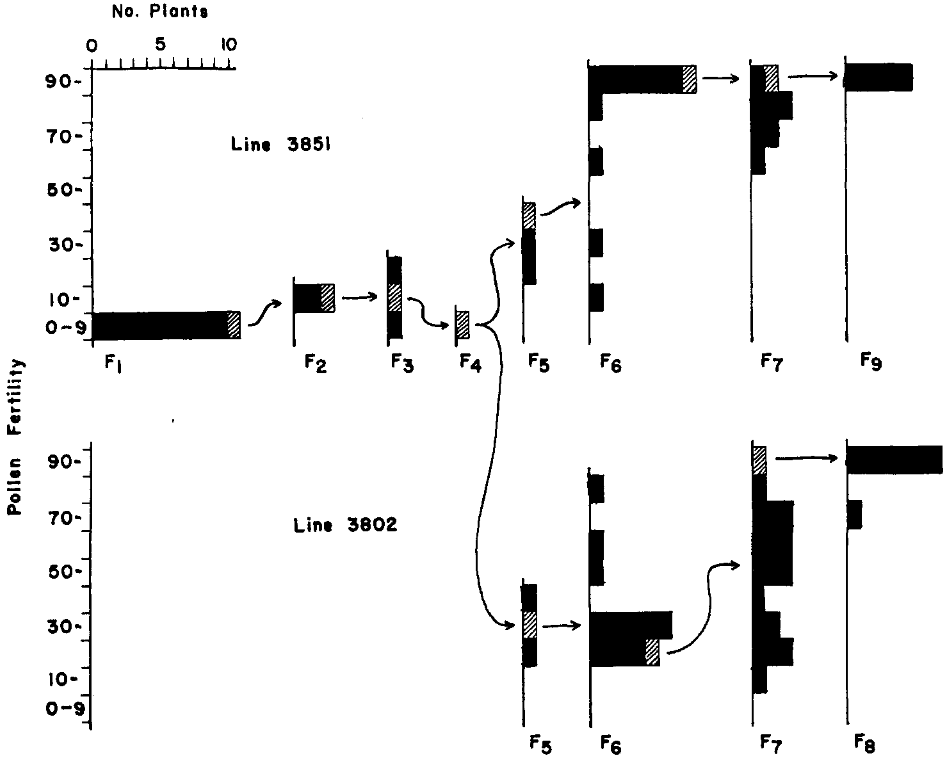


FIGURE 3.—Frequency distribution of individuals with different pollen fertilities in successive generations of two lines belonging to Branch II. Individuals selected as parents of later generations are indicated as cross-hatched areas in the bar graphs.

families in the final generations were then uniform for high pollen fertility. Some typical results are diagrammed in Figure 3.

That the observed rise in pollen fertility is due to selection for this character is confirmed by a comparison of two lines, 3851 and 3802, descended from the same F_4 parent, but from sister individuals with different pollen fertilities in F_5 (Figure 3). Line 3851 was selected for high pollen fertility and responded accordingly. Line 3802, on the other hand, was selected in the reverse direction for semisterility of pollen in F_5 and F_6, and responded by producing F_6 and F_7 families with variable fertility in the semisterile range. When the direction of selection was reversed again in this line in F_7, high pollen fertility was established in the daughter families belonging to the final generations (see Figure 3).

Changes in chromosome number: It will be recalled that meiosis in pollen

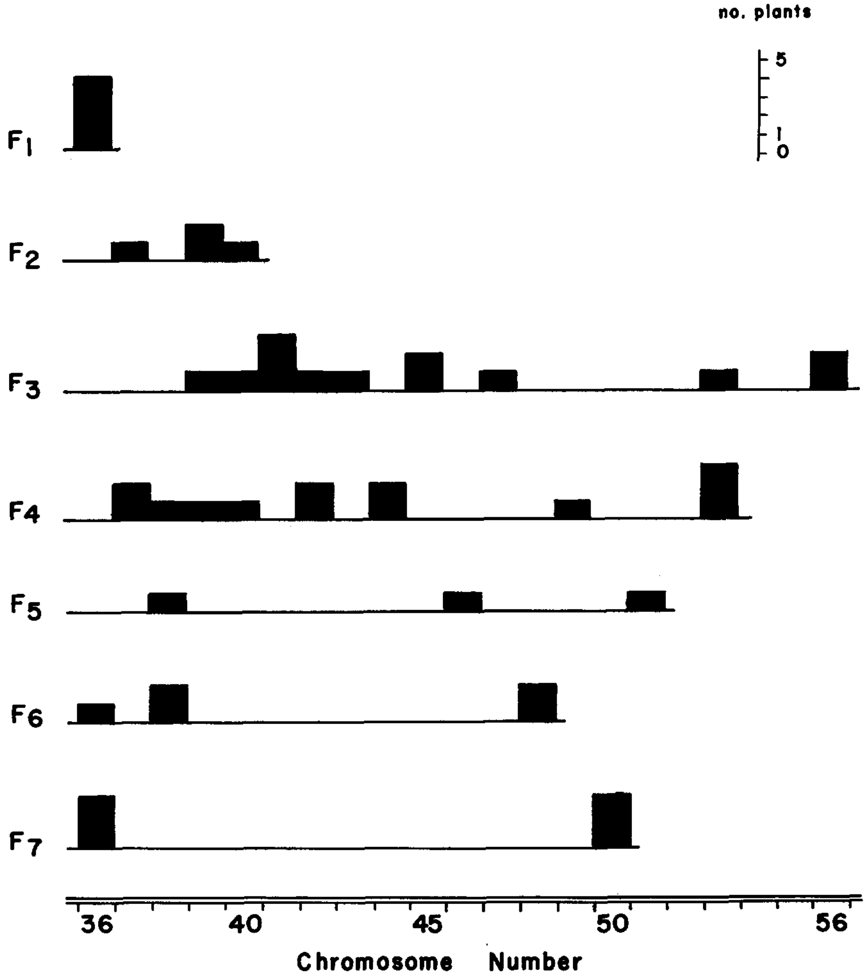


FIGURE 4.—Frequency distribution of plants with different chromosome numbers in successive generations derived from the cross of *G. malior* ($2n = 36$) × *G. modocensis* ($2n = 36$).

