GENETIC REACTIONS IN NICOTIANA. II. PHENOTYPIC REACTION PATTERNS

E. M. EAST

Harvard University, Bussey Institution, Jamaica Plain, Massachusetts

Received March 10, 1935

In a paper entitled "Genetic Reactions in Nicotiana. I. Compatibility," the hybrid compatibility of twelve species groups of *Nicotiana* was reported. Reasonably adequate tests were made on all the possible combinations. Complete, but less extensive, tests were described on three other species. Of the 132 unions possible in the first group, 52 hybrids were obtained which grew to maturity. In the other tests, 4 mature hybrids were produced, in addition to *nudicaulis* × *trigonophylla*. If the two tests are added, the result is 56 flowering hybrids out of 210 possible unions.

In this paper, I shall describe the phenotypic reaction patterns of these hybrids. (So far as I know, this apt term was first used by SINNOTT in his work on the shapes of cucurbits.) My object is not simply to describe the various hybrids, but rather to inquire as to how a given species reacts with various other species in regard to any stated characteristic. This is a method of study, thus far neglected, that ought to give us genetic information of considerable value.

As a necessary preliminary to these discussions, a few words should be said about the stocks used for the crosses and about the inheritance of certain obvious character differences in varietal crosses.

As GOODSPEED (1933) has stated, most of the *Nicotiana* species give evidence of polymorphism. *N. sylvestris* and *N. paniculata* are exceptions. Our own stocks, however, had all been inbred until they had reached a high degree of uniformity before they were used in the crossing experiments, aside from *N. Sanderæ*, which is self-sterile. Several genotypes were used as foundation stocks, however, in the species *N. Sanderæ*, *N. Langsdorffii*, *N. longiflora*, *N. glauca*, *N. glutinosa*, *N. paniculata*, *N. trigonophylla*, *N. Bigelovii*, *N. rustica*, and *N. Tabacum*. I think it may be assumed, therefore, that the species involved in the crosses, always excepting *N. Sanderæ*, are as nearly homozygous as one can expect in such experiments. Naturally, in an investigation extending over a quarter of a century, it is impossible to control matters in an ideal way. But several of our reciprocals were made with identical plants. In other instances, a given plant was crossed with several other species.

Much of the *Nicotiana* polymorphism is exhibited as size differences. One is not far wrong in saying that *N. Sanderæ* and *N. Tabacum* are the only species where numerous qualitative variations are found. In *N. Sanderæ* these qualitative differences are confined, in general, to genes affecting

corolla color. Some idea of the genetic behavior of the anthocyan-producing genes of *N. Sanderae* may be found by referring to papers cited by EAST (1928); but much remains to be done. *N. Tabacum* varieties vary especially in flower color and in leaf shape. Tobacco flowers are either red or white. The reds are of several kinds, and are due to the influence of two main genes and at least two modifying genes, as reported by SETCHELL, GOOD-SPEED and CLAUSEN (1922). Our own results from numerous varietal crosses are essentially the same. There is also a set of genes controlling leaf shape, petioled types being partially dominant. Three such genes are described in the paper cited above as having the major influence in determining the differences between petioled and sessile forms. There is little doubt that three gene pairs are the main determiners of naked petiole, fringed petiole, sessile, and auricled leaves; but we feel, from our own experience, that there is a very large series of gene differences which have various modifying effects.

EXPERIMENTAL WORK

General plant reactions

It is not easy to generalize on the way these species react with each other when considering characteristics which may be grouped under the somewhat unsatisfactory phrase *habit of growth*.

All species of *Nicotiana* form a rather definite rosette from which an erect stem emerges. But the *longiflora* group has a rosette that is markedly flattened, and the *Sanderae* group and *Langsdorffii* have one that is noticeably flattened; while the remaining species exhibit more spacing between the early leaves. Where *longiflora*, *Sanderae*, and *Langsdorffii* are brought together (crosses 1, 2, 5, 7), the hybrids invariably show the flat rosette tendency. Where the genomes of these three types are united with the genomes of other species, however, the reaction is a variable one which is not closely correlated with the number of chromosomes contributed by the two parents. *Longiflora* with *suaveolens*, for example, produces more of a characteristic flat rosette than *longiflora* with *glauca*. Again, *Sanderae* and *Langsdorffii*, when combined with *Tabacum*, give flatter rosettes than when combined with some of the 12-chromosome species.

Similar remarks cannot be made regarding stems. The simple ascending stem which appears after a month or so in the *Nicotianas* rapidly becomes diffuse to a greater or less degree in *Sanderae*, *Langsdorffii*, *paniculata*, and *suaveolens*. In other species the stem remains single, though the development of lateral shoots may give the appearance of diffuseness in such species as *glutinosa*, *Bigelovii*, and *rustica*. The diffuseness of *suaveolens* is apparent in all combinations, although there is a reasonable degree of variability in the different types of hybrids. With this exception, the

single erect stem is, as one may say, dominant in combinations where *glauca*, *tomentosa*, or *Tabacum*, is one of the parents.

Of the species being considered here, the only one that is truly arborescent is *glauca*; but all the species are suffrutescent to a greater or less degree. It is interesting to note that hybrid combinations made with *glauca*, of which there are fourteen, all show a definite and individually characteristic reaction pattern which can more easily be referred to the type of the other parent than to its chromosome number. Thus, *tomentosa*, which is more woody and tree-like than *Tabacum*, yields hybrids with *glauca* which are more tree-like than those with *Tabacum*; while the hybrids with *Sanderæ* and *Langsdorffii* have but a slight tendency to become woody and are too stunted to exhibit any tendency to arborescence if they possess it.

The young stems of all hybrids where *glauca* is one parent are hairy, though there is a difference both in the size and the frequency of the hairs in different combinations. *Longiflora* $\times$ *glauca* even shows the prickles characteristic of *longiflora*, though they are small and somewhat sparse. In every hybrid, however, the hairs have a tendency to fall off the stems as the stems become older. This trend toward a bald condition begins at different ages in the different hybrids. The stems rarely become truly smooth, and new stem growths are always hairy. The bloom of *glauca* does not appear in hybrids.

