THE ORIGIN OF A NEW SPECIES OF GILIA IN A HYBRIDIZATION EXPERIMENT

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Received June 20, 1966

The formation of a new species isolated by a sterility barrier is perhaps the evolutionary change of largest magnitude that can be retraced experimentally. In plants this process has been extensively studied in the special case of allopolyploidy. Allopolyploidy is in fact the only mode of speciation, in either plants or animals, which has been thoroughly studied as a process in the laboratory or breeding plot. It is very desirable to investigate by similar methods the formation of new species without change in ploidy.

The purpose of the present paper is to describe such a case of speciation under experimental conditions in the plant genus Gilia. This case involves a derivative of the sterile hybrid *Gilia malior* × *G. modocensis*. It will be shown that this hybrid derivative, designated Branch III, has recovered full fertility without change in ploidy, has attained a new combination of morphological characters, and is isolated by a strong sterility barrier from the parental species and from the other hybrid derivatives.

MATERIALS AND METHODS

The materials for this experiment are the autogamous annual tetraploid (2n = 36) plants, *Gilia modocensis* and *G. malior*, and their hybrid derivatives. These plants have been grown over a period of 16 years at the Rancho Santa Ana Botanic Garden, Claremont, California. The experiment to be described here was begun in 1956 and concluded in 1966.

The artificial hybrid of *Gilia malior* ♀ × *modocensis* ♂ had been produced and analyzed earlier. Three significant background facts about this cross were known by 1956 (but published later). (1) The F₁ hybrid is highly sterile with an average of 2% well formed pollen grains and a seed fertility of 0.007% (Grant 1964, 1966a). (2) Chromosome pairing is much reduced in the F₁, the number of bivalents per pollen mother cell (PMC) ranging from 1 to 10 and averaging 6.0, where 18 would represent complete pairing (Grant 1964). The available cytogenetic evidence suggests strongly that the observed reduction in pairing is due mainly, though not entirely, to structural differences between the genomes of the parental species (Grant and Grant 1960; Day 1965; Grant 1966a). (3) The few F₂ progeny of this chromosomally sterile hybrid were not, contrary to expectation, doubled in chromosome number and fertile, but instead were essentially tetraploid with one to four extra chromosomes and highly sterile (Grant 1966a).

The cross of *Gilia malior* × *modocensis* thereupon appeared to furnish suitable material for testing the hypothesis of Müntzing, Stebbins and others (see Discussion) that a chromosomally sterile hybrid can give rise to new fertile types on the homoploid level by recombination of the preexisting sterility factors. The new, structurally homozygous, recombination types are expected to be fertile themselves but intersterile with their parents and most of their siblings.

1 This study has been supported in part by grant GB-3620 from the National Science Foundation.

The first and hardest step was to produce by inbreeding and selection an array of fertile and meiotically normal lines. For this purpose the plants were grown in an insect-proof screenhouse, selected artificially for fertility in each generation, allowed to set seeds autogamously, and propagated as a series of inbred lines. With one generation per year, this phase of the operation required quite a few years to complete. The details have been described elsewhere (Grant 1966a). Three fertile lines, designated as branches, were obtained in the advanced generations of selection from three different $F_2$ individuals (see Figure 1).

The next and final step was to cross the fertile lines with one another and with the parental species, and to analyze the outcross and backcross hybrids cytologically. Fertile plants in the $F_6$ to $F_9$ generations were used as parents in these crosses. The meiotic behavior of the plants was studied in squash preparations of PMC's by phase and/or bright-field microscope.

**RESULTS**

*Fertility and cytology of Branch III:* The pedigree of Branch III is shown in Figure 1. The chromosome number of the parental species and $F_1$ hybrid was $2n = 36$. The $F_2$ plant used as the parent of Branch III was not counted, but four
sister plants in the F2 generation ranged from 2n = 37 to 40. Two plants in the F3 generation of Branch III had 39 and 40 chromosomes. The 39-chromosome individual (Plant 3123–1 in Figure 1) gave rise to the main surviving line of Branch III. Its F4 descendant (Plant 3301–1) which became the parent in turn of all subsequent members of Branch III had 2n = 38. This latter chromosome number was the only one encountered in periodic checks in the F6, F7, and F10 generations. The main line of Branch III evidently became stabilized at 2n = 38 from F4 on.