mother cells in the F_1 hybrid was characterized by low chromosome pairing, lagging, and irregular chromosome distribution to the poles. Most of the products of this meiosis would be expected to carry deficiencies, and, consequently, to develop into inviable pollen grains, which they did.

The most likely way of getting a meiotic product with a full complement of

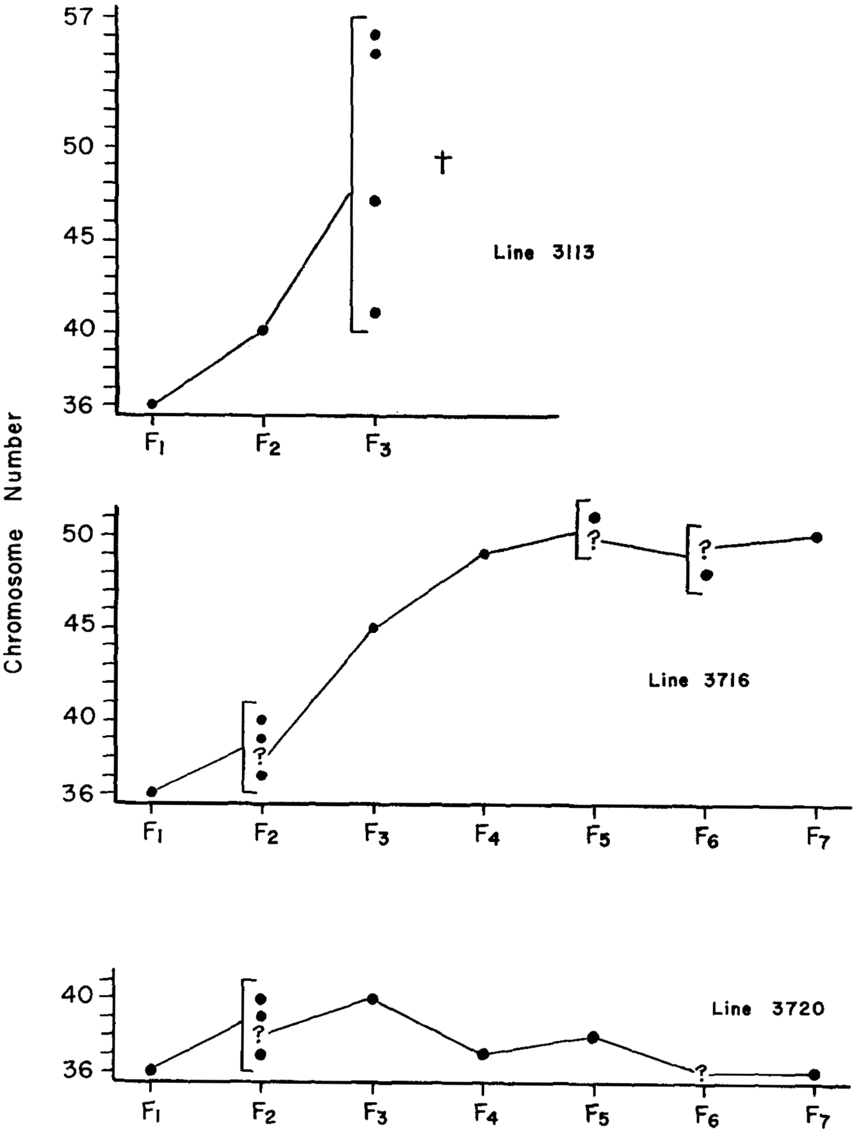


FIGURE 5.—Changes in chromosome number during successive generations in three lines. In several cases the chromosome number of a parental individual is not known, but that of sister individuals belonging to the same family is; in such cases the latter are plotted as dots and the former as question marks.

genes, apart from doubling, is by the accumulation of extra chromosomes. The male gametophytes are much more tolerant of duplications than of deficiencies. Furthermore, the tetraploid plants used in this study can tolerate duplications in their gametophytes more readily than could diploid plants. We would expect therefore that most of the functional (but undoubled) gametes produced by the hybrid *Gilia malior* × *modocensis* would carry extra chromosomes and give aneuploid progeny. This was the case.

The chromosome numbers found in a sample of the hybrid progeny are summarized in Figure 4. This graph shows that the F_1, like the parental species, had 36 chromosomes, but gave rise to F_2 individuals with $2n = 37$, 39, and 40. A larger number of individuals were determined for chromosome number in the F_3 and F_4 generations, and were found to form an aneuploid series from $2n = 37$ to 56.

In later generations, with continued selection for improved vegetative vigor and pollen fertility, the intermediate aneuploids disappeared. Some lines reverted to $2n = 36$ or 38, while others became stabilized at $2n = 50$.

These differences were characteristic of whole branches. Three lines belonging to Branch II all levelled off at $2n = 36$. Branch III came to have $2n = 38$. Three lines in Branch I all reached a new balanced condition of $2n = 50$.