Color reactions

Purple anthocyan appears in the stems of certain populations of *Sanderæ*. It is due to a gene *C* which is linked with the self-sterility gene *S*. *Langsdorffii* plants apparently are always *c*. Hybrids between the two species are purple or non-purple, depending on whether *C* has entered from *Sanderæ*. Certain other species, such as *longiflora* and *glauca*, usually (perhaps always) have purple anthocyan spots either at the base of the stem or at the leaf axils. *Tomentosa* and *Tabacum* varieties may have such spots. I have never seen this color, however, in *glutinosa*, *paniculata*, *sylvestris*, *Bigelovii*, or *rustica*. I have followed the transmission of color with particular care in the *glauca* hybrids. There the purple color from *glauca* appears in all hybrids except the *glauca*-*Langsdorffii* and the *glauca*-*glutinosa* combinations. In the *glauca*-*Tabacum* combinations, the color not only appears, but the amount appearing is much greater than that found in *glauca*, even when the variety of *Tabacum* used shows no trace of stem color. Instead of the color being confined to the leaf axils, as in *glauca*, the petioles and veins of the leaves are a bright purple, which, however, gradually fades.

One must suppose, therefore, that *glauca* and most other species show

an antagonism to the full development of stem anthocyan, which is increased by *glutinosa* and *Langsdorffii* genoms to the point of full inhibition, and that these antagonistic genes are absent in at least some of the varieties of *Tabacum*. Since *tomentosa* hybrids made with *glauca* develop the spots of color, the release to full development should come from *sylvestris* genes (if *sylvestris* is a component of *Tabacum*).

In other instances anthocyan appears to be transmitted as if there were no or very little antagonism to its development. White-flowered *Sanderæ* plants transmit no color when combined with species having no anthocyan. But red-flowered or purple-flowered *Sanderæ* reproduce their colors, albeit as if somewhat diluted, in combination with non-anthocyan species. The same may be said of the various reds in *Tabacum*. GOODSPEED and CLAUSEN (1922) found that in crosses of five different *Tabacums* with *sylvestris*, the particular color type of *Tabacum* was transmitted, though there was perhaps a smaller quantity of the coloring material. We have corroborated their results with four different *Tabacum* varieties. When a white-flowered *Tabacum* was used, no flower color appeared in the hybrid.

In many of the white-flowered *Sanderæ*s (the so-called *alatas*) and in the *longiflora* group, a slight tinge of purple appears on the back of the corolla. This is never extended in hybrids, though it is present.

The experiences given above hold for all anthocyan-colored flowers, including those of *tomentosa* and *glutinosa*, where dull reds appear, when combinations are made with white-flowered types such as *Sanderæ-alata*, *longiflora*, *sylvestris*, *suaveolens*, and *Bigelovii*. They also hold when species with greenish-yellow flowers (the flowers of *tomentosa* and *glutinosa*, and the outer veins of the flowers of *Bigelovii* develop a little chlorophyll) are used, such as *Langsdorffii*, *glauca*, *paniculata*, and *rustica*, but with some modification.

When *Tabacum* is involved, its red anthocyan is transmitted with very little dilution, no matter which other species is used. When the anthocyan-producing genes enter a cross from species having 9 or 12 chromosomes, however, there is much variation in the way they act. *Sanderæ-Langsdorffii* hybrids, where both species have 9 chromosomes, behave as in varietal crosses except for the addition of chlorophyll. The color follows *Sanderæ*. *Paniculata* × *Sanderæ* behaves similarly, but here the anthocyan is dulled, even though there is less green in the hybrid flowers than when *Langsdorffii* is used. The flowers are larger than in *Sanderæ-Langsdorffii* and appear as if a given quantity of anthocyan and of chlorophyll were spread out over a larger surface than in the parents. If white *Sanderæ*s are used, the hybrid flowers are white, with no more green on the exterior of the tube than is often present in the pure (= *alata*) type. In combination with *glauca*, which has a yellow flavone color in addition to the green

chlorophyll, the reds of *tomentosa* and *glutinosa* are modified in quantity, type, and distribution more than are those of *Sanderæ*. *Glutinosa* $\times$ *glauca* has slight flakes of red color, while *glauca* $\times$ *tomentosa* has only the slightest suggestion of color.

The brighter flavone yellow of *glauca* appears in crosses with *paniculata*, but is not obvious in crosses with *Langsdorffii*. Other combinations of green types with green types have their own characteristics and usually show the influence of both parents.

One gets the idea, therefore, that anthocyanins are due to a very limited number of genes whose normal activity is not significantly distorted by the genes of other species, but that chlorophyll production in the greenish-yellow flowered species is highly complex and can be modified in numerous ways, though not prevented, by other gene complexes.

Flower reaction patterns

In table 1 a list of the fifty-two hybrids which attained maturity in the primary investigation is given. For forty-six of the hybrids, certain flower measurements are given which permit comparisons. The length of the pistil is recorded where the pistil is longer than the corolla in one of the parents. The length of the flower, as listed, is taken from the lower end to the constriction of the throat just below the corolla limb. The breadth is the greatest breadth across the corolla lobes. The fourth and fifth measurements are the length of the largest corolla lobe and the length of the adjacent sinus.

These measurements have a certain importance in that they are the only quantitative characters thus far noted which are, for all practical purposes, unaffected by environment (see EAST 1916). Naturally there are variations in flower size on any given plant, but the modal condition always shows a sharp peak. And the frequency distributions of a pure line of plants, when each plant is represented by modal measurements for that plant, are almost identical when the population is grown in small pots where the plant food is restricted and when it is grown under the best of field conditions.

The first point to which I wish to draw attention is that reciprocal crosses are as nearly identical as are the plants of a given parental population. There are seventeen pairs of reciprocals, having the chromosome numbers shown.

