COMPATIBILITY OF CERTAIN NICOTIANA SPECIES

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INTRODUCTION

The genus *Nicotiana* has been the subject of many cytological and genetic investigations. Much yet remains to be done, however, before the phylogeny of this rather large group of species is well understood. At the outset of the work herein reported it was thought advisable, first, to extend the available information on the compatibility and relationships of the numerous species of the genus by making a number of crosses, especially some not previously attempted. The second problem undertaken has grown out of the first. In the course of the crossing together of different species a partially fertile triple species hybrid was produced (*rustica* var. *humilis* $\times$ *paniculata*) $\times$ *Langsdorffii*. A discussion of the cytological and genetic nature of these plants together with a brief discussion of their progeny, will be published as a separate paper. The third and last problem to be undertaken as a part of this study, while more distinct, is still concerned with a study of interspecific hybrids. Some hybrids are very vigorous and also fertile. Others are scarcely able to germinate, or, if they grow at all, they are always weak and unproductive. In the case of other crosses capsules are formed but no viable seeds are produced. Several questions may be raised about the development of hybrid embryos in these different categories. A third paper, also in preparation, will discuss this phase of the work.

The investigations were begun in the fall of 1927 but had to be discontinued from August 1928 until September 1930, when another leave of absence from his regular work made it possible for the writer to resume his studies. Nearly all the F_1 seed was produced and many hybrids grown in 1928, but several lots of the F_1 seed were not planted until October 1930. This lapse of two years, then, must be borne in mind in considering the viability of these hybrids, and will be referred to again in that connection.

MATERIALS AND METHODS

All the plants and seeds used in these investigations were obtained at Bussey Institution, HARVARD UNIVERSITY, where the work was done. Most of the species had been under observation there for several years

and have been described in numerous papers. All the plants used were grown in a screened greenhouse, and the usual precautions taken to prevent accidental crossing. All the pollinations were made by hand except as otherwise noted.

Seeds were germinated in sterilized soil in the seed bench, and the seedlings allowed to grow there until large enough to transplant.

EXPERIMENTS AND RESULTS

All of the very considerable amount of work done on this subject from the time of KÖLREUTER and GAERTNER up to 1928 has been summed up and brought together in one comprehensive paper by EAST (1928). This publication gives the authority for all species used except one, *Cavanillesii*, Dun. A few other publications will be referred to later.

The work of the present writer on the genus began with a number of F_1 hybrids he obtained from Mr. M. CHRISTOFF. These were described by CHRISTOFF (1928), but had not been adequately tested for fertility. During the fall of 1927 and the following winter these plants of 11 hybrid combinations were selfed and backcrossed to both parents, the number of pollinations of each kind varying usually from 10 to 110, 25 to 50 being the more common number. All of these hybrids failed to produce any viable seed. They were as follows: *suaveolens* $\times$ *longiflora*, *suaveolens* $\times$ *glutinosa*, *paniculata* $\times$ *glutinosa*, *plumbaginifolia* $\times$ *Langsdorffii*, *nudicaulis* $\times$ *trigonophylla*, *paniculata* $\times$ *Sanderae*, *Bigelovii* $\times$ *Tabacum* var. *macrophylla*, *longiflora* $\times$ *alata*, *glutinosa* $\times$ *nudicaulis*, *suaveolens* $\times$ *plumbaginifolia*, and *plumbaginifolia* $\times$ *alata*.

My own crosses in some cases were a repetition of the work of others, but for the most part new combinations were tried. Altogether 195 combinations were made between 21 species. In a few cases some of these might be considered duplicates since more than one variety of *rustica* and *Tabacum* were crossed with the same species, not always with exactly the same result, however. These 21 species used, their gametic chromosome numbers, and a summary of results of the crosses made, are shown in table 1. The number of pollinations made in each case was from 1 to 14, probably averaging about 5.