The trends in chromosome number from generation to generation within particular lines can be seen in Figure 5. Line 3113 is an example of one in which the chromosome number increased but failed to reach a new genic balance, for it did not get beyond the F_3 generation. Line 3720 (in Branch II), after an initial rise in chromosome number, returned to the parental condition of $2n = 36$ by F_7. Line 3716 (in Branch I), by contrast, gradually stepped up to $2n = 50$.

CLAUSEN (1926, 1931) and WINGE (1940) have observed a similar tendency in interspecific hybrids in Viola and Erophila. There also the chromosome number rose above the F_1 level in the F_2 and later generations.

Meiotic behavior: The pollen sterility of the hybrid and its derivatives is determined by abnormal meiotic behavior, and this in turn appears to be due to factors of two sorts. Some aberrant features, such as low chromosome pairing and chain formation, evidently stem from structural differentiation between the parental chromosomes, which is known to occur here (see MATERIALS and METHODS). Other disturbances like stickiness, clumping and asynapsis are probably genically determined. Selection for pollen fertility is therefore in effect selection for the structural and genic constitutions which permit normal meiosis. The expected improvements of meiotic behavior in response to selection for pollen fertility did occur.

Let us first examine the increase in bivalent formation in successive generations. The modal numbers of bivalents per PMC in different F_1 individuals were 5 and 7. The modal numbers of bivalents for different individuals rose to 7-13 in F_2. In the F_3 generation in Branches II and III the mode rose again to 13-16 bivalents, and in F_4 to 17-21 bivalents. Only minor lapses of bivalent formation were seen in F_5. From F_6 onwards there was complete or nearly complete bivalent pairing. (Copies of reference tables giving the details as to meiotic behavior

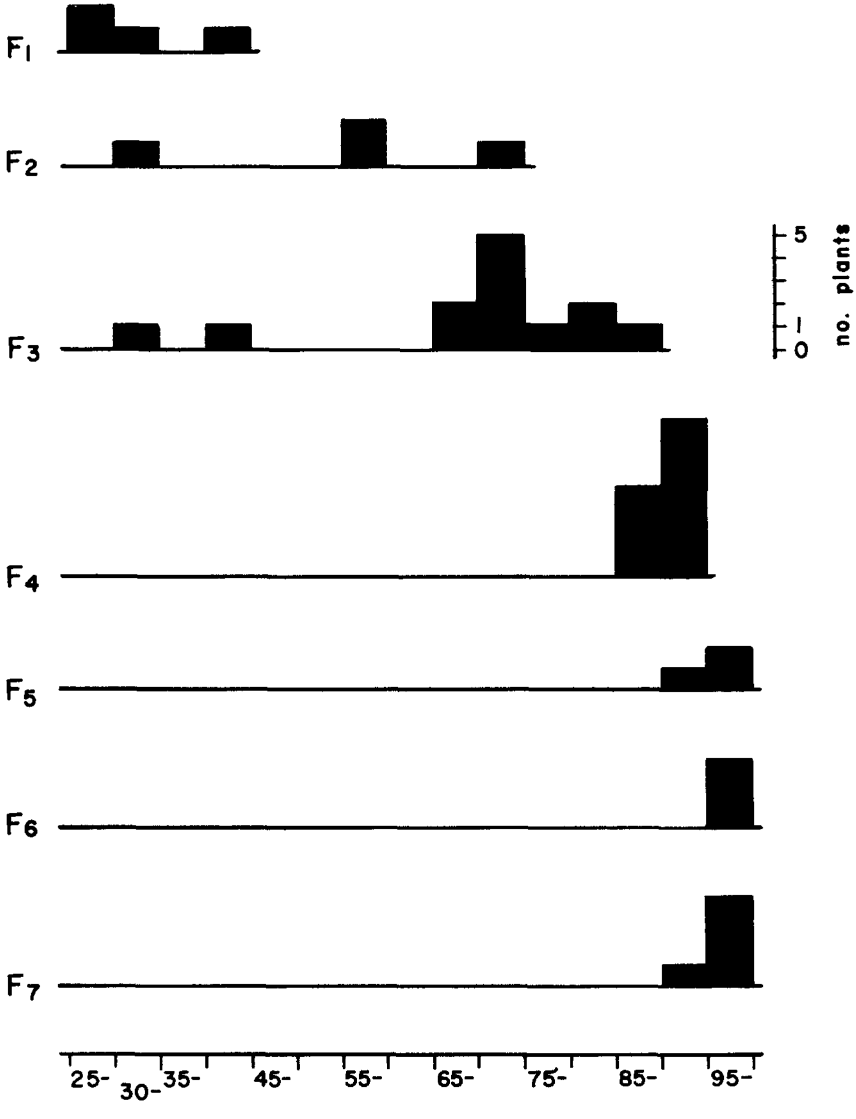


FIGURE 6.—Frequency distribution of individuals with different average percentages of their chromosomes paired in bivalents or chains in successive generations.

and fertility of individual plants have been placed on file in the editorial office of GENETICS.)

It is desirable to compare on a common denominator the amount of chromosome pairing in individuals and lines differing in chromosome number and in frequency of chains. The average percentage of the total number of chromosomes that are paired in a plant, either in bivalents or higher associations, makes such direct comparisons possible. These percentages are shown in Figure 6.

The bar graph shows that low pairing and high individual variation in the amount of pairing were characteristic of the F_2 and F_3 generations. The low variable pairing disappeared by F_4. And from F_5 onwards the chromosome pairing was complete or nearly so in most individuals.

