and criticize the manuscript. The neamine base used in these experiments was a gift from Dr. W. T. Sokolski, The Upjohn Company, Kalamazoo, Michigan.

[1] Sager, R., and Z. Ramanis, these PROCEEDINGS, **50,** 260 (1963).
[2] *Ibid.,* **53,** 1053 (1965).
[3] Gillham, N. W., *Genetics,* **52,** 529 (1965).
[4] *Ibid.,* **48,** 431 (1963).
[5] Levine, R. P., and W. T. Ebersold, *Z. Vererbungslehre,* **89,** 631 (1958).
[6] Ebersold, W. T., and R. P. Levine, *Z. Vererbungslehre,* **90,** 74 (1959).

EVIDENCE FOR THE SELECTIVE ORIGIN OF INCOMPATIBILITY BARRIERS IN THE LEAFY-STEMMED GILIAS*

BY VERNE GRANT

RANCHO SANTA ANA BOTANIC GARDEN, CLAREMONT, CALIFORNIA

The hypothesis that natural selection will build up hybridization-preventing barriers between sympatric species where hybridization has deleterious effects in F_1 and later generations[1] has hitherto been supported almost entirely by evidence concerning ethological isolation in *Drosophila* and other animals.[2] Much of this evidence, particularly in the case of the earlier studies, is inadequate in one way or another, as has been pointed out by several critics,[3] the best evidence being that reported recently by Dobzhansky and his co-workers[4] for the *Drosophila paulistorum* group. It is desirable, therefore, to seek critical evidence in plants bearing on the hypothesis of selection for reproductive isolation. The purpose of this paper is to present comparative data on the strength of incompatibility barriers in the plant genus *Gilia* which strongly support the hypothesis of a direct selective origin of these barriers.

Materials.—The leafy-stemmed gilias (Gilia section *Gilia,* Polemoniaceae) form a natural group of ten species possessing characteristics favorable for testing the hypothesis of selection for isolation. Nine of the ten species have been studied genetically. These are sexually reproducing, and either diploid ($2n = 18$, six species) or tetraploid ($2n = 36$, three species). The plants are annual herbs which die as individuals when they go to seed, so that the loss in seed output involved in the production of sterile interspecific hybrids must have a high selective disadvantage.

Twenty-two of the 36 possible crosses between the nine species have been attempted. Nine of the crosses failed, and the remaining 13 yielded F_1 hybrids. These hybrids were highly or completely sterile with reduced chromosome pairing and other disturbances of meiosis. It can be demonstrated on indirect evidence that the crosses which failed or were not attempted would produce chromosomally sterile F_1 hybrids. All of the genetically known species of leafy-stemmed gilia are thus intersterile.[5]