As may be seen from the table, 15 of the 195 crosses made, marked (v), gave vigorous hybrids. These were as follows: *Langsdorffii* $\times$ *glaucal*, *glaucal* $\times$ *plumbaginifolia*, *paniculata* $\times$ *glaucal*, *Palmeri* $\times$ *Rusbyi*, *Palmeri* $\times$ *tomentosa*, *trigonophylla* $\times$ *tomentosa*, *paniculata* $\times$ *rustica* var. *humilis*, *suaveolens* $\times$ *glaucal*, *nudicaulis* $\times$ *glaucal*, *nudicaulis* $\times$ *Palmeri*, *nudicaulis* $\times$ *Rusbyi*, *Bigelovii* $\times$ *glaucal*, *Tabacum* var. *macrophylla* $\times$ *glutinosa*, *trigonophylla*

× *Palmeri*, and *Tabacum* var. *macrophylla* × *tomentosa*. Nine of these crosses are believed to be here reported for the first time, *Langsdorffii* × *glauca*, *glauca* × *plumbaginifolia*, *Palmeri* × *Rusbyi*, *Palmeri* × *tomentosa*, *suaveolens* × *glauca*, *nudicaulis* × *Palmeri*, *nudicaulis* × *Rusbyi*, *Bigelovii* × *glauca*, and *trigonophylla* × *Palmeri*.

Four other hybrid combinations, *plumbaginifolia* × *glauca*, *glutinosa* × *glauca*, *glutinosa* × *sylvestris*, and *glutinosa* × *tomentosa*, designated by (y), gave plants which seemed to be vigorous but which were discarded in an

FIGURE 1.—Deformed plant, *Bigelovii* × *glauca*.

immature condition when the work was discontinued, and this point could not be exactly determined. They had, however, already definitely demonstrated their hybrid nature by certain paternal characters. The first and last of this group are new hybrids.

Some of these 19 hybrids will be described later but it is interesting to note here in passing that five of the group grown in 1928, *Langsdorffii* × *glauca*, *glauca* × *plumbaginifolia*, *Bigelovii* × *glauca*, *glutinosa* × *glauca* and *nudicaulis* × *glauca*, showed a very noticeable lack of uniformity among the

plants grown, of which there were usually five. Another lot of *Bigelovii* × *glauca* seed grown in 1931 showed again the same kind of variation. The differences involved practically all leaf characters and in some cases were quite striking. In *Bigelovii* × *glauca* some of the plants had numerous thick, rubbery, dark colored, curly, asymmetrical leaves growing close together. These plants made a very poor growth and had a dwarfed, distorted appearance. Such a plant is shown in figure 1. The growth indicated here was accomplished only after liberal applications of sodium nitrate. *Nicotiana glutinosa* × *glauca* and *nudicaulis* × *glauca* exhibited similar abnormalities in the texture and symmetry of the leaves. It is interesting to note further that each of these five hybrids had *glauca* as one parent. There appears to be nothing in our observations to explain the variability noted.

This group of 19 hybrids includes one pair of reciprocals, the crosses between *plumbaginifolia* and *glauca*.

Seven other combinations, indicated in the table by (w), produced hybrids which made only a weak growth, namely, *glutinosa* × *Rusbyi*, *Bigelovii* × *tomentosa*, *nudicaulis* × *sylvestris*, *rustica* var. *humilis* × *Langsdorffii*, *Tabacum* var. *angustifolia* × *glutinosa*, *nudicaulis* × *Tabacum* var. *purpurea*, and *suaveolens* × *Tabacum* var. *purpurea*. The first three of these also are believed not to have appeared in the literature before. The other four had already been produced by EAST, CHRISTOFF, and KOSTOFF (EAST 1928). The *Tabacum* × *glutinosa* cross has usually given vigorous plants, but my two year old seeds may have lost some of their vigor. It was at first thought that possibly some unfavorable environmental factor was stopping the growth of these seedlings, so more were transplanted as a check. Altogether 18 were tested and some given special care. Nevertheless all died at the same young seedling stage, except one. This one plant which made a normal growth, and which proved to be a haploid *Tabacum* with all the maternal characters, has already been described (McCRAE 1932).