The percentage of well formed and well stained pollen grains, taken as a fairly close measure of gametic fertility, showed the following range among sister plants in successive generations. The number of plants tested is given in parenthesis.

<table>
<thead>
<tr>
<th>Generation</th>
<th>Range</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1–5%</td>
<td>(11)</td>
</tr>
<tr>
<td>F2</td>
<td>10–13%</td>
<td>(3)</td>
</tr>
<tr>
<td>F3</td>
<td>9–30%</td>
<td>(5)</td>
</tr>
<tr>
<td>F4</td>
<td>13–69%</td>
<td>(6)</td>
</tr>
<tr>
<td>F5</td>
<td>15%</td>
<td>(1)</td>
</tr>
<tr>
<td>F6</td>
<td>8–74%</td>
<td>(4)</td>
</tr>
<tr>
<td>F7</td>
<td>8–82%</td>
<td>(5)</td>
</tr>
<tr>
<td>F8</td>
<td>46%</td>
<td>(1)</td>
</tr>
<tr>
<td>F9</td>
<td>84–91%</td>
<td>(4)</td>
</tr>
<tr>
<td>F10</td>
<td>35–89%</td>
<td>(9)</td>
</tr>
</tbody>
</table>

**Figure 2.**—Degree of chromosome pairing in typical PMC's in the parental species, F1 and F2 hybrids, and successive generations in Branch III. Bivalents are shown black and univalents white. From camera lucida drawings.
By $F_7$ some individual segregates appeared with nearly normal pollen fertility, and by $F_9$ these fertile types had become characteristic of whole families. This trend can be attributed to the artificial selection for pollen fertility.

The same selective process brought about, as expected, a correlated improvement in meiotic behavior in PMC's. The trend is shown graphically in Figure 2. In the early ($F_3$ and $F_4$) generations in Branch III there was considerable reduction in pairing and some lagging. Chains were occasionally seen in these generations. In $F_9$ meiosis was almost normal with 17 to 19 bivalents per cell, and in subsequent generations became quite normal.
Seed production was low in the early generations (F, to F,) of Branch III. The plants in the F, to F, ranged from semifertile to highly fertile as to seeds. All vigorous plants in F, were fully seed fertile.

**Vigor:** From F, to F, inclusive the families of Branch III consisted predominantly or entirely of weak individuals. The more robust individuals were selected as parents during these generations. Response to this selection for vigor has become apparent in recent years. In F, one small family contained five vigorous and four stunted individuals. A larger family in F, contained 68 vigorous plants, 32 runts, and 18 plants of intermediate vigor.

As of F,, therefore, Branch III appears to have broken through the subvitality barrier, but has not attained uniformly high vigor in the experimental environment. The line is being continued from a vigorous parental individual in order to determine whether it will continue to segregate for vigor or not.

**Morphological characters:** The parental species differ in at least 16 quantitative characters in all parts of the plants. Eleven of these proved to be useful in practice for scoring the parents and hybrid progenies. They are listed in Table 1 and shown in Figures 3 and 4.

The F, hybrid was intermediate between the parental species in all characters measured (no data on earliness). There was segregation for these characters in the early generations (before F,). The parental individuals used to propagate the lines were selected for fertility and vigor, but not for any morphological traits.

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**Figure 4.—Branching pattern, leaf form, and inflorescence in Branch III and the parental species.** The circles represent each flower in a typical inflorescence (above), and the first flower to develop in the habit diagrams (below). Traced from live specimens grown in Claremont.
TABLE 1

Morphological and developmental characters of Gilia malior, G. modocensis and Branch III

