GENETIC REACTIONS IN NICOTIANA. I. COMPATIBILITY

E. M. EAST

Harvard University, Bussey Institution, Jamaica Plain, Mass.

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GOODSPEED (1933) has summarized his conclusions as to the cytogenetic relationships of some 40 species of *Nicotianas* which he has had under observation at the UNIVERSITY OF CALIFORNIA for a number of years. The American species possess 9, 10, 12, or 24 pairs of chromosomes. [There is no dispute about the chromosome numbers for the various species except a so-called *N. viscosa* listed by CHRISTOFF (1928) as having 24 pairs. I suspect that the form was wrongly identified but can not suggest the correct designation. Though the work of CHRISTOFF was done in this laboratory, I have no notes on these plants and have no seeds from which to grow them]. The Australasian species, all apparently closely related, possess 16, 20, 22, 24, or 32 pairs. After considering both the genetic and the taxonomic evidence, he groups the species as follows. There is an *alata* group, including *N. alata* Link and Otto, *N. Langsdorffii* Weinm., *N. Sanderæ* Hort., and *N. bonariensis* Lehm. ($n=9$); a *longiflora* group, consisting of *N. longiflora* Cav. and *N. plumbaginifolia* Viv. ($n=10$); a *tomentosa* group, including *N. tomentosa* Ruiz and Pavon, *N. tomentosiformis* Goodspeed, *N. glutinosa* L., and *N. wigandioides* Koch and Fint. ($n=12$); a *glauca* group, containing *N. glauca* Grah., *N. Raimondii* Macbride, *N. paniculata* L., *N. solanifolia* Walp., *N. cordifolia* Phil., and *N. undulata* Ruiz and Pavon ($n=12$); a *Cavanillesii* group, including *N. Cavanillesii* Dun., and *N. pampasana* O. Kuntze ($n=12$); an *acuminata* group, containing *N. acuminata* (Grah.) Hook., *N. caudigera* Phil., and *N. attenuata* Torr. ($n=12$); and a *repanda* group, consisting of *N. repanda* Willd., *N. Stocktoni* Brandg., and *N. nesophila* Johnston ($n=24$). There is also a natural Australasian group as noted above, typified by *N. suaveolens*. Certain, if not all, of the 24-chromosome species are believed to be allopolyploids. For example, there is considerable evidence that *N. Tabacum* derives from hybrids between a member of the *tomentosa* group and *N. sylvestris*; and there is somewhat less convincing evidence that *N. rustica* involves two species of the *glauca* group, of which *N. paniculata* is almost certainly one, and that *N. Bigelovii* S. Wats. involves *N. attenuata* Torr. and possibly *N. trigonophylla* Dun.

Since GOODSPEED and CLAUSEN have concentrated their attention upon this genus for more than twenty years, and since their studies have involved several Australasian and American species not used by other workers, one may accept the above idea of the evolution of the group as the best available. I may add that my own experience, though more casual,

leads me to endorse it in all its essential points. I should not distinguish *N. plumbaginifolia* Viv. from *N. longiflora* Cav. or *N. tomentosiformis* Goodspeed from *N. tomentosa* Ruiz and Pavon as being forms worthy of specific rank, and I should question several other points; but these are minor details. [The stocks which I have had of *N. plumbaginifolia* cross readily with *N. longiflora*, and those of *N. tomentosiformis* (called *N. Rusbyi* in the previous publications from this laboratory) cross readily with *N. tomentosa*. The F_1 plants from both crosses are perfectly fertile].

In general, then, there is an agreement as to the probable evolution of the *Nicotiana* species, which rests upon as extensive a series of investigations as has been recorded. I do not intend to discuss this problem directly, therefore, either in this paper or in the related papers which will follow. What I propose to do at this time is to present certain data bearing on the reactions of a representative group of these *Nicotiana* species with each other. This is done as a matter of record preliminary to a discussion of patterns which reacting genomes produce after union in hybrids. These reactions, in turn, form part of the foundation supporting conclusions that I have been driven to make regarding the type of variation which appears to me to play the major rôle in natural evolution.

EXPERIMENTAL WORK

Observations upon the compatibilities of some of the species selected for study have been reported by several of my students, among whom may be mentioned KOSTOFF, CHRISTOFF, BRIEGER, and MCCRAY. I have used their work freely for comparison; but my observations, in nearly every case, have been independent and have been made upon different material. Cytological examinations have also been made upon all the pure species and upon about half of the possible hybrids. Some considerable repetition has thus ensued; but this seemed desirable, since the studies of THOMPSON (1930), WATKINS (1932), and MÜNTZING (1933), on compatibility, have been based, to a considerable extent, upon *Nicotiana*.

The 12 species groups (table 1) selected for systematic study need a little explanation. Under *Sanderae*, *alata* is included, on the ground that nearly all the *alata* plants in cultivation are merely white-flowered segregates from the *Sanderae* group. The two types, though self-sterile, are perfectly fertile together in the sense that the term is ordinarily used. It is probable that all such segregates react similarly to other species, except that long-flowered plants are not dependable as females on account of the mechanical difficulties involved. Similarly, *longiflora* and *plumbaginifolia* are grouped under *longiflora*; *tomentosa* and *tomentosiformis* (previously called *Rusbyi* in publications from this laboratory) under *tomentosa*; and *Bigelovii* and *quadrivalvis* under *Bigelovii*. Each pair has been proven to hybridize

TABLE 1

Compatibilities of certain Nicotiana species.

	N. San.	N. Lang.	N. long.	N. glauc.	N. glut.	N. pan.	N. tom.	N. syl.	N. suav.	N. Big.	N. rust.	N. Tab.	
N. Sanderæ 9	—	X	HL	X	HM	WS	HM	X		X	P	X	HD
N. Langsdorffii 9	HL	—	X	X	HS	X	X	X	X	P	X	X	X
N. longiflora 10	HM	X	—	WE	HS	X	X	P	X	X	X	X	WE
N. glauca 12	WE	HS	WE	HS	—	X	X	HM	X	WS	X	X	WE
N. glutinosa 12	X	P	WS	X	P	WE	HD	WE	WS	HM	X	X	WE
N. paniculata 12	WE	HM	WS	HD	X	WS	—	X	X	P	WE	HS	WE
N. tomentosa 12	X	P	X	X	HM	WE	HS	X	WE	WS	X	HM	X
N. sylvestris 12	X	P	X	X	HM	WE	—	X	HM	X	WE	WS	WE
N. suaveolens 16	P	WS	X	WS	HM	X	X	—	X	X	X	WE	HL
N. Bigelovii 24	X	P	X	X	HM	X	P	X	—	HM	X	X	WE
N. rustica 24	WE	HS	WE	HM	X	WE	HM	X	WE	—	X	HM	WS
N. Tabacum 24	X	HD	WE	HS	WE	WS	HM	HS	X	WE	HS	—	WE

Females

freely and to produce perfectly fertile hybrids. Moreover, each pair is practically identical, taxonomically, except in certain size relations.

To term this group of species "representative," may seem somewhat optimistic. There are no forms from the groups designated by GOODSPEED as *corymbosa*, *Cavanillesii*, *acuminata