<i>Sanderæ</i> $\times$ <i>Langsdorffii</i>	9 and 9
<i>Sanderæ</i> $\times$ <i>longiflora</i>	9 and 10
<i>Sanderæ</i> $\times$ <i>paniculata</i>	9 and 12
<i>Sanderæ</i> $\times$ <i>Tabacum</i>	9 and 24

<i>Langsdorffii</i> × <i>glauca</i>	9 and 12
<i>longiflora</i> × <i>glauca</i>	10 and 12
<i>glauca</i> × <i>tomentosa</i>	12 and 12
<i>glauca</i> × <i>Tabacum</i>	12 and 24
<i>glutinosa</i> × <i>Bigelovii</i>	12 and 24
<i>glutinosa</i> × <i>Tabacum</i>	12 and 24
<i>paniculata</i> × <i>rustica</i>	12 and 24
<i>tomentosa</i> × <i>sylvestris</i>	12 and 12
<i>tomentosa</i> × <i>Tabacum</i>	12 and 24
<i>sylvestris</i> × <i>Tabacum</i>	12 and 24
<i>suaveolens</i> × <i>Bigelovii</i>	16 and 24
<i>Bigelovii</i> × <i>Tabacum</i>	24 and 24
<i>rustica</i> × <i>Tabacum</i>	24 and 24

Measurements were recorded for only one cross in the combinations *glauca-tomentosa* and *tomentosa-sylvestris*, but my notes show that the reciprocals were so similar that they could not be told apart. If, then, these two pairs are included in the total, there are thirteen pairs where the hybrids are identical, for all practical purposes. Seven pairs were made in which the parents used were the same plants, although the measurements of the parental flowers do not check in every case because they were sometimes taken at different flowering periods. In most of the other instances sister plants were used. The four apparent exceptions are not true exceptions. In general characteristics the reciprocals considered as units were similar to each other, but the flower measurements are different because different varieties were used. The *Sanderæ* genotypes were not at all alike in the combination *Sanderæ-Langsdorffii*, though they were similar in the combinations *Sanderæ-paniculata* and *Sanderæ-longiflora*. The cross *tomentosa* × *Tabacum* was made with *tomentosiformis*, while the reciprocal, so-called, was made with *tomentosa*. Two different varieties of *Tabacum* were used in the *Bigelovii-Tabacum* unions, and two different varieties of *rustica* were used in the *rustica-Tabacum* unions.

There are also two identical reciprocals that are not included in table 1 (see Genetic Reactions in Nicotiana. I.). They are the *caudigera-acuminata* combinations and the *Bigelovii-trigonophylla* combinations. One of the latter is represented in figure 3. Thus there are fifteen reciprocals that are comparable genetically, and each member of every pair is identical with the other.

In view of the general interest in reciprocal crosses because of possible plasmatic influence, it is necessary to emphasize, at this point, that in characteristics other than the flower measurements, these reciprocals are also identical when comparable. In other words, reciprocal crosses in

TABLE 1 (Continued)
Flower measurements in various hybrid combinations (millimeters).

HYBRID COMB.	MATERNAL PARENT					HYBRID					PATERNAL PARENT				
	L. PIS.	L. COR.	BR. COR.	MAX. LOBE	ADJ. SINUS	L. FIS.	L. COR.	BR. COR.	MAX. LOBE	ADJ. SINUS	L. FIS.	L. COR.	BR. COR.	MAX. LOBE	ADJ. SINUS
27. <i>toment.</i> × <i>Tab.</i>	36	24	18	6	4	49	33	26	11	7	43	42	24	10	5
28. <i>syloes.</i> × <i>toment.</i>		88	30	15	9	64	60	21	15	9	36	25	19	7	4
29. <i>syloes.</i> × <i>Tab.</i>		92	31	16	10		62	33	16	6		53	29	13	5
30. <i>suav.</i> × <i>long.</i>		43	33	16	12		70	38	18	7		91	53	24	8
31. <i>suav.</i> × <i>glauca</i>		42	32	15	11		32	19	8	7		30	12	4	4
32. <i>suav.</i> × <i>glut.</i>		43	33	16	12		43	35	17	13		26	—	—	—
33. <i>suav.</i> × <i>Big.</i>		42	32	15	11		40	41	20	13		20	26	11	7
34. <i>Big.</i> × <i>glauca</i>		24	23	11	6		24	14	6	5		28	10	3.5	3.5
35. <i>Big.</i> × <i>glut.</i>		25	28	12	6		26	29	13	6		26	—	—	—
36. <i>Big.</i> × <i>toment.</i>	26	25	25	12	6	32	26	25	12	6	36	25	19	7	4
37. <i>Big.</i> × <i>suav.</i>		20	26	11	7		41	42	21	13		42	32	15	10
38. <i>Big.</i> × <i>Tab.</i>		25	28	12	6		35	24	12	6		40	22	10	5
39. <i>rust.</i> × <i>Sand.</i>															
40. <i>rust.</i> × <i>Langs.</i>		16	15	5	4		18	16	5	4		19	14	6	5
41. <i>rust.</i> × <i>glauca</i>															
42. <i>rust.</i> × <i>pan.</i>		15	15	5	4		20	16	5	4		30	10	3	3
43. <i>rust.</i> × <i>Big.</i>		15	14	4.5	4		25	23	10	8		25	24	12	7
44. <i>rust.</i> × <i>Tab.</i>		15	15	5	4		30	26	9	8		50	29	12	4
45. <i>Tab.</i> × <i>Sand.</i>		36	20	9	4		40	27	14	7		64	40	19	7
46. <i>Tab.</i> × <i>Langs.</i>															
47. <i>Tab.</i> × <i>glauca</i>		43	24	10	5		42	24	9	4		28	10	4	4
48. <i>Tab.</i> × <i>glut.</i>		42	30	12	6	41	36	30	13	7		25	—	—	—
49. <i>Tab.</i> × <i>toment.</i>	44	42	24	10	5	60	40	31	13	8	48	28	28	9	7
50. <i>Tab.</i> × <i>syloes.</i>		51	28	12	4		61	31	15	5		90	30	16	10
51. <i>Tab.</i> × <i>Big.</i>															
52. <i>Tab.</i> × <i>rust.</i>		46	29	11	4		33	24	9	7		19	19	7	5

Nicotiana, no matter what the chromosome numbers happen to be, show no significant differences. In figure 8, two flowers are shown representing reciprocals between *glauca* and *longiflora* (*plumbaginifolia*).

The flower measurements are spread on the record, with the hope that they may be useful, but I am not at all confident that statistical data of this kind will yield many acceptable generalizations. It seems to me that the situation here is similar to that with which geneticists were confronted at the beginning of this century, when the application of methods of gross analysis merely resulted in restricting progress.

The development of the flower can presumably be expressed by some type of exponential curve. One surmises, however, that the growth-curve of the flower is somewhat different from most growth-curves in that there is a marked activity at the opening of the flower, followed by a sudden cessation, rather than a gradually diminishing growth force. What we have here is a set of final measurements of total growth obtained when genoms $A+A$, $A+B$, and $B+B$ are involved.