The pattern of geographical distribution of the nine species makes it possible to compare sympatric and allopatric species with respect to the strength of incompatibility barriers. Five of the species—*G. tricolor* (2x), *G. angelensis* (2x), *G. capitata* (2x), *G. achilleaefolia* (2x), and *G. clivorum* (4x)—occur in the foothills and valleys of coastal California and adjacent regions. The areas of these species overlap extensively (Fig. 1, *left*), and individuals belonging to two or more species grow side by side in many places (Fig. 2). Four other species—*G. millefoliata* (2x), *G. nevinii* (4x), *G. lomensis* (4x), and *G. valdiviensis* (2x)—occur on the Pacific coastal strand in
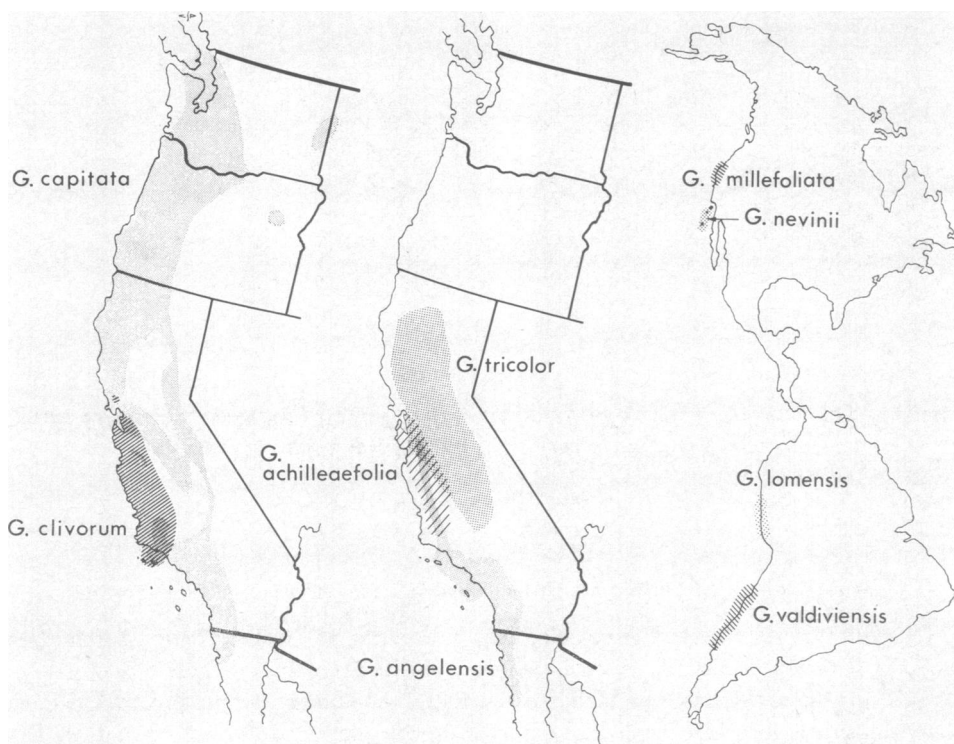
FIG. 1.—Geographical distribution of nine species of leafy-stemmed gilia. *Left,* the California foothill-and-valley species; *right,* the strictly maritime species.
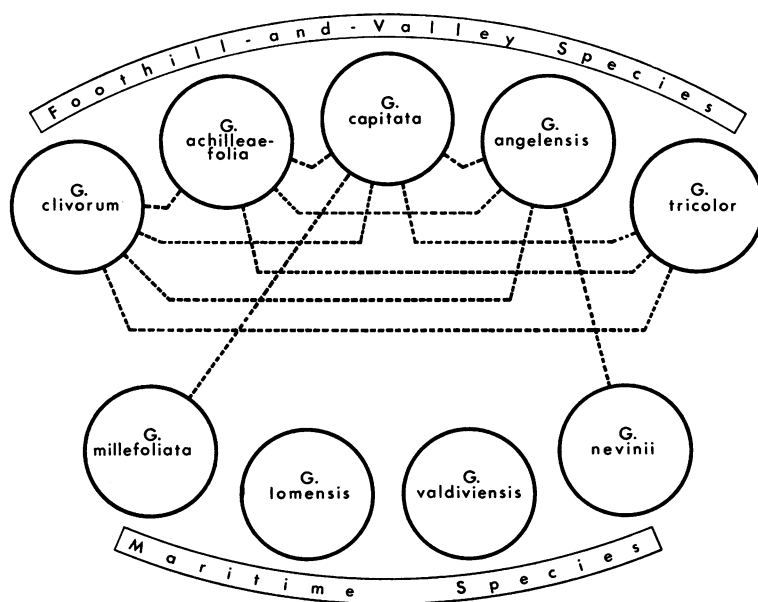

FIG. 2.—Paired combinations of species of leafy-stemmed gilia which have been found growing side by side in nature.

four widely separated areas of North and South America (Fig. 1, *right*). The maritime species are completely allopatric with respect to one another, and come into contact only rarely or not at all with the foothill-and-valley species (Fig. 2).

The areas of these species have probably undergone migrational shifts with the climatic changes during and since the Pleistocene. However, maritime habitats have always occupied a peripheral position in relation to mediterranean habitats in the general area in which the leafy-stemmed gilias have developed. It is likely, therefore, that the broad geographical relationships now found in this group, though not necessarily the detailed ranges, have prevailed during much of the phylogenetic history of the group. The foothill-and-valley species probably have a long history of frequent sympatric contacts, leading to possibilities of interspecific hybridization, while the maritime species by contrast have probably enjoyed a long history of geographical isolation.