Eleven other combinations, marked (s) in the table, produced seed which has not been tested. In several cases data were already available from the work of other investigators, showing that hybrids are obtained from these crosses. This is not true, however, so far as I am aware, in the case of the seeds obtained from the cross *caudigera* × *acuminata*. The viability of this cross remains unknown, but from the similarity of the parents one might expect it would be successful.

Fifty-one other crosses, indicated by (d), produced some seed which, however, either failed to grow or was so evidently parthenocarpic that it was discarded. Five of these lots did give some plants but they were pure

maternals. In the 108 cases designated by (o) the flowers dropped before maturity.

Descriptions are available for the 11 new hybrids which made any considerable growth and will be given in the order in which they were originally named.

Langsdorffii × *glauca* plants made a tall vigorous growth. Three of them were viscid like *Langsdorffii* and had comparatively thick soft leaves. Two others were non-viscid, with a very short pubescence, and leaves much

FIGURE 2.—a, flower of *Palmeri* × *Rusbyi*; b, flower of *Palmeri*.

thinner and smoother than the others, resembling in these traits *glauca* somewhat more than *Langsdorffii*.

N. glauca × *plumbaginifolia* likewise produced vigorous plants showing marked variations. Three were slightly viscid with a fairly thick pubescence. Two others had little or none of either condition. In leaf form they resembled both parents. The leaves were rather broadly ovate with either acute or obtuse tips and bases, and were sessile with a narrow wing running down the midrib, showing clearly here the influence of the male parent. As in the preceding cross the plants differed considerably also in leaf texture. One had quite thick leaves, one very thin, while three were

intermediate in this respect. On one of these plants the leaves were rather bullate and rough to the touch. The flowers of this cross resembled the male parent somewhat more closely, being green in color and having the corolla limb sharply lobed. The length of the lobes, however, was only about 4 mm so the flower was narrow as in *glauca*.

The plants of *Palmeri* × *Rusbyi* are vigorous and have a long, fine, woolly pubescence, and large leaves, up to 7½ inches in length, pubescent on both

FIGURE 3.—a, flower of *suaveolens*; b, flower of *suaveolens* × *glauca*; c, flower of *glauca*.

sides. In these traits they stand somewhat closer to *Rusbyi*. In other leaf characters the parents are similar. The flowers of this cross are compared with those of *Palmeri* in figure 2.

The cross *Palmeri* × *tomentosa* closely resembles the last, as would be expected from the similarity of *Rusbyi* and *tomentosa*. Where the parents differ much in leaf and stem characters the hybrids resemble *tomentosa* somewhat more closely. They did not flower during the period of observation.

The plants of the cross *suaveolens* × *glauca* were like *suaveolens* in most leaf characters, but showed the *glauca* influence by having the base of the leaf reduced to a very narrow wing so the leaves were almost petiolate. The flowers of the hybrid and its parents are illustrated in figure 3.

Nicotiana nudicaulis × *Palmeri* produces hybrid offspring much more like *nudicaulis* than like *Palmeri*, but they resemble the latter in having somewhat lanceolate leaves and pointed lobes on the corolla limb, instead of rounding as in *nudicaulis*.

Nicotiana nudicaulis × *Rusbyi* is like *Rusbyi* in practically all vegetative characters, but is intermediate in flower size and color, and in the shape

FIGURE 4.—B, flower of *Bigelovii*; B × Gc, flower of *Bigelovii* × *glauca*; Gc, flower of *glauca*.

of the corolla lobes. Two flowers averaged 12 mm across the corolla limb and 18 mm in length. Corresponding measurements for *nudicaulis* and *Rusbyi*, respectively, are 8 × 14 mm and 28.5 × 40 mm. The corolla tubes are green, more like *nudicaulis*, but the limb has a faint pink color from the *Rusbyi* parent. In pure *nudicaulis* the lobes of the corolla limb are rounded and shallow. In *Rusbyi* they are deeper and acute pointed. A combination of these traits makes the lobes in the hybrid shallow with acuminate tips.