The close correlation between chromosome pairing and pollen fertility is revealed by the next graph (Figure 7). There are, to be sure, some exceptional individuals with high pairing but low pollen fertility, but the correlation coefficient is high ($r = +0.694$) and significant ($P < .01$).

Sticky chromosomes occurred in several F_2 and F_3 individuals but were not of general occurrence. Clumping was a feature peculiar to a few F_3 and F_4 individuals. Certain F_3 plants were asynaptic or semi-asynaptic. These aberrations, which were absent in the P and F_1 generations, present in some members of the early segregating generations, but absent again in sister plants grown under the same conditions are attributed to disharmonious interspecific gene recombinations.

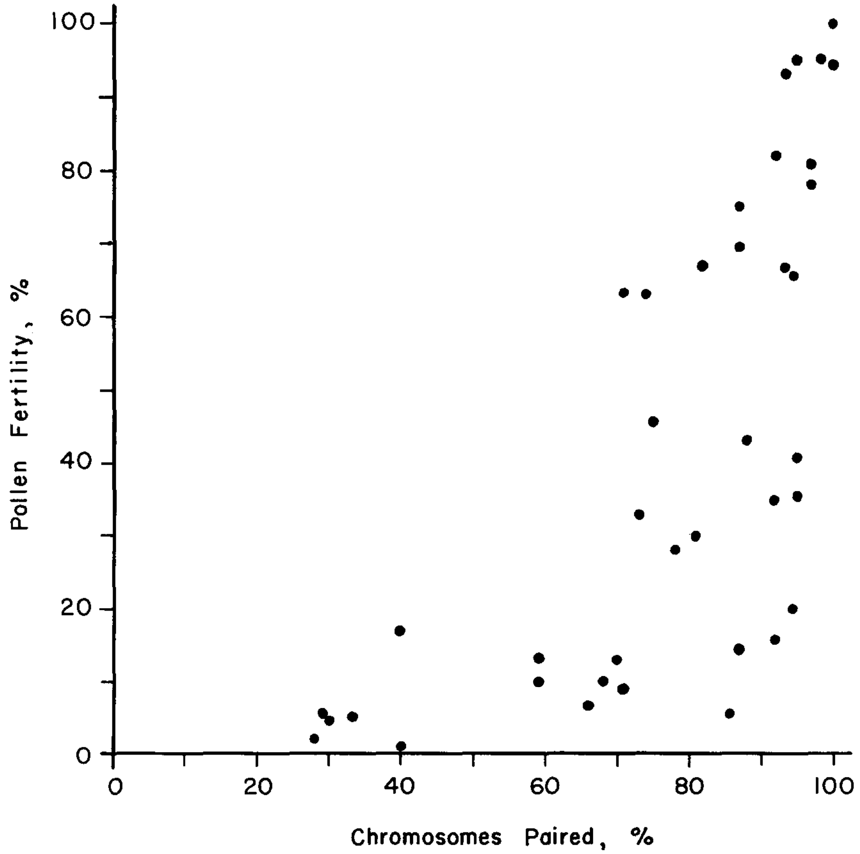


Figure 7.—Correlation between chromosome pairing in PMCs and pollen fertility in 39 individuals belonging to seven generations.

Sticky chromosomes, clumping, and asynapsis were always associated with a pollen fertility lower than that found in sister plants lacking these conditions. It is not surprising, therefore, that these disturbances of meiosis became rare by F_4 and were not seen thereafter.

Fixation of structural homozygosity by inbreeding and selection for pollen fertility: Failure of bivalent pairing is the most important single cause of pollen sterility in the hybrids and hybrid-progeny of *Gilia malior* × *modocensis.* The failure of bivalent formation is due, in turn, largely if not primarily to structural differences between the chromosomes of the parental species and hence to structural heterozygosity in the early hybrid generations.

Selection for pollen fertility, then, is expected to lead to the establishment of structural homozygosity in the fertile lines. There is some cytogenetic evidence to indicate that the lines did become structurally homozygous as they became bivalent-forming and fertile.

Although parental individuals with incomplete bivalent pairing and some good pollen have repeatedly produced progeny showing full or high bivalent formation, in no case has a plant with complete bivalent pairing given rise, by self-pollination, to progeny segregating for this trait. Furthermore, segregation for morphological characters, which was rampant in the early generations, was either absent or very subdued in the progeny of plants with full bivalent pairing. The one-way direction of these trends is consistent with the view that structural homozygosity, once attained in the inbred lines, becomes fixed and is retained thereafter.

Closely related individuals with good bivalent formation are expected to be structurally homozygous for the same combination of segmental arrangements. The bivalent-forming plants belonging to distantly related lines, on the other hand, are likely to possess different structurally homozygous complements. To test these assumptions two crosses were made with fertile F_6 or F_7 plants.

Two sister individuals belonging to the same family (3612) in F_6 of Branch II, both of them completely fertile as to pollen and hence undoubtedly normal in meiosis, produced an F_1 hybrid with complete pairing and full pollen fertility (92%). Among ten PMCs nine had 18 bivalents and the tenth had 17 bivalents plus 2 univalents. Chromosome separation at anaphase was also regular.

A fertile and meiotically normal individual in the F_7 generation of Branch II was outcrossed to a fertile and presumably meiotically normal plant in F_7 of Branch III. Their F_1 hybrid had greatly reduced pairing with a mode of 11 bivalents and range of 10 to 14 bivalents. A chain of three chromosomes was present in some cells. The pollen fertility of this hybrid was 9%.