It is possible to compare the strength of incompatibility barriers between species in the two geographical classes of leafy-stemmed gilias by using the following measure. The output of hybrid seeds by a fair number of flowers emasculated and cross-pollinated under insect-free conditions gives a good estimate of the degree of cross-compatibility. The average number of plump hybrid seeds produced per flower pollinated will be designated as S/Fl. The hybrid constitution of the seeds is confirmed by identification of the F_1 plants grown from them.

Results.—The data for 22 interspecific crosses, many of which have been carried out in both reciprocal directions and replicated with different strains,[5] are presented in Table 1 and summarized in Table 2.

The tables show that hybridization between the sympatric foothill-and-valley species is blocked by very strong incompatibility barriers. The seed output from artificial hybridizations ranges from 0.0 to 1.2 S/Fl in different combinations and averages 0.2 S/Fl for all interspecific crosses combined. One species in this class, *Gilia tricolor*, is isolated from all other species by an incompatibility block which has never been breached in numerous attempts.[6]

The allopatric maritime species, on the other hand, although as well differentiated from one another morphologically and cytogenetically as the foothill-and-valley species, are not separated by any significant incompatibility barrier at all. The values of S/Fl range from 7.7 to 24.8 and average 18.1 for five interspecific combinations (Table 2). These values are comparable to those obtained in intraspecific crosses.

This is true even in the case of diploid-tetraploid combinations. For example, *Gilia millefoliata*, a diploid on the California coastal strand, and *G. lomensis*, a tetraploid of the Peruvian coast, cross in either direction about as easily as two sister plants from the same population.[7] The figures are 18.3 S/Fl for *G. lomensis* (4x) ♀ × *millefoliata* (2x), and 15.6 S/Fl for *G. millefoliata* ♀ × *lomensis*.

Crosses between the two geographical classes of species, finally, show incompatibility barriers of intermediate strength. The mean of means for the various crosses of foothill species × maritime species is 3.2 S/Fl. The variation from cross to cross is considerable, as the figures in Table 1 show.

It is of interest to know whether the incompatibility block observed in foothill × maritime species crosses can be correlated with the female reproductive functions of the foothill species or not. Three pairs of species consisting of a foothill species and a maritime species which do not differ in flower size or ploidy have been crossed in both reciprocal directions. In each of the three comparisons the hybrid seed set is about equally low in the two reciprocal crosses. For example, the cross *G. clivorum* (4x) ♀ × *lomensis* (4x) yielded 0.00 S/Fl, and *G. lomensis* ♀ × *clivorum* 0.06 S/Fl. The available evidence thus indicates that the incompatibility barrier is

TABLE 1

Seed Production in Interspecific Crosses in the Leafy-Stemmed Gilias

Cross	No. flowers pollinated	No. hybrid seeds	Av. no. seeds per flower (S/Fl.)
(A) *Crosses between foothill-and-valley species*			
G. tricolor × capitata	42	0	0.00
G. tricolor × achilleaefolia	72	2	0.03
G. tricolor × clivorum	30	0	0.00
G. tricolor × angelensis	23	0	0.00
G. capitata × achilleaefolia	231	14	0.06
G. capitata × clivorum	85	ca. 12	ca. 0.14
G. capitata × angelensis	23	10	0.43
G. achilleaefolia × clivorum	45	2	0.04
G. achilleaefolia × angelensis	90	105	1.17
(B) *Crosses between maritime species*			
G. millefoliata × nevinii	29	570	19.65
G. millefoliata × lomensis	30	500	16.67
G. millefoliata × valdiviensis	38	832	21.89
G. nevinii × valdiviensis	40	990	24.75
G. lomensis × valdiviensis	60	460	7.67
(C) *Crosses between A and B species*			
G. millefoliata × tricolor	24	3	0.12
G. millefoliata × capitata	39	Few	—
G. millefoliata × achilleaefolia	126	525	4.17
G. millefoliata × angelensis	11	75	6.82
G. millefoliata × clivorum	51	145	2.84
G. nevinii × achilleaefolia	40	205	5.12
G. valdiviensis × achilleaefolia	33	Many	—
G. lomensis × clivorum	36	1	0.03

of approximately the same strength whether the foothill species behaves as a female or as a male parent.