A partial description of *Bigelovii* × *glauca* has already been given in noting some of the differences between the plants. The leaves were

smooth, more like *glauca*. The inflorescence also was more like that of *glauca*, each branch bearing a small panicle. The flowers were intermediate in length of corolla tube, and length, width, and shape of the limb, as shown in figure 4. In color, however, they were white like *Bigelovii*.

Nicotiana trigonophylla and *Palmeri* are so very similar that it was to be expected, as pointed out by EAST (1928), that they should cross easily and produce at least partially fertile hybrids. Such is the case. At least, each pollination made between them, crossing both ways, produced a good sized capsule of seed in 1928. When planted in 1930, the *Palmeri* $\times$ *tri-*

FIGURE 5.—a, flower of *Tabacum* var. *macrophylla*; b, flower of *Tabacum* var. *macrophylla* $\times$ *glutinosa*; c, flower of *glutinosa*.

gonophylla seed failed to germinate. One of the reciprocal hybrids, however, germinated, grew vigorously, and bore numerous capsules apparently full of seed, both from hand selfing and from natural selfing. This plant naturally resembled both parents closely. About the only distinctive difference between the parents is a larger flower size in *Palmeri*. This trait is also displayed by the hybrid.

Nicotiana plumbaginifolia $\times$ *glauca*, like *suaveolens* $\times$ *glauca*, showed the *glauca* influence by having the bases of its sessile leaves reduced to a very narrow wing, making the leaves almost petiolate. They seemed to be more upright in growth also than is *plumbaginifolia*.

Nicotiana glutinosa $\times$ *tomentosa* is also distinguished from its maternal parent by leaf shape. The latter has petiolate leaves with a cordate base, while the hybrid has sessile leaves like *tomentosa*.

Another hybrid which has been described before is pictured in figures 5 and 6, which show flowers and leaves of the cross *Tabacum* var. *macrophylla* $\times$ *glutinosa*, and of its parents.

FIGURE 6.—a, leaf of *Tabacum* var. *macrophylla*; b, leaf of *Tabacum* var. *macrophylla* $\times$ *glutinosa*; c, leaf of *glutinosa*.

Only two of my hybrids, *nudicaulis* $\times$ *glauca* and *paniculata* $\times$ *glauca*, had matured when left in 1928, and as most of the hybrids produced that season did not grow again in 1930, the tests of fertility are very limited. The former of these two was pollinated only with *glauca* pollen, 4 times. Two capsules resulted, one of which was about $\frac{1}{4}$ full of seeds. They failed to germinate in 1930. *N. paniculata* $\times$ *glauca* was pollinated 16 times with *paniculata* pollen, 11 times with *glauca* pollen, and selfed once, but produced no capsules at all.

One *Bigelovii* × *glauca* plant was selfed once and backcrossed 11 times to the female parent and 3 times to the male. No capsules resulted.

The apparent fertility of *trigonophylla* × *Palmeri* has already been mentioned. The seeds produced by selfing have not yet been tested.

DISCUSSION

Where the crosses made here parallel other investigations, no important differences in the results were found. Quite likely some of the hybrid seed which failed to grow in 1930 might have grown in 1928. Out of 35 kinds of seed planted in October 1930, 12 which had grown before failed to germinate. Two others, *glutinosa* × *sylvestris* and *Tabacum* var. *angustifolia* × *glutinosa*, developed only into weak unsuccessful plants, whereas earlier tests have given vigorous hybrids in these cases. But at the same time so many of these F_1 seeds failed, in 5 cases out of the 35, maternals were produced in 1930 and these plants were vigorous and made a normal growth. This would seem to indicate that pure seed in these species retains its viability longer than hybrid seed.