The fertility relationships of the extracted lines will be described in more detail in a future paper. The results given here are, however, in accord with the interpretation that one structurally homozygous genome was fixed by inbreeding in the members of one line, and another different one in a separate inbred line.

Seed fertility: Seed fertility is a complex trait determined by several different conditions. Gametic fertility, already discussed, is one of these conditions, but there are others. Some plants with high pollen fertility during a brief period of anthesis were male sterile and produced only abortive anthers during most of the flowering season. Lack of vegetative vigor is another condition contributing to poor seed production.

An interesting cause of seed sterility in the early segregating generations was failure of autogamous self-pollination in certain recombination-type flowers. The stamen length and style length are mutually adjusted in each parental species so as to ensure automatic deposition of the pollen on the stigma. But the parental species differ in the length of these organs. In some of their recombination products, the stamens overshoot the stigma, while in others the style is elevated above the anthers.

Such plants were seed sterile in the breeding cage even though they produced good pollen and ovules. For example, plant 3481–49 (in F_5 of Branch I) had 74% good pollen and quite regular meiosis, but produced almost no seeds. Gametically fertile recombination types of this sort would be seed sterile in nature also, since the flowers are small, inconspicuous, and unattractive to insects.

Instances of imperfect correlation between pollen fertility and seed fertility have been noted in the progeny of interspecific hybrids in other plant groups, i.e., Galeopsis (MÜNTZING 1930), Rubus (VAARAMA 1954), and Elymus-Sitanion (STEBBINS and VAARAMA 1954).

The F_1 hybrids in the present study were all highly or completely seed sterile. The F_2 plants varied from sterile to semisterile. In the F_3, F_4 and F_5 there was segregation in seed production from completely sterile through semisterile to more or less fertile plants. (Data on file in the editorial office of GENETICS.) Twenty out of 107 individuals in F_3, F_4 and F_5 were more or less fertile as to pollen but sterile as to seeds.

By F_6 most vigorous plants produced abundant sound seeds, and from F_7 on all vigorous plants were seed fertile. Selection was evidently successful in weeding out the various causes of poor seed production.

The combination of vigor and fertility: Fertility, as we have seen, is a genetically and developmentally complex trait, and so is vigor. Although the genotypes of the parental species bring about normal vigor and fertility, these balanced genotypes were broken up by hybridization, and numerous inviable and/or sterile types appeared. Only a minority of the hybrid progeny in the early generations of the experiment were either vigorous or fertile, and fewer still were both.

In F_2 an estimated 18% of the germinable seeds developed into vigorous individuals, and none of these also had high pollen fertility (in the 80 to 100% range). In subsequent generations the corresponding proportions of vigorous individuals and of both vigorous and pollen fertile individuals were as follows:

F_3 24% vigorous, 0% vigorous-fertile
F_4 25% vigorous, 5% vigorous-fertile
F_5 22% vigorous, 4% vigorous-fertile

From F_1 to F_5, therefore, only 1% or less of the zygotes were both vigorous and pollen fertile.

TABLE 1

The proportion of individuals that were both vigorous and fertile or semifertile as to seeds in successive generations

Generation	Estimated percent of germinable seeds that developed into vigorous individuals	Percent of vigorous individuals that were fertile	Estimated percent of germinable seeds that were both vigorous and fertile
F_1	100	0	0
F_2	18	0	0
F_3	24	45	11
F_4	25	83	21
F_5	22	66	15
F_6	37	71	26
F_7	80	96	77
F_8	97	100	97
F_9	100	100	100

The estimates of the frequency of the biologically significant combination of vigor and seed fertility in various generations are given in Table 1. This table shows a low proportion of vigorous, seed fertile individuals in the early generations. It was this low proportion of individuals which selection for the combinatin of vigor and fertility had to work with.

The lines were propagated from single plant selections in the vigorous, fertile fraction of each generation. The performance of the elite individuals chosen as parents, in terms of seed output and number of vigorous fertile progeny, is summarized in Table 2. It will be seen that most parental plants in the F_1 to F_5 produced few seeds each (3 to 200) and even fewer vigorous fertile progeny (0 to 20).

Line 3851 in Branch II is an example of one which managed to live through

TABLE 2

Reproductive potential of individuals chosen as parents in different generations

Parental generation	Seed output of parent plants	Number of vigorous and fertile progeny
F_1	3, 4, 10, 10, 15, 15	0, 0, 0, 0, 0, 0
F_2	5, 7, 8, 9, 11, 26, 49, 50, 51, 82	0, 0, 1, 1, 1, 2, 2, 3, 3, 4
F_3	no data	1, 2, 3, 4, 6, 6, 6, 29
F_4	7, 15, 30, 36, 63, 75, 96, 378, 400, 400	0, 0, 0, 0, 1, 2, 3, 5, 14, 28
F_5	20, 34, 34, 42, 50, 98, 109, 130, 151, 181, 234, 245, 369, 415	0, 0, 0, 0, 0, 0, 0, few, few, few, 15, 16, 19, ca 60
F_6	45, 55, 55, 60, 65, 138, 139, 145, 177	0, 0, 2, few, ca 20, ca 24, 30, ca 30, many
F_7	4, 191, 250, 250	0, 2, 8, ca 92
F_8	140, 891	ca 33, ca 311

The number of seeds and number of vigorous fertile progeny per parent plant are listed in magnitude array.

the series of bottlenecks in the early generations and emerge successful in later generations. The four successive parental individuals in this line from F_1 to F_4 never produced more than 49 seeds each or more than four vigorous fertile progeny; and some parents produced as few as seven seeds and one vigorous fertile offspring. The seed output of the parental plant in the next generation (F_5) was moderately high (181 seeds), but the number of vigorous fertile progeny (in F_6) was fairly low (15 individuals). From F_7 on, however, the line was normal in fertility and vigor. (The F_8 family in this line was small, but this was due to poor conditions of seed germination that year.)