<table>
<thead>
<tr>
<th>Character</th>
<th>G. malior</th>
<th>G. modocensis</th>
<th>F\textsubscript{10} Branch III</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Length of central leader</td>
<td>short</td>
<td>erect and long</td>
<td>erect and long</td>
</tr>
<tr>
<td>(2) Length of lateral branches</td>
<td>long</td>
<td>short</td>
<td>intermediate</td>
</tr>
<tr>
<td>(3) Orientation of lateral branches</td>
<td>spreading</td>
<td>ascending</td>
<td>ascending</td>
</tr>
<tr>
<td>(4) Point of departure of lateral branches</td>
<td>arising near base of plant</td>
<td>arising in upper part of plant</td>
<td>arising in upper part of plant</td>
</tr>
<tr>
<td>(5) Thickness of stem</td>
<td>slender, 1 to 2 mm diam; wiry</td>
<td>stout, 2 to 3 mm diam; succulent</td>
<td>intermediate, &lt;2 to 2.5 mm diam; succulent</td>
</tr>
<tr>
<td>(6) Length of lobes of lower leaves</td>
<td>long</td>
<td>short</td>
<td>long</td>
</tr>
<tr>
<td>(7) Angle of divergence of leaf lobes</td>
<td>pointing forward</td>
<td>at right angles to rachis</td>
<td>intermediate</td>
</tr>
<tr>
<td>(8) Inflorescence</td>
<td>loose; first flower of a cluster pedicelled</td>
<td>glomerate; first flower of a cluster sessile</td>
<td>intermediate</td>
</tr>
<tr>
<td>(9) Flower size</td>
<td>small; corolla limb 3 to 4 mm diameter</td>
<td>large; corolla limb 6 to 7 mm diameter</td>
<td>small; corolla limb 3 to 4 mm diameter</td>
</tr>
<tr>
<td>(10) Pigmentation of corolla</td>
<td>pale violet</td>
<td>deep violet</td>
<td>deep violet</td>
</tr>
<tr>
<td>(11) Earliness</td>
<td>early; in full bloom ± 76 days after seed sowing</td>
<td>late; in full bloom ± 102 days after seed sowing</td>
<td>early; in full bloom ± 87 days after seed sowing</td>
</tr>
</tbody>
</table>

per se. The families belonging to the later inbred generations (after F\textsubscript{5}) were relatively uniform morphologically, and differ markedly from one another (GRANT 1966b).

The characters of vigorous plants in the F\textsubscript{10} of Branch III are shown and compared with those of the parental species in Table 1 and Figures 3 and 4. It can be mentioned here that the character combination in this line has not changed in any important way since F\textsubscript{5}.

Branch III is like G. modocensis in four of the 11 characters scored, like G. malior in three, and like the F\textsubscript{1} hybrid in four. It has, for example, some of the branching characters of G. modocensis combined with certain leaf and floral features of G. malior, and is intermediate in various other features. Branch III thus possesses a new combination of the parental characteristics.

*Fertility relationships of Branch III:* Two fully fertile individuals in the F\textsubscript{5}
generation of Branch III were crossed successfully with *Gilia malior*. The crosses were Plant 3928-1 (Br. III) ♀ × *G. malior* ♂ and 3928–2♀ × *G. malior* ♂ (see pedigree in Figure 1). The first of these replicate crosses yielded one F₁ hybrid and the second yielded three hybrid plants.

The backcross hybrids were highly sterile. The percentage of well formed pollen on the four hybrid plants was 4%, 10%, 13%, and 18%. The hybrids flowered and self-pollinated for seven weeks and produced four or five plump seeds per plant. The seed fertility is estimated to be 0.4%.

One hybrid individual was analyzed cytologically. It had 2n = 37 as expected from parents with 2n = 38 and 36 respectively. Chromosome pairing was greatly reduced at metaphase in PMC's (Figure 5). In 23 cells the number of bivalents ranged from 10 to 15 and averaged 12.9 per cell. The remaining chromosomes appeared as univalents (Figure 5). Lagging chromosomes were seen in cells at anaphase and telophase.

Branch III was outcrossed to Branch II using F₁ individuals with normal fertility in each line as parents. The cross was Plant 3720–94 (Branch II) ♀ × 3723–15 (Branch III) ♂. The Branch III pollen parent was fertile (82% good pollen) but weak. A single hybrid plant was obtained from this cross.

The F₁ hybrid was vigorous but highly sterile. It had 9% well formed pollen. A few flowers out of many on the plant produced a total of 16 plump seeds,

![Figure 5](image)

_Figure 5._ Chromosome pairing in PMC's in backcross and outcross hybrids of Branch III. Bivalents shown black, univalents white, and chains stippled. 2n = 37 in these hybrids.
giving an estimated seed fertility of 1.3%. The one vigorous F₁ plant obtained from these seeds had 29% well formed pollen and was thus semisterile.