It has been suggested (CHRISTOFF 1928) that in this genus the inter-specific hybrids are easier to make if the species with the larger chromosome number is used as the female, and that (EAST 1928) the hybrid plants tend to resemble the species with the larger number of chromosomes. Some support is given the first claim by the data here presented. The two parallel lines in table one enclose crosses between species having 12 chromosomes each. Even a casual inspection of the table reveals the fact that most of the hybrids produced lie either in this rectangle or immediately below it, where the female parent has a larger chromosome number than the male. More explicitly, out of 23 hybrids produced, 11 had the higher chromosome species as the female parent. In 9 cases each species had 12 chromosomes, and in only 3 the male parent had the higher chromosome number. To look at the same problem in another way, in seven cases involving two different chromosome numbers the crosses were successful when the female had the larger chromosome number, but in only one case, *glauca* × *plumbaginifolia*, was the reciprocal, *plumbaginifolia* × *glauca*, obtained.

A more complete test of this hypothesis is secured by considering the 79 hybrids charted in EAST's table 2, together with my 14 new hybrids, which gives a total of 93 crosses between 22 species. The number of crosses entered into by each species is shown in table 2.

Twelve of these 22 species varying in chromosome number all the way from 9 to 24 showed about the same compatibility with other species

TABLE 2

Compatibility of certain Nicotiana species used as ♀ and as ♂.

SPECIES MORE OFTEN SUCCESSFUL AS ♀				SPECIES MORE OFTEN SUCCESSFUL AS ♂			
SPECIES	CHROMOSOME NUMBER	CROSSES AS ♀	CROSSES AS ♂	SPECIES	CHROMOSOME NUMBER	CROSSES AS ♀	CROSSES AS ♂
<i>rustica</i>	24	7	1	<i>Rusbyi</i>	12	3	6
<i>glutinosa</i>	12	8	4	<i>tomentosa</i>	12	3	9
<i>paniculata</i>	12	9	3	<i>Langsdorffii</i>	9	4	8
<i>suaveolens</i>	16	8	4	<i>sylvestris</i>	12	2	4
<i>nudicaulis</i>	24	7	2	<i>Sanderae</i>	9	2	7

Species about equally successful whether used as ♀ or as ♂.

<i>Tabacum</i>	24	11	9	<i>Bigelovii</i>	24	6	7
<i>glauca</i>	12	7	10	<i>longiflora</i>	10	3	4
<i>alata</i>	9	5	7	<i>plumbaginifolia</i>	10	4	3
<i>acuminata</i>	12	0	1	<i>trigonophylla</i>	12	2	1
<i>caudigera</i>	12	0	0	<i>Palmeri</i>	12	2	2
<i>Cavanillesii</i>	12	0	0	<i>attenuata</i>	24	0	1

whether used as male or as female, and may therefore be left out of account. But not so with the other ten. Five of these species, *rustica*, *glutinosa*, *paniculata*, *suaveolens* and *nudicaulis*, succeed from 2 to 7 times as often in producing hybrids when used as the female as when used as the male. It will be noted that all of these species have from 12 to 24 chromosomes. Five other species, *Rusbyi*, *tomentosa*, *Langsdorffii*, *sylvestris* and *Sanderae*, succeed from 2 to 3 times as often as males as they do as females. And these have only from 9 to 12 chromosomes. In other words species with large chromosome numbers show somewhat more compatibility with other species when used as the female; and species with low chromosome numbers show somewhat more compatibility when used as males. Or, as CHRISTOFF suggests, crossing in *Nicotiana* is somewhat more likely to succeed when the species with the larger chromosome number is used as the female.

The evidence on the relation of chromosome number and resemblance of the hybrid to its parents is not so clear. Five of my crosses definitely resembled one of the parents more than the other. Three of these, *Bigelovii* × *glauca*, *nudicaulis* × *Palmeri*, and *nudicaulis* × *glauca*, appeared like the parent with the larger chromosome number, while the other two, *nudicaulis* × *Rusbyi*, and *Langsdorffii* × *glauca*, resembled more closely the species having the smaller chromosome number. But considering all the 93 crosses already mentioned, there are 22 which, in the opinion of the ob-

servers, resembled more closely the parent having the larger chromosome number; and only 3 which showed more resemblance to the parent with the lower chromosome number, *paniculata* × *alata* being the lone exception in addition to my two crosses just mentioned.