Now when we examine the situation presented by the measurements listed in the table, certain matters are obvious; and they are obvious no matter how the figures are treated, whether arithmetically or logarithmically, whether as straight linear measurements or as combination indices. We see a developmental pattern always influenced in some respect by both parents. Sometimes the influence of one parent is hardly discernible in length of corolla or in spread of corolla or in the amount of lobing; but there is a harmonious development of the organ group as a whole, in which the activity of the genes of each parent can be traced. There is no heterosis (unless one so interprets the *Bigelovii* hybrids). I should interpret this fact as an indication, first, that hybridity is not accompanied here by some mysterious growth force inexplicable by gene activity, as has been suggested by some writers in an endeavor to explain heterosis; and, second, that there is seldom, if ever, a dominant action of genes in the production of a quantitative growth-pattern. Gene actions are additive in the sense that each would seem to have its effect on the final outcome of any particular part of the pattern; but they are not additive in the sense that two genes A have twice the effect of a single gene A .

When one compares the various crosses with *Tabacum*, it is clear that the twenty-four chromosomes of *Tabacum* sometimes appear to have an overwhelming effect on the end result when pitted against the effect of a smaller number of chromosomes, but this is, I think, illusory. And there are numerous examples where there is no such apparent effect. Consider, for example, the *Sanderæ-Tabacum* union, where the nine chromosomes of *Sanderæ* have such a distinct and positive effect. The same thing may be said, in general, when one takes any given species and compares the various

hybrids obtained with other species. No chromosome generalization holds. The pattern is the thing.

There is also a second point upon which there is substantial evidence.

FIGURE 1.—*N. rustica humilis*; *N. rustica humilis* × *N. paniculata*; *N. paniculata*. ×1.

In work with qualitative characters one often finds gene combinations that are epistatic to others, gene combinations that shut out the effects of other genes, genes that are suppressors, and genes which, conversely, have a

FIGURE 2.—*N. glauca*; *N. glauca* × *N. Langsdorffii*; *N. Langsdorffii*. ×1.

releasing effect on the activity of other genes. Little of this is in evidence in the behavior of the gene combinations which produce the flower patterns. The reactions are harmonious. There is only one exception in the table. The variety of *Bigelovii* used most frequently in crosses is the small-

flowered variety, *quadrivalvis*. The typical *Bigelovii*, however, has very much larger flowers (see figures 3 and 4). In crosses with *Bigelovii quadrivalvis* the hybrids obtained had larger flowers than one would expect, on the basis of what was found in hybrids between other species. It seems,

FIGURE 3.—*N. trigonophylla*; *N. trigonophylla* × *N. Bigelovii quadrivalvis*, *N. Bigelovii quadrivalvis*. ×3/4.

FIGURE 4.—*N. suaveolens*; *N. suaveolens* × *N. Bigelovii quadrivalvis*; *N. Bigelovii quadrivalvis*;
N. rustica texana × *N. Bigelovii quadrivalvis*; *N. rustica texana*. ×3/4.

therefore, as if *Bigelovii quadrivalvis* has a tendency for larger flowers that is suppressed in the pure variety but is released in species hybrids.

The exact reaction pattern that results in the particular type of hybrid flower obtained is more clearly seen in the photographs. Visualization of

the flowers from the measurements is much more difficult, since one can

FIGURE 5.—*N. longiflora*; *N. longiflora* × *N. Sanderæ*; *N. Sanderæ*. ×3/4.

FIGURE 6.—*N. Bigelovii quad.*; *N. Bigelovii quad.* × *N. Tabacum Havana*; *N. Tabacum Havana*; *N. Tabacum Havana* × *N. glutinosa*; *N. glutinosa*. ×3/4.

not get a clear idea of the width of the tube and has no data from which to reconstruct the shape of the tube.

Let us consider, first, the hybrids between the species which produce

the yellow-green flowers. These flowers are tubular without much spread to the limb. The three primary species which we have used are: *N. Langsdorffii* (9) of the *Sanderæ* group, and *N. glauca* (12) and *N. paniculata* (12), of the *glauca* group. The *paniculata* $\times$ *glauca* hybrid is, as one might expect, of an intermediate character, for the flowers of the parents are similar.

FIGURE 7.—*N. paniculata*; *N. Sanderæ alata* $\times$ *N. paniculata*; *N. Sanderæ alata*; *N. Langsdorffii* $\times$ *N. Sanderæ alata*; *N. Langsdorffii*. $\times 3/4$.

The size is approximately that of the *glauca* parent, the spread of the corolla being even slightly greater. The *glauca* (12) $\times$ *Langsdorffii* (9) hybrid (figure 2) is also intermediate with slightly more influence of *glauca* seen in the size and shape of the tube and in the characteristics of the calyx; but with the *Langsdorffii* influence obvious in the shape and size of the limb. The *rustica* (24) $\times$ *paniculata* (12) hybrid is shown in figure 1. *Paniculata* is presumably one of the components of *rustica*; and, owing possibly to the effect of the twenty-four other chromosomes, the hybrid

appears to stand somewhat closer to *rustica*. It is not certain, however, that this admission is in accordance with the facts, though a marked influence of *rustica* in this case would not invalidate our position any more than the marked influence of *alata* (9-chromosome) in crosses with species having higher chromosome numbers would demonstrate it. At all events, when the influence of the two parents of this hybrid is analyzed, length

FIGURE 8.—Upper row: *N. tomentosiformis*; *N. trigonophylla* (Palmeri) $\times$ *N. tomentosiformis*; *N. trigonophylla* (Palmeri); *N. glauca*; *N. tomentosa* $\times$ *N. glauca*; *N. tomentosa*. Lower row: *N. tomentosiformis* $\times$ *N. Bigelovii* quad.; *N. Bigelovii* quad.; *N. nudicaulis*; *N. tomentosa* $\times$ *N. nudicaulis*. $\times 3/4$

and breadth taken on a logarithmic basis and various minor effects considered, it is not too certain where the hybrid stands. Moreover, precise analysis of the situation would require a knowledge of the second constituent of *rustica*. Of it we know nothing, though it may be assumed, I believe, that the second component has a corolla limb that is more lobed than *paniculata*. No lobing is apparent in the *glauca-paniculata* or *glauca-Langsdorffii* unions; but whenever a pattern tendency for lobing is put

into a cross with one of these species, the effect of it is clear. The result is seen in figure 7, where *Sandera alata* is crossed both with *paniculata* (12) and with *Langsdorffii* (9). One may note: first, the similar corolla lobing in the two hybrids; second, the size and shape of the tube, with individual influences for *paniculata* and for *Langsdorffii*; third, the difference in the