The F₁ hybrid of Branch II × III had 2n = 37 chromosomes as expected. Chromosome pairing was strongly reduced at metaphase (Figure 5). Sixteen PMC’s had between 10 and 14 bivalents per cell (average 11.6). A chain of three chromosomes was seen in some cells (Figure 5). Some of the univalents often lagged at anaphase.

The backcross hybrid of Branch III × G. modocensis has not yet been available for analysis. This hybrid is being produced currently by Miss Ruth Wilson. However, Branch II has previously been shown to be interfertile and chromosomally homologous with G. modocensis (Grant 1966b). The sterility and reduced pairing in the outcross hybrid between Branch III and Branch II is therefore indicative of the fertility relationships of Branch III with G. modocensis.

**DISCUSSION**

Several authors have proposed and developed the hypothesis that two plant species differing with respect to two or more independent segmental rearrangements, and chromosomally intersterile on this account, can give rise by hybridization, recombination and inbreeding to new fertile types which are on the same ploidy level as the parental species but are separated from these by sterility barriers (Müntzing 1929, 1930, 1938; Gerassimova 1939; Stebbins 1942, 1950, 1957, 1959; Grant 1956, 1958, 1963, pp. 469-481). It is convenient to refer to this hypothetical process here as recombinational speciation.

The simplest case would be that of two parental types differing by two independent translocations, and having the genomic constitutions AA BB CC DD and A_b A_a B_b B_a C_c C_a D_d D_d. Their F₁ hybrid is partially sterile with an expected 75% of abortive pollen if the deficiency-duplication products are inviable in the gametophyte stage. The progeny of this hybrid can be expected to include some new homozygous recombination types for the translocations (i.e., AA BB C_c C_c D_D_d) which are fully fertile themselves but partially intersterile with both parental types. Parental plants differing in two independent sets of transpositions or reinversions will produce an F₁ hybrid with a similar degree of sterility, if crossing over occurs regularly between the transpositions or within the reinversions on each chromosome pair, and can likewise give rise to new fertile recombination types in F₂ or later generations.

A larger number of heterozygous segmental rearrangements will of course give rise to chromosomal sterility barriers comparable in strength to those commonly found between well isolated plant species. For example, two species differing by six independent rearrangements of the types and under the conditions mentioned above will yield an F₁ hybrid with a gametic fertility of 1.56%. This sterile hybrid can then go on to produce fertile recombination types in later generations which are isolated by strong sterility barriers from both parental species. The most common type of fertile recombination product expected from this hybrid would yield backcross hybrids with either parental species which have a gametic fertility in each case of 12.5%.
This hypothesis has been developed and advocated in the hope that it would provide a partial solution for the common occurrence in many plant groups of morphologically similar species on the same ploidy level separated by chromosomal sterility barriers. A single pair of genomically differentiated species can theoretically give rise to one or more new species by hybridization and recombination without change in ploidy.

There have been three previous attempts to verify this hypothesis experimentally, and each of these has been successful in one respect or another. Two additional hybridization experiments in Erophila and Phaseolus confirm similar but somewhat different hypotheses of hybrid speciation (Winge 1940; Lamprecht 1941).

Gerassimova (1939) was able to derive fertile homozygous types of *Crepis tectorum* differing from one another as well as from the parental population by single translocations and semisterility barriers. These two types then produced by recombination a third fertile form designated *Crepis nova* which contained both translocations in homozygous condition and was even more intersterile with the original parental type of *Crepis tectorum*.

The F₁ hybrid of *Nicotiana langsdorffii × sanderae* is semisterile with 45 to 55% well formed pollen and slightly irregular meiosis. Smith (1954) and Smith and Daly (1959) derived several inbred lines from this hybrid. The diploid derivatives with short corollas (which are the ones of main interest to us here) became almost fully fertile in later generations (76 to 80% good grains in F₂ to F₅). These short-flowered derivatives produced semisterile hybrids with both parental species; the backcross hybrids with *N. langsdorffii* had 46 to 58% good pollen and those with *N. sanderae* had 60 to 61%. This fertile hybrid derivative is thus as intersterile with the two parental species as these are with one another (Smith and Daly 1959).