A third question might be raised, namely, how much correlation there is between the taxonomic relationship of the species and compatibility. I have tested the matter by classifying the 93 hybrids as shown in table 3, using for the purpose the taxonomic classifications followed by EAST, putting all the species into three groups, namely, *Tabacum*, *Rustica* and Petunioides. It should be noted here however that this writer questions the justification for the last two groups, and especially for Petunioides, and suggests that more complete information may warrant several subdivisions of the genus.

TABLE 3
Taxonomic relationship and compatibility of species.

NUMBER OF F ₁ HYBRIDS SECURED BETWEEN SPECIES OF THE THREE GROUPS OF NICOTIANA				
♀ \ ♂	<i>Tabacum</i>	<i>Rustica</i>	PETUNIOIDES	TOTAL
<i>Tabacum</i>	5	5	7	17
<i>Rustica</i>	8	9	18	35
Petunioides	11	12	18	41
Total	24	26	43	93

It will be seen from the table that there is little or no relationship between the present taxonomic standing of these species and their compatibility. The species of one group apparently cross about as freely with those of another group as with other members of their own group. And, in as much as many of these hybrids are the result of crossing very unlike species, it does not seem that the mere ability to produce hybrid progeny upon crossing would serve much better as a test of relationship of species in this genus. If a genetic basis of such classification were to be employed perhaps fertility of the hybrid would have to be used.

To summarize, the ability of *Nicotiana* species to produce F₁ hybrids seems to depend more upon the chromosome numbers involved than upon anything else; and the phenotype of the hybrid when produced usually is more like that of the parent having the higher chromosome number. Probably we may also conclude then that the resemblance of a hybrid to the parent species is not often determined by just a few dominant factors but rather by a relatively large number of factors scattered through the

chromosomes; and that, on the whole, the more chromosomes a species has the more likely is it that its hybrid progeny will exhibit its somatic characters.

Three species included in the investigation, namely, *Rusbyi*, *Palmeri* and *tomentosa*, have been used but little in earlier studies. It will be in order then to call special attention to their behavior in crossing with other species. *Rusbyi*, the hybrids of which with *Tabacum* have been made the subject of intensive study by BRIEGER (1928), was here crossed with 15 species as female, and with 14 as male. Only 3 hybrids resulted, *glutinosa* × *Rusbyi*, which was weak, and *Palmeri* × *Rusbyi* and *nudicaulis* × *Rusbyi*, which have already been described.

Nicotiana tomentosa, used in exactly the same number of crosses, and in most instances with the same species, produced 5 hybrids, and, as in the case of *Rusbyi*, always when it was used as the pollen parent. *Palmeri* × *tomentosa*, *trigonophylla* × *tomentosa*, and *Tabacum* var. *macrophylla* × *tomentosa* were vigorous plants, *Bigelovii* × *tomentosa* weak, and *glutinosa* × *tomentosa* apparently vigorous, but they did not mature during the period of observation.

Palmeri succeeded twice as female and twice as male in producing hybrids, which were vigorous in each case. Altogether the pollen of 16 species was used upon it, and its pollen was used on 18 other species. The 4 hybrids, *Palmeri* × *tomentosa*, *Palmeri* × *Rusbyi*, *nudicaulis* × *Palmeri*, and *trigonophylla* × *Palmeri*, have already been described.

The last combination is interesting because of the interfertility of these species. *Palmeri* and *trigonophylla* are so nearly alike in all somatic characters except flower size that they would defy differentiation; and a high degree of compatibility, if not fertility, was to be expected. If one may judge from the behavior of the single plant, the strains of these species used seem to breed together as freely as varieties, resembling, in this respect, *longiflora* and *plumbaginifolia* (EAST 1928, and other papers).