The differences between the means of the three classes of interspecific crosses given in Table 2 are statistically significant in all paired combinations ($P < 0.01$). In fact, the seed output in species crosses in the maritime class could be 20 per cent less than the observed amount, and the difference in mean values between the maritime and foothill classes of crosses would still be significant at the 1 per cent level.

If incompatibility barriers between plant species arose only as by-products of evolutionary divergence, we would expect the maritime and the foothill-and-valley species of leafy-stemmed gilia to be separated by crossing barriers of comparable strength, for the species in the two classes are differentiated morphologically and cytogenetically to a similar extent. The facts in this case are opposed to an explanation of the origin of interspecific incompatibility in terms of divergence *per se.* The same facts are in agreement with expectation on the alternative and supplementary hypothesis that incompatibility is sometimes built up by natural selection for reproductive isolation under conditions of extensive sympatric contact between species.

TABLE 2

Degree of Crossability as Correlated with Geographical Relationships between Species of Leafy-Stemmed Gilia

Geographical relationship of parental species	No. combinations of parental species	Mean of means for different combinations (S/Fl)	Range of means (S/Fl)
California foothill-and-valley species *inter se*	9	0.2	0.0–1.2
Maritime species *inter se*	5	18.1	7.7–24.8
Foothill species × maritime species	8 (complete data for 6)	3.2	0.0–6.8

Summary.—Nine intersterile species of annual gilias in Pacific North and South America, belonging to the same natural group (leafy-stemmed gilias), fall into two classes with regard to geographical distribution. Five species in the California foothills and valleys are sympatric. Four maritime species are completely allopatric in relation to one another, and either completely or mainly allopatric in relation to the foothill-and-valley species. The sympatric foothill species are isolated by very strong incompatibility barriers, whereas the allopatric maritime species can be crossed *inter se* with the greatest of ease. The observed correlation between cross-ability and geographical distribution is interpreted as evidence in favor of the hypothesis of a selective origin of hybridization-preventing mechanisms in annual plants under conditions of sympatry.

[1] Wallace, A. R., *Darwinism: An Exposition of the Theory of Natural Selection* (London: Macmillan, 1889), pp. 173 ff.; Fisher, R. A., *The Genetical Theory of Natural Selection* (Oxford: Clarendon, 1930), pp. 130 ff.; Dobzhansky, Th., *Genetics and the Origin of Species* (New York: Columbia University Press, 1941, 1951), eds. 2 and 3, pp. 209 ff.

[2] Recent reviews by Mayr, E., *Animal Species and Evolution* (Cambridge, Mass.: Harvard University Press, 1963), pp. 548 ff.; and Grant, V., *The Origin of Adaptations* (New York: Columbia University Press, 1963), ch. 17.

[3] Volpe, E. P., *Am. Nat.*, **89,** 303–317 (1955), Moore, J. A., in *The Species Problem*, ed. E. Mayr (Washington, D.C.: Am. Assoc. Adv. Sci., 1957), pp. 325–338; Clausen, J., R. B. Channell, and U. Nur, *Rhodora*, **66,** 32–46 (1964).

[4] Dobzhansky, Th., L. Ehrman, O. Pavlovsky, and B. Spassky, these PROCEEDINGS, **51,** 3–9 (1964).

[5] Grant, V., *Aliso*, **3,** 35–49 (1954); *Heredity*, **20,** in press.

[6] Grant, V., *Aliso*, **2,** 375–388 (1952).

[7] Grant, V., *Heredity*, **20,** in press.

EFFECT OF SIMPLE SUGARS ON THE MORPHOLOGY AND GROWTH PATTERN OF MAMMALIAN CELL CULTURES*

BY RODY P. COX† AND BERTRAM M. GESNER

DEPARTMENT OF MEDICINE, NEW YORK UNIVERSITY SCHOOL OF MEDICINE

Components of mammalian cell surfaces contain a variety of sugars including D-mannose, L-fucose, D-galactose, amino sugars, and sialic acid.[1] These sugars form heterosaccharides which are synthesized under genetic control and are able to interact with structures in the environment. Their physiological function is not known.

In the present study, mammalian cells were grown in the presence of simple sugars found in heterosaccharides of mammalian cell surfaces. It has been observed that there is a selective effect of individual sugars on the cell morphology and pattern of growth of certain cell lines.