In Line 3611, also in Branch II, we have an example of a related line with a fecundity comparable to that of Line 3851 in the early generations, which however died out in F_6. There were four vigorous fertile plants in the F_3 family in Line 3611; six vigorous fertile plants in the F_4 family; and in the F_5 family there was one vigorous semisterile plant which produced 20 seeds. The 20 seeds yielded one vigorous F_6 individual which was sterile. This sterile plant was the end of the line.

The success or failure of lines like the foregoing which are represented by very small numbers of vigorous fertile individuals during several successive generations evidently depends partly on chance. Drift, as well as selection, is a factor determining the fate of such lines.

Of the 28 lines handled during the course of this experiment, 18 became extinct between F_3 and F_6. Two other lines were kept alive and became fertile but remained vegetatively weak up to the end of the experiment. Constitutional defects of one sort or another were characteristic of most of these 20 unsuccessful lines. This conclusion is supported, first, by the appearance of similar inviability or sterility phenomena in successive generations in the same line, and second, by the failure in certain instances of all related lines branching off from a common parental family. Selection for vigor and fertility was obviously powerless in lines which had become constitutionally and characteristically weak or infertile.

Eight of the original 28 lines emerged vigorous and fertile in the F_7 and later generations. These eight lines all belong to Branches I and II and hence descended from two of the 11 F_2 individuals which were progeny tested. It would appear in retrospect that they had possibilities of succeeding from the start. However, the success of selection for vigor and fertility in these eight lines was by no means a sure thing. They had to run the gauntlet of several successive generations in which only a few vigorous fertile individuals appeared. Related lines in the same branches with apparently similar possibilities of succeeding failed to run the same gauntlet. Under these circumstances the establishment of vigor and fertility in the eight successful lines should be attributed, not to selection alone, but to the combination of selection and drift.

OTHER EXAMPLES

Previous workers have recovered fertile, true-breeding progeny from interspecific hybrids without change in ploidy in various plant groups. The cases

TABLE 3

Plant species hybrids which have produced fertile derivatives without doubling

Cross	Fertility of F_1		Reference
	Pollen	Seed	
Nicotiana rustica × *paniculata*	3–10%	3–4%	East 1921
Nicotiana langsdorffii × *sanderae*	average 55%	semifertile	Smith and Daly 1959
Polemonium mexicanum × *pauciflorum*	semifertile	semifertile	Ostenfeld 1929
Galeopsis tetrahit × *bifida*	average 59%	33–36%	Müntzing 1930
Galeopsis pubescens × *speciosa*	average 9%	fairly sterile	Müntzing 1930
Tragopogon pratensis × *porrifolius*	....	8%	Winge 1938
Erophila confertifolia × *violacea-petiolata*	low	1%	Winge 1940
Phaseolus vulgaris × *multiflorus*	10–30%	moderately sterile	Lamprecht 1941, 1944
Godetia amoena × *whitneyi* (= *Clarkia rubicunda* × *amoena*)	6%	3%	Hiorth 1942; Håkansson 1947
Elymus glaucus × *Sitanion jubatum*	<1%	0.01%	Stebbins and Vaarama 1954; Stebbins 1957

known to me are listed in Table 3 (see also Stebbins 1950, pp. 286–287; Lewis and John 1963, p. 334).

The previous studies have mostly dealt with sterility barriers which are relatively weak, or at least not extremely strong, and have mostly recorded only the general results. Only for *Galeopsis tetrahit* × *bifida* does one find fairly complete data, and this hybrid was semifertile. Only the Elymus × Sitanion hybrid was extremely sterile, but the full details regarding its return to fertility are not available.

A detailed study of the recovery of fertility and vigor in the undoubled progeny of a highly sterile hybrid has apparently not been reported heretofore. Nor is there a previous example, to my knowledge, in which the rise in fertility and vigor can be connected with artificial selection for these traits per se. The hybridization experiment described in this paper was undertaken, in the first place, to provide such a detailed and controlled case history.

A second objective of this study, and an objective of some of the others, was to determine the morphological and genetic relationships of the new stable fertile lines. This aspect of the problem will be dealt with in future papers.

I am indebted to Dr. Kenneth Lewis, Oxford, for reviewing the manuscript and making a number of very helpful suggestions.

SUMMARY

The cross between two autogamous annual species of Gilia (Polemoniaceae), namely *G. malior* and *G. modocensis* (both $2n = 36$), yields a highly sterile F_1 hybrid with low chromosome pairing. The undoubled later-generation progeny are preponderantly, but not exclusively, inviable or sterile.—Artificial selection for vigor and fertility was carried out in 28 inbred lines derived from the F_2

generation. The artificial selection for vigor and fertility within each line was superimposed on the inevitable natural selection for the same traits.—Response to selection was rapid in the five generations from F_2 to F_6. Further slight improvements occurred in F_7. By F_8 and F_9 full vigor, normal chromosome pairing, and full fertility had been recovered in the few successful lines.—The selective elimination accompanying these changes was very great during the critical early generations as the various inviability and sterility factors sifted out together. About 75% of the seeds expected to germinate in the F_2 to F_6 either failed to germinate or gave inviable or sub-vital seedlings. Of the remaining fraction of vigorous plants, only a small proportion were also fertile or semifertile as to seeds. In the F_1 to F_6 generations inclusive, an estimated 2% of the zygotes were both vigorous and fertile.—A precariously low number of vigorous fertile segregates appeared in each of the early generations in any single line. For example, two related lines had, in the four generations from F_2 to F_5, only one to six plants per family which were both vigorous and fertile or semifertile. In F_6 one of these lines had no vigorous fertile segregate and became extinct; whereas the other line had 15 vigorous fertile plants in F_6, and went on to attain normal fecundity in F_7.—It is concluded that the eight successful lines which got through the bottleneck of the early generations and emerged with full vigor and fertility owe the fixation of their beneficial characteristics to the joint action of selection and drift.