SUMMARY

1. The compatibility of 21 *Nicotiana* species is reported, 195 different combinations between them having been tried, many of them hitherto unattempted.

2. Fourteen new hybrids are listed, namely, *Langsdorffii* × *glauca*, *glauca* × *plumbaginifolia*, *Palmeri* × *Rusbyi*, *Palmeri* × *tomentosa*, *suaveolens* × *glauca*, *nudicaulis* × *Palmeri*, *nudicaulis* × *Rusbyi*, *Bigelovii* × *glauca*, *trigonophylla* × *Palmeri*, *plumbaginifolia* × *glauca*, *glutinosa* × *tomentosa*, *glutinosa* × *Rusbyi*, *Bigelovii* × *tomentosa*, and *nudicaulis* × *sylvestris*. Of these

the first 9 at least and probably 11 are vigorous in growth. The last three are weak. The first 11 are described, although not fully in all cases as some of them were immature when studied.

3. Supporting earlier observations, it seems that crosses are more likely to be successful if the species with the larger chromosome number is used as the female. There were seven cases in which two species with different chromosome numbers were crossed both ways, and in which the cross was successful when the female had the larger chromosome number. Only one of the reciprocal crosses produced a hybrid, *plumbaginifolia* $\times$ *glauca*. Out of 23 hybrids reported, 9 were produced by crossing a species of smaller chromosome number on a species with a larger chromosome number. Only 3 crosses were of the opposite type. In the 11 other cases the two species had the same chromosome number.

Evidence secured from a study of 79 other hybrids included in EAST's summary also shows a strong tendency for species in this genus to enter into interspecific crosses more readily when the species having the larger chromosome number is used as the female. Five of the 22 species involved in the combined list of 93 different hybrids having 12 to 24 chromosomes made many more hybrids when used as females; and 5 others having only 9 to 12 chromosomes produced many more hybrids when used as males.

4. The suggestion that the hybrids between these species more often bear a close resemblance to the parent having the higher chromosome number than to the other parent is also supported. The matter could be tested in only 5 hybrids among those produced by the writer, but including again the 79 others recorded by EAST, 22 of the combined group of 93, according to the observers, looked more like the parent with the larger number of chromosomes, and only 3 looked more like the other parent.

5. When these 93 hybrids were classified in a frequency table according to the taxonomic position of the parent species, no correlation could be detected between taxonomic relationship and ability to cross with other species. Inter-group crosses are about as numerous as crosses between species within a group.

6. Among the 21 species tested are 3 which have been used but little in earlier studies of compatibility, *Rusbyi*, *tomentosa*, and *Palmeri*. *Rusbyi* was crossed with 15 species as female and with 14 as male. Only 3 hybrids resulted, *glutinosa* $\times$ *Rusbyi*, which was weak, and *nudicaulis* $\times$ *Rusbyi* and *Palmeri* $\times$ *Rusbyi*, both of which were vigorous. *Nicotiana tomentosa*, used in the same number of crosses, produced 5 hybrids, always when used as male, *Palmeri* $\times$ *tomentosa*, *trigonophylla* $\times$ *tomentosa*, *Tabacum* var. *macrophylla* $\times$ *tomentosa*, and *glutinosa* $\times$ *tomentosa*, which were strong, and *Bige-*

lovii × *tomentosa* which was weak. *Palmeri* succeeded in producing 4 crosses, 2 when used as female and 2 when used as male. All of these hybrids, *Palmeri* × *tomentosa*, *Palmeri* × *Rusbyi*, *nudicaulis* × *Palmeri*, and *trigonophylla* × *Palmeri*, were vigorous. It was used 16 times as female and 18 times as male.

7. The species entering into the most crosses are *Tabacum*, *glauca* and *Bigelovii*, which accounted for 20, 17 and 13 respectively of the total of 93 crosses. Six other species, *glutinosa*, *paniculata*, *suaveolens*, *tomentosa*, *Langsdorffii* and *alata* participated in 12 crosses each.

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