FIGURE 9.—Upper row: *N. Tabacum mac.*×*N. tomentosiformis*; *N. Tabacum mac.*×*N. tomentosa*; *N. Tabacum mac.*×*N. glauca*; *N. nudicaulis*×*N. tomentosa*; *N. nudicaulis*×*N. tomentosiformis*; *N. Bigelovii quad.*×*N. tomentosiformis*. Lower row: *N. glauca*×*N. longiflora plumbaginifolia*; *N. longiflora plumbaginifolia*×*N. glauca*; *N. suaveolens*×*N. glauca*; *N. glutinosa*×*N. sylvestris*; *N. nudicaulis*×*N. trigonophylla* (Palmeri); *N. trigonophylla* (Palmeri)×*N. tomentosiformis*. ×3/4.

amount of hairiness (the *paniculata* being practically without hairs); and fourth, the different effects produced by the decidedly different calyxes of *paniculata* and of *Langsdorffii*.

Figures 3 and 4 show the flowers of the hybrids and of the parents when *Bigelovii quad.* (24) is crossed with the three species *trigonophylla* (12), *suaveolens* (16), and *rustica* (24). The great limb spread in the hybrid

corollas, mentioned earlier, is the most obvious point to be observed. The size is even larger where *trigonophylla* (12) enters the hybrid than it is where *rustica* (24) enters. The individual influences of *trigonophylla*, *suaveolens*, and *rustica* are easy to detect, however, in the shape and form of the limb and in the shape and form of the calyx. It is less easy to see how *trigonophylla* influences the shape of the corolla lobes, but it is clear in the

FIGURE 10.—Upper row: *N. paniculata*; *N. paniculata* × *N. Tabacum mac.*; *N. Tabacum max.* × *N. glauca*; *N. glauca*. Middle row: *N. rustica texana*; *N. rustica texana* × *N. Tabacum Cuba*; *N. Tabacum macrophylla*; *N. Tabacum mac.* × *N. tomentosiformis*; *N. tomentosiformis*. Lower row: *N. Tabacum Cuba* × *N. sylvestris*; *N. sylvestris*. ×3/4.

case of *suaveolens*. It is also clear in the slight lobing of the *rustica* hybrid, though the photograph does not show this characteristic very well.

Figure 5, the *longiflora* (10) × *Sanderæ* (9) hybrid, is a combination of members of two groups where the organ patterns are similar. The hybrid is consistently intermediate. In the photograph only the relative influences

of the two parents on size and shape of the tube are apparent; but in the living flowers, the influence of both parents on the texture is obvious.

One of the most interesting series (figures 5, 8, and 9) is the one illustrating hybrid unions made with *tomentosa* and *tomentosiformis*. In figure 9, upper row, flowers 1 and 2 are hybrids between *Tabacum macrophylla* (24) and *tomentosiformis* and *tomentosa*; while in the same row flowers 4 and 5

FIGURE 11.—Upper row: *N. paniculata*; *N. paniculata* × *N. Bigelovii*; *N. Bigelovii*; *N. Bigelovii* × *N. Tabacum lanc.*; *N. Tabacum lanc.* Center row: *N. Tabacum Cuba*; *N. Tabacum Cuba* × *N. paniculata*; *N. paniculata*. Lower row: *N. Sanderæ alata*; *N. Langsdorffii* × *N. Sanderæ alata*; *N. Langsdorffii*. ×3/4. Except center row. ×9/16.

are hybrids between *nudicaulis* (24) and *tomentosa* and *tomentosiformis*. In both instances the hybrids are similar in character. The sole distinguishing feature is the greater size of the flower where *tomentosa* is used. This is the result one should expect from the two species, as may be seen by referring to the flowers of the two parental types in figure 8 (flowers 1

and 6, upper row). Such results, plus the fact that *tomentosa* and *tomentosiformis* give hybrids which are perfectly fertile, would appear to be conclusive evidence that the two are simply varieties of one species. One or the other of these varieties is also shown in combination with *trigonophylla* (Palmeri) (12), *glauca* (12), and *Bigelovii* quad. (24) in figure 8. The points to be noted are the specific influences of *trigonophylla* (Palmeri), *glauca*, *Bigelovii*, *nudicaulis*, and *Tabacum* on shape and constriction of calyx, length, and breadth of the tube, location and type of swelling of throat, type of limb, shape of petal lobes, and height of pistils and stamens.

From these same figures, one may also see the reactions of *glauca* with *tomentosa* (figure 8), *longiflora plumbaginifolia*, *suaveolens*, and *Tabacum*. Figures of the reciprocals between *glauca* and *longiflora plumbaginifolia* are given in order that it may be seen how nearly identical they are.

The last and largest series is the group of crosses with *Tabacum*, shown in figure 10. There are combinations with five other species. To these figures, three other crosses are to be added—those with *glutinosa* and *Bigelovii* quad. in figure 6, and that with *glauca* in figure 9. A flower of *Tabacum macrophylla* is figured in figure 10. But, unfortunately, though I have made the cross between *Tabacum macrophylla* and *sylvestris*, I have no photograph of it. The hybrid shown is with the *Tabacum* variety Cuba, where the flower is nearly like that of the Havana variety illustrated in figure 6, but with slightly deeper lobes. The lobes of the actual Cuba flowers are seen in figure 11.

It is regrettable that I obtained no photographs of *Tabacum* hybrids with *Sanderæ* and with *Langsdorffii*, the 9-chromosome group. The *Tabacum-Langsdorffii* hybrid had flowers approaching *Tabacum* in size and shape. They were approximately three-fourths size, with very little chlorophyll discernible. Of all our photographs, they were most similar to the *paniculata-Tabacum* hybrid, though smaller. The *Tabacum-Sanderæ* (*alata*) hybrid is figured in EAST and HAYES (1912). They were one-fourth longer than those of the *Tabacum* parent, with the limb reminiscent of *Sanderæ-alata*, but somewhat narrowed. The swelling at the throat showed *Tabacum* influence, as did the texture. The fresh flowers resembled *Tabacum* somewhat more than the illustration shows. One could imagine that if the hybrids had been made with a smaller-flowered variety of *Sanderæ*, they would have resembled *Tabacum* still more closely. And this was actually the case in a hybrid produced here by CHRISTOFF, though one could still call them intermediate.