LITERATURE CITED

Clausen, J., 1926 Genetical and cytological investigations on *Viola tricolor* L. and *V. arvensis* Murr. Hereditas **8:** 1–156. —— 1931 Cyto-genetic and taxonomic investigations on Melanium violets. Hereditas **15:** 219–308.

Day, A., 1965 The evolution of a pair of sibling allotetraploid species of Cobwebby Gilias (Polemoniaceae). Aliso **6:** 25–75.

East, E. M., 1921 A study of partial sterility in certain hybrids. Genetics **6:** 311–365.

Grant, V., 1954a Genetic and taxonomic studies in Gilia. V. *Gilia clivorum*. Aliso **3:** 19–34. —— 1954b Genetic and taxonomic studies in Gilia. VI. Interspecific relationships in the Leafy-stemmed Gilias. Aliso **3:** 35–49. —— 1964a Genetic and taxonomic studies in Gilia. XII. Fertility relationships of the polyploid Cobwebby Gilias. Aliso **5:** 479–507. —— 1964b The biological composition of a taxonomic species in Gilia. Advan. Genet. **12:** 281–328. —— 1965 Species hybrids and spontaneous amphiploids in the *Gilia laciniata* group. Heredity **20:** 537–550.

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

Håkansson, A., 1947 Contributions to a cytological analysis of the species differences of *Godetia amoena* and *G. whitneyi*. Hereditas **33:** 235–260.

Hiorth, G., 1942 Zur Genetik und Systematik der *amoena*-Gruppe der Gattung Godetia. Z. Ind. Abst. Vererb. **80:** 289–349.

Lamprecht, H., 1941 Die Artgrenze zwischen *Phaseolus vulgaris* L. und *multiflorus* Lam. Hereditas **27:** 51–175. —— 1944 Die genisch-plasmatische Grundlage der Artbarriere. Agri Hortique Genetica **3/4:** 75–142.

Lewis, K., and B. John, 1963 *Chromosome Marker*. Little, Brown, Boston.

Müntzing, A., 1930 Outlines to a genetic monograph of Galeopsis. Hereditas **13**: 185–341.

Ostenfeld, C. H., 1929 Genetic studies in Polemonium. II. Experiments with crosses of *P. mexicanum* Cerv. and *P. pauciflorum* Wats. Hereditas **12**: 33–40.

Smith, H. H., and K. Daly, 1959 Discrete populations derived by interspecific hybridization and selection in Nicotiana. Evolution **13**: 476–487.

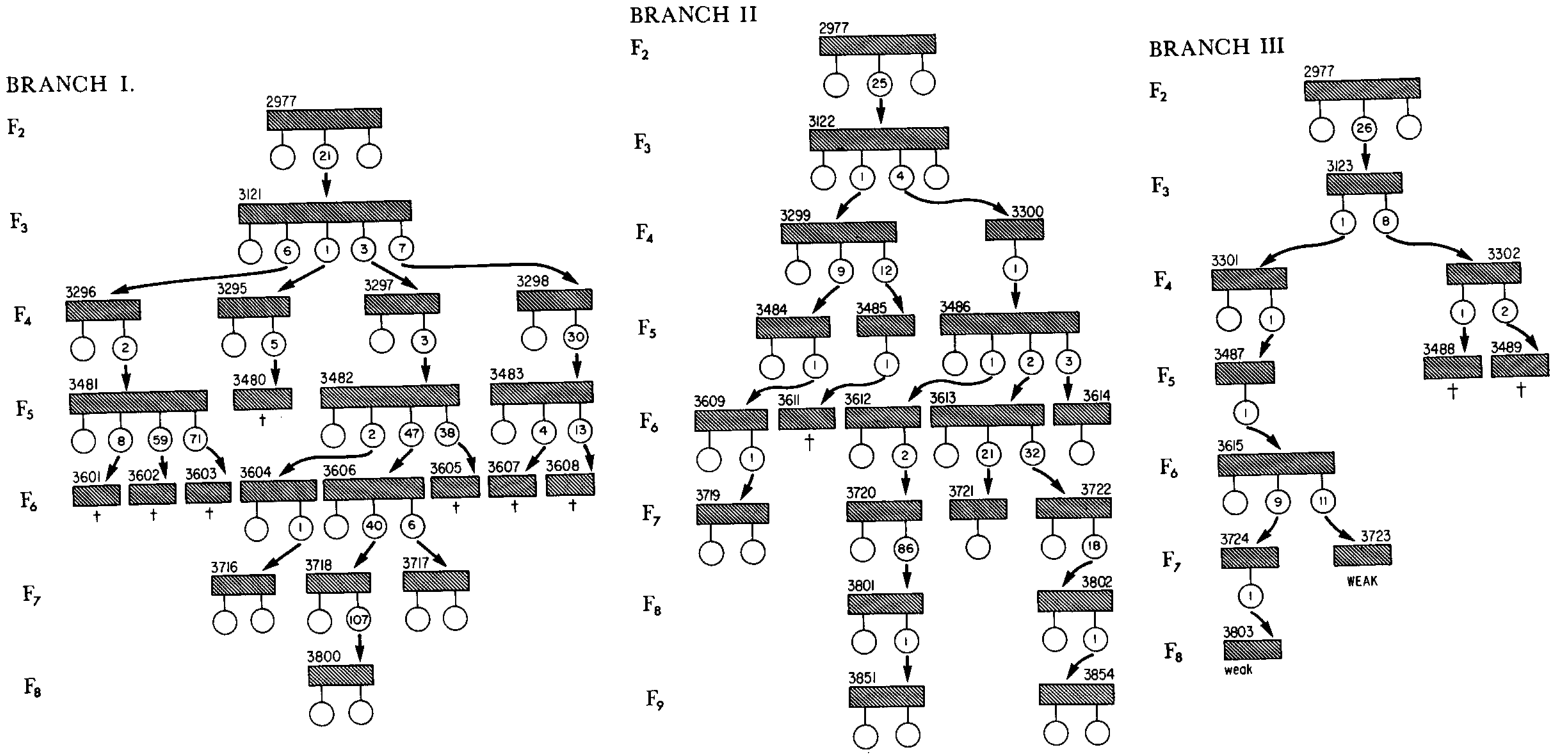
Stebbins, G. L., 1950 *Variation and Evolution in Plants.* Columbia University Press, New York. —— 1957 The hybrid origin of microspecies in the *Elymus glaucus* complex. Proc. Intern. Genet. Symp. (Cytologia, Suppl. Vol.) 336–340.