The F₁ hybrid of *Elymus glaucus × Sitanion jubatum* is highly sterile with < 1% good pollen. Chromosome pairing is fairly normal in the hybrid, but structural differences are inferred to exist from the behavior of the allopolyploid derivatives (Stebbins and Vaarama 1954). By backcrossing the hybrid with *Elymus glaucus* and selfing the B₁ plant, Stebbins (1957) obtained fully fertile homoplloid progeny in F₁ and F₂. The derived line formed a hybrid with *Elymus glaucus* which was highly sterile with 0 to 3% good pollen (Stebbins 1957).

The Crepis and Nicotiana experiments confirm the hypothesis in terms of weak sterility barriers. The Elymus experiment is the first one involving strong sterility barriers. Here the fertile hybrid derivative was obtained by backcrossing, which, in these predominantly autogamous plants, might or might not occur in nature. The Gilia experiment now provides a case of a new, fertile, strongly isolated type which has emerged from a highly sterile hybrid by the normal pathway of straight inbreeding.

Our final task is to attempt to assess the importance and extent of recombinational speciation in nature in the light of the experimental evidence. Theoretically this process can be compared with allopolyploidy which represents an alternate pathway from a chromosomally sterile hybrid to a new fertile species. Allopolyploidy...
ploid speciation is known to be of common occurrence in plants on the basis of experimental and cytotaxonomic evidence, and therefore we might logically expect recombinational speciation to have occurred frequently too, but to remain undetected cytotaxonomically.

The possibility that recombinational speciation may be common in plants has been in my mind during the years of my experimental work on this problem. Similar views have been expressed by Stebbins (i.e., 1966, pp. 122–123). I am now inclined to think that recombinational speciation involving strong sterility barriers is a real and interesting but relatively rare process in nature.

The Gilia experiment was conducted with one aspect of the situation, hybrid sterility, mainly in mind. Allopolyploidy is a way out of the impasse of chromosomal sterility in a hybrid plant, as Darlington (1932, 1958) and others have pointed out; and recombination of separable chromosomal sterility factors could be considered an alternative way out of the same impasse. A second important aspect of the situation, namely hybrid breakdown, was, however, neglected in predicting the experimental results. For allopolyploidy is a way around both chromosomal sterility and hybrid breakdown; but recombinational speciation circumvents only the first of these barriers.

In the event, the Gilia experiment was needed to reveal the full force of the hybrid breakdown barrier. The vast preponderance of F,

\[ \text{Gilia malior} \times \text{modocensis} \]

were subvital, sterile, or both (Grant 1966a). Two of the three vigorous and fertile lines derived from the hybrid turned out to be reversions to one parental type (Grant 1966b). The only fertile derivative that possessed a new combination of sterility factors was weak during many generations and could be kept alive only with difficulty.

Among the numerous sterile species hybrids of Gilia raised in our experimental garden over the years, seven have doubled spontaneously to produce fertile or semifertile allopolyploid progeny, and some of these hybrids have doubled repeatedly in replicate cultures (Grant and Grant 1960; Grant 1965; Day 1965). The new allopolyploid plants showed good general vigor from the start. Other workers have had similar experiences in other plant groups. One gets the definite impression from such experiences that speciation accompanied by strong sterility barriers may take place fairly easily by the allopolyploid route, but only with difficulty and under exceptional circumstances by the recombinational route.

The cross-pollinations were made by Dr. Alva Day and Mrs. Karen A. Grant. The latter also helped with the scoring of morphological characters, and critically read the manuscript. Jeanne R. Janish made the drawings for Figure 3, and Charles Popp the chart for Figure 1. The help of these workers and the financial assistance of the National Science Foundation are gratefully acknowledged.

**SUMMARY**

One of the selection products of a chromosomally sterile species hybrid in Gilia became fully fertile without change in ploidy. This fertile hybrid derivative has its own distinctive character combination and is intersterile with both
parental species. It represents an experimental case of the hybrid origin of a new isolated species without chromosome doubling.

LITERATURE CITED