In a casual examination, one would be likely to say that the hybrids with *Langsdorffii* (9), *paniculata* (12), and *glauca* (12), appear to stand very close to *Tabacum*, but this is only because the flower types of these three species have less to add in changing the form than have some of the

other species. The *Tabacum-Sandera* hybrid, where the pattern could be changed more abruptly, exhibited a marked influence by the 9-chromosome species. And one can trace the influence of the greenish-yellow flowered species in the calyxes and tube shapes of the hybrids. The influences of *glutinosa* and of *tomentosa* are observable in the zygomorphism of the flowers, in the length of the pistils and stamens, in the shape of the limb and the type of the corolla lobes, in the length of the tube, and particularly in the proportions of constricted and of expanded tube. In combinations with *sylvestris*, the other 12-chromosome species that is thought to be a component of the allotetraploid *Tabacum*, the influence of *sylvestris* is seen chiefly in the texture of the corolla, length of tube, the form of the swollen portion of the tube, and the constriction at the throat.

The very different influences exerted by *Bigelovii* (24) and *rustica* (24) in unions with *Tabacum* need no special emphasis. But it is very doubtful whether these species show more influence in the developed characters of the hybrids than do the species having smaller chromosome numbers, when the possibilities of changing the pattern are analyzed closely. *Glaucia* and *paniculata* do not possess marked traits which can exert such influence. *Tomentosa*, *sylvestris*, *Bigelovii*, and *rustica* do possess characteristic patterns, and these patterns have their effect. To show what I mean by these statements, let us consider the two crosses with *paniculata* and with *rustica*. *Paniculata* and *rustica* have 12 and 24 chromosomes, respectively, and *paniculata* is a component of *rustica*. Moreover, they have somewhat similar flower patterns except for length and breadth of corolla. Does *rustica* appear to exert more influence on the form of the hybrid flower than *paniculata* in unions with *Tabacum*? After a casual examination, any observer would say that it does. But I believe that this is an illusion. The two hybrids both show the corolla limb of *Tabacum* with but slight modification. Where they differ is in the effect which *rustica* has in shortening and broadening the corolla tube. *Paniculata* has a slight swelling of the tube below the throat swelling. This is traceable in the hybrid. *Paniculata* has a more constricted throat than *rustica*. This pattern influence is also discernible in the hybrid.

Again, compare the combinations of *Tabacum macrophylla* with *glauca* (12) and with *tomentosiformis* (12). *Glaucia* has a straight tube (the figure is slightly flattened), with a funneled, unlobed limb. The calyx tube is also straight. And both calyx tube and corolla tube are almost without hairs. The length of the flower is about the same as that of *paniculata*. One should expect that the hybrid, therefore, should resemble *paniculata-Tabacum*, as to size and shape, but with the calyx modified toward that of *glauca*. Hairiness should be restricted more than in *paniculata-Tabacum*, as *paniculata* has more hairs on the calyx, though the corolla is as smooth as *glauca*.

These modifications are precisely what are obtained. *Tomentosiformis*, on the other hand, has numerous identifying characters, which may be noted in calyx shape, sepal lobes, open throat with exceptionally marked swelling, peculiar pointed corolla lobes, and extruded stamens and pistils. There is an opportunity, therefore, for modification of the flower pattern in numerous ways. And all these modifications can be traced in the hybrids.

Or, one may compare the crosses of *Tabacum Havana* with *glutinosa* (12) and *Bigelovii quad.* (24), as shown in figure 6. Size relations are intermediate in both hybrids. But *Bigelovii* has a more hairy calyx than *glutinosa*. The calyxes of the two hybrids differ as expected in this respect. And *glutinosa* has a very hairy corolla, while *Bigelovii* is nearly smooth. The corollas of the hybrids exhibit this same reversal of conditions. The most conspicuous character of *Bigelovii*, however, is the funnel-shaped flower, while the most conspicuous character of *glutinosa* is the zygomorphic flower, with its peculiarly swollen throat. The hybrids stand intermediate with regard to these characteristics. *Bigelovii* has no more influence than *glutinosa*.

Thus one may analyze the entire series of hybrids; and, when this is done, an unexpected result is obtained. *All constituents of the pattern are active in modifying the pattern of the hybrids, and the grade of these modifications is little affected by differences in the amount of chromatin contributed.*

Figure 11 is given in order that the reader may trace modifications in the corolla limb. The cross between *Tabacum Cuba* and *paniculata* is figured because it illustrates one of the few cases where the use of two rather similar varieties of a given species gives different hybrid patterns. The corolla limb of the hybrid with *Tabacum Cuba* shows only a faint lobing, while that of the hybrid with *Tabacum macrophylla* (figure 10) shows considerable lobing.

Leaf reaction patterns

Leaf reaction patterns are traceable as the effects of the genom activity of the constituents of these hybrids just as are the flower reaction patterns, but several matters must be taken into consideration if one is to get a clear idea of the situation. The size of the leaves is influenced markedly by external conditions. Leaf-size is also influenced decidedly by internal conditions. If the hybrids are vigorous, the leaves may be large; if the hybrids are weak, the leaves may be small. It seems as if the machine does not run properly in certain combinations. It is also to be noted that the determination of sessile or of petioled leaves appears to be controlled by relatively few genes, as in varietal crosses between sessile and petioled *Tabacum* varieties. The reaction pattern that seems to be comparable with the flower patterns is that which controls the shape or proportions of the

leaves. Evidence that this is true appears in figures 12-16, which were kindly drawn from life, under my supervision, by my assistant, Miss ESTELLA HUMPHREY. Relative size is merely indicated.