Stebbins, G. L., and A. Vaarama, 1954 Artificial and natural hybrids in the Gramineae, tribe Hordeae. VIII. Hybrids and allopolyploids between *Elymus glaucus* and *Sitanion spp.* Genetics **39**: 378–395.

Vaarama, A., 1954 Inheritance of morphological characters and fertility in the progeny of the hybrid *Rubus idaeus* × *R. arcticus.* Proc. 9th Intern. Congr. Genet. (Caryologia Suppl. Vol.) 846–850.

Winge, Ö., 1938 Inheritance of species characters in Tragopogon. A cytogenetic investigation. Compt. Rend. Trav. Lab. Carlsberg, Ser. Physiol. **22**: 155–194. —— 1940 Taxonomic and evolutionary studies in Erophila based on cytogenetic investigations. Compt. Rend. Trav. Lab. Carlsberg, Ser. Physiol. **23**: 41–74.

See following pages for Appendix I and Appendix II



Appendix I.—*Pedigrees of the later generation derivatives of* Gilia malior × modocensis. The three branches (I, II, and III) are derived from different F_2 individuals as shown. Each family is represented by a horizontal cross-hatched bar with the family number listed above and the individual plant numbers below. Only the individual plants which were selected as parents are indicated by number.

APPENDIX II

Size and vigor of families in progeny of Gilia malior × modocensis

Generation and family		No. seeds sown	No. seeds germinated	No. vigorous seedlings	No. plants both vigorous and seed fertile
Early generations					
F_1	2143	203	51	51	few
F_2	2977	522	26	24	6
F_3	3113	51	16	12	3
	3114	8	3	1	1
	3115	5	1	0	0
	3116	11	6	3	2
	3117	82	16	1	1
	3118	7	1	1	1
	3119	50	12	8	3
	3120	9	2	0	0
	3121	500	12	9	5
	3122	49	20	10	4
	3123	26	8	2	2
F_4	3293	few	0	0	0
	3294	few	0	0	0
Branch I					
F_3	3121	see above			
F_4	3295	some	18	11	6
	3296	some	9	8	4
	3297	much	10	9	6
	3298	much	36	31	29
F_5	3480	30	1	0	0
	3481	378	117	18	5
	3482	400	57	40	28
	3483	400	21	15	14
F_6	3601	98	3	0	0
	3602	34	10	1	0
	3603	34	4	0	0
	3604	234	20	16	16
	3605	42	3	0	0
	3606	415	109	60	ca. 60
	3607	109	22	0	0
	3608	50	5	0	0
F_7	3716	145	35	30	ca. 30
	3717	55	28	24	ca. 24
	3718	138	21	20	ca. 20
F_8	3800	250	92	92	ca. 92
Branch II					
F_3	3122	see above			
F_4	3299	much	15	13	6
	3300	some	1	1	1
F_5	3484	96	3	2	2
	3485	63	5	3	0
	3486	7	5	3	3
F_6	3609	151	31	31	few
	3611	20	1	1	0

APPENDIX II—Continued

Size and vigor of families in progeny of Gilia malior × modocensis

Generation and family		No. seeds sown	No. seeds germinated	No. vigorous seedlings	No. plants both vigorous and seed fertile
	3612	181	20	15	15
	3613	369	38	37	few
	3614	245	21	19	19
F_7	3719	65	12	12	few
	3720	177	30	30	30
	3721	45	2	2	2
	3722	139	49	47	many
F_8	3801	191	2	2	2
	3802	250	8	8	8
F_9	3851	891	311	ca. 311	ca. 311
	3854	140	33	ca. 33	ca. 33
Branch III					
F_3	3123	see above			
F_4	3301	some	4	3	3
	3302	few	3	3	2
F_5	3487	75	11	1	1
	3488	15	1	0	0
	3489	36	0	0	0
F_6	3615	130	13	13	few
F_7	3723	55	6	0	0
	3724	60	24	0	0
F_8	3803	4	3	0	0