Figure 12 illustrates two hybrids between *paniculata* (12), and *glutinosa* (12) and *rustica* (24). The *paniculata-glutinosa* hybrid shows the influence of both parents in the proportions above and below the point where the broadest part of the leaf comes, and also in the shape at the base. The

FIGURE 12.—Leaves of crosses with *N. paniculata*.

rustica-paniculata hybrid is somewhat more like *rustica* than it is like *paniculata*. This is particularly true at the base, where there is no tendency toward a cordate condition, and also in the texture, which is thick and smooth like the *rustica* parent. There is a tendency, however, for the broader part of the hybrid leaf to be shifted somewhat toward the petiole, as in *paniculata*. This tendency is not difficult to see when a goodly number of plants is examined, though it is not so obvious in the leaf from which the drawing is made. The latter is as close to the modal condition as I could find.

Figure 13 pictures the leaves of three hybrids made with *suaveolens* (16)

as one of the parents. The other parents are *longiflora* (10), *glutinosa* (12), and *Bigelovii* (24). The modification toward an obovate leaf, through the influence of *longiflora*, is easily seen. It is also obvious that the effect of *glutinosa* has been to shift the point of greatest breadth well toward the base of the leaf. The hybrid has a distinct petiole, though the proportion of blade to petiole has been increased by the influence of *suaveolens*. The base is presumably what one should expect in a combination of the tendencies of the two parents. The extraordinary thing in this figure is the

FIGURE 13.—Leaves of crosses with *N. suaveolens*.

apparent lack of influence of the 24-chromosomed *Bigelovii* in its combination with *suaveolens*. *Bigelovii* has a very striking type of leaf, ovate with a constricted base possessing auricles. The leaf of the hybrid is very nearly like that of *suaveolens*, though intermediate in texture and having the disagreeable odor of *Bigelovii*. I have no explanation for this exception to the general rule. It matches the *paniculata* situation in the other direction.

Figure 14 shows the leaves of four hybrids where *paniculata* (12) was combined with species having sessile leaves. The other parents are *Langsdorffii* and *Sandera alata*, with 9 pairs of chromosomes, and *Bigelovii* and *Tabacum*, with 24 pairs of chromosomes. Both *Langsdorffii* and *Sandera*

alata are markedly rugose, with great fullness of tissue between the veins. *Paniculata* is relatively smooth in this respect, although covered with downy hairs. The hybrids are intermediate in both of these conditions. *Langsdorffii* differs from *Sanderæ alata*, however, in having a blunter tip. Comparable leaves of these species also show that the latter commonly have a greater proportion of narrowed blade than the former. The hybrids show the specific influence of the 9-chromosome parents in both of these

FIGURE 14.—Leaves of crosses with *N. paniculata*.

respects. The *Bigelovii* hybrid apparently resembles *Bigelovii* more than *paniculata* in shape and in basal constriction, though auricles are less in evidence. It should be noted, nevertheless, that though *Bigelovii* and *paniculata* are vastly different in relative length and breadth, they are rather similar in the proportionate amount of blade above and below the greatest breadth, if one imagines the proportions of *paniculata* drawn out into a sessile leaf. One may think of the sessile condition of the *Bigelovii* leaf as being partially dominant in this cross, while the petioled condition of *paniculata* is partially dominant in the *Tabacum* cross.

In figure 15, the leaves of parents and hybrids are reproduced for five crosses with the smooth, glaucous, petioled *glauc*. The other parents are *Langsdorffii* (9), *longiflora* (10), *glutinosa* (12), *tomentosiformis* (12), and *suaveolens* (16). None of the hybrids had bloom on the leaves, though where the texture of the leaf-tissue in the two parents differed, the hybrids were intermediate.

The leaves of *Langsdorffii* and of *longiflora* are superficially rather

FIGURE 15.—Leaves of crosses with *N. glauca*.

similar. Both are rugose. They differ especially in three characteristics: *Langsdorffii* is soft, downy-haired, and has almost an ovate leaf, if one disregards the narrower part; *longiflora* is harsh, prickly-haired, and has the greatest breadth somewhat further toward the tip. *Glauc* × *Langsdorffii* leaves have soft hairs, particularly when the leaves are young. They are attenuated more nearly to a petiole than *Langsdorffii*, and have the relative proportion of blade from the tip to the greatest breadth intermediate between the characteristics of the parents. *Glauc* × *longiflora* leaves have a few prickly hairs. They have a goodly portion of the total leaf length attenuated toward a petiole. And the relative proportion of

the blade from the tip to the greatest breadth is less than in the other hybrid (influence of *longiflora*).

The *glutinosa-glauca* hybrid has something of a type of its own, as if it had been stretched out. The part having the greatest breadth comes at about the point one should expect, but the leaf then narrows unexpectedly. There is no trace of the cordate condition of the two parents. This condition is not inexplicable, however, if one considers the venation of the two parents. *Glauca* is pinnately veined; *glutinosa* has a tendency toward radiate venation at the base, which gathers the tissue of the base into an extremely cordate form. Now if the effect of *glauca* on the leaf pattern is to draw out the radiate venation at the base into more of a pinnate form, the result is that presented in the hybrid.

The influence of both parents is obvious in the leaves of *glauca* × *tomentosiformis*. It is also traceable in those of *suaveolens* × *glauca*. But there is less influence of *suaveolens* in determining the point of greatest breadth than one would ordinarily expect. The leaf-tissue, however, is narrowed to a fringed petiole throughout over one-third of the total length. This tendency exists in *suaveolens*, and of course there is a true petiole in *glauca*. Taking the total proportions into consideration, therefore, one can see that the point of greatest breadth is actually shifted toward the tip by the influence of *suaveolens*.

Figure 16 illustrates various crosses made with *Tabacum*. The variety of *Tabacum* illustrated is Cuban, as four of the seven crosses represented were made with that variety. In the other three crosses, with *glutinosa*, *paniculata*, and *sylvestris*, respectively, the *Tabacum* variety used was Havana. Any one interested can find a photograph of a Havana leaf (together with a Cuban leaf) in HAYES, EAST and BEINHART (1913) on Plate XII. The Havana leaf is longer than the Cuban, and the place of greatest breadth is a little nearer the tip.

Four of these crosses are with species having petioled leaves, where the influence of the members of the parental pairs is not easy to analyze. I may point out, however, that the reactions of these four petioled types, *paniculata*, *glauca*, *glutinosa*, and *rustica texana*, with the sessile *Tabacum*, are unlike. *Glauca* × *Tabacum* has a true petiole; *paniculata* × *Tabacum* has almost a petiole, but the pseudo-petiole is fringed to the base and there expands into noticeable auricles; *Tabacum* × *glutinosa* is sessile, though narrowed at the base like several of the other species; and *Tabacum* × *rustica texana* (24 chromosomes), though influenced by the *rustica* shape, is narrowed to a short fringed petiole, followed at the base by fair-sized auricles.

The influences of *Sandera alata*, *longiflora*, and *sylvestris* are traceable in the form of the patterns, if one considers particularly the shape of the

base and the relative length of the blade to the point where the greatest breadth is seen. Individual influence is still more obvious in matters of

FIGURE 16.—Leaves of crosses with *N. Tabacum*.

texture, venation, color, rugosity, and hairiness, which are points that do not show in the illustrations. There appears to be no material quantita-

tive difference in the effects produced by the 9-chromosome species (*Sandera alata*), the 10-chromosome species (*longiflora*), the four 12-chromosome species (*glauca*, *glutinosa*, *paniculata*, and *sylvestris*), and the 24-chromosome species (*rustica*).

DISCUSSION AND SUMMARY

In this paper, data are submitted on about sixty hybrids between *Nicotiana* species. No attempt was made to describe each of these hybrids in minute detail, as might have been done had the objective been taxonomic. Such description as seemed essential has been given, plus photographs and measurements from which the reader may draw his own inferences, with the definite idea of trying to see whether useful conclusions could be drawn regarding the problem of phenotypic behavior in hybrid combination. The objective, in other words, is genetic. The treatment is the analysis of what occurs when a given species is crossed with several other species having the same or different chromosome numbers.

Four conclusions emerge. The first is reasonably conclusive; the other three, though somewhat heterodox, are strongly indicated.

1. On the basis of data from 15 pairs of genetically comparable reciprocals—that is, reciprocals made with identical pure parents or with sister plants belonging to near-homozygous lines—it may be stated that reciprocal hybrids in *Nicotiana* are as nearly identical as are plants in a pure line of a given *Nicotiana* species. This is true whether the parental forms have the same or different chromosome numbers.

In spite of the facts, therefore, that the genus has become highly differentiated and has developed forms having several different chromosome numbers, there is no evidence that the species have distinctive plasmons in the sense of VON WETTSTEIN.

2. The gene changes producing the qualitative effects, which differentiate minor varieties and are so largely used in genetic research, appear to be different, as a general group, from the gene changes which accumulate into specific differences. The latter are usually quantitative and are difficult of demonstration for this reason. The qualitative variations are usually easy to detect because they so often cause distortions, restrictions, or stoppage of what have come to be normal processes through natural selection. In their inheritance one finds these strange peculiarities: dominance, where *A* is sufficient to make the process go on almost as well as *AA*, but *a* restricts it; inhibitors; several genes necessary for the production of a detectable difference; and so on. Such genes, I am inclined to believe, are, by their nature, usually incapable of playing a part in natural evolution, though they may be very advantageous in building up knowledge of the heredity mechanism.

When such genic differences do not tend to depart too abnormally in their control of the life processes, they appear to be transmitted in species crosses as in variety crosses (color reactions). But this may not be the case if processes concerned happen to be of the more essential type that can be influenced materially by a large number of genes (sessile and petioled leaves). Here one finds phenomena such as FISHER imagined in his writings on the evolution of dominance. But the important point is that the effect of different residual germ plasma is detectable in various manners. There appears to be no effect of the type required to pass in one direction and evolve dominance, but rather an effect which produces a "pattern" that is different.

3. When one studies this series of hybrids by taking each species in turn and asking what is the effect on form when various other species are combined with it, one is considering combinations of gene processes that have survived natural selection. Each genome has possibilities of normal orderly processes rather than restricted, inhibited, inefficient processes. And when one does this, he is forced to the conclusion that the various genes of each genome are active in producing slight changes in developmental patterns, whether these patterns be in flowers, leaves, or habit of growth. There is, therefore, hardly any evidence of the phenomenon of dominance which is more or less characteristic of the other group of gene changes.

4. A detailed analysis of pattern effects gives *no* support to the idea that the influence of a genome is in some way proportional to its contribution of chromatin. Reactions are varied, it is true, but in a 9-24-chromosome combination, special effects are as likely to be exerted by the 9-chromosome parent as by the 24-chromosome parent. In the idea, as generally held, that chromatin has somewhat of a mass action, it would seem that there is a postulate of numbers of actively different genes in proportion to amount. It is obvious that this view is unsound if one stops to think a moment. A 9-chromosome *alata* plant has the ability to produce a complete *Nicotiana* with the special features of *N. alata*, just as the 24-chromosome *rustica* plant has the ability to produce a complete *Nicotiana* with the special features of *N. rustica*. The reason we get pattern effects here and dominant (often) effects in certain varietal crosses is, as I believe, inherent in the type of reaction.

The current view would probably not have been advanced if a reasonably large series of species hybrids had been analyzed in the manner suggested here.

LITERATURE CITED

- EAST, E. M., 1916 Significant accuracy in recording genetic data. *Amer. J. Bot.* **5**: 211-222.
1928 The genetics of the genus *Nicotiana*. *Bibliogr. Genet.* **4**: 243-320.
EAST, E. M. and HAYES, H. K., 1912 Inheritance in maize. *Conn. Agric. Expt. Sta. Bull.* **167**: 1-142.

- GOODSPEED, T. H., 1933 Chromosome number and morphology in *Nicotiana*. VI. Chromosome numbers of forty species. *Proc. Nat. Acad. Sci. Washington* **19**: 649-653.
- GOODSPEED, T. H. and CLAUSEN, R. E., 1922 Interspecific hybridization in *Nicotiana*. I. On the results of backcrossing the F₁ *sylvestris-Tabacum* hybrids to *sylvestris*. *Univ. California Publ. Bot.* **11**: 1-30.
- HAYES, H. K., EAST, E. M. and BEINHART, E. G., 1913 Tobacco breeding in Connecticut. *Conn. Agric. Expt. Sta. Bull.* **176**: 1-68.
- SETCHELL, W. A., GOODSPEED, T. H. and CLAUSEN, R. E., 1922 Inheritance in *N. Tabacum*. I. A report on the results of crossing certain varieties. *Univ. California Publ. Bot.* **5**: 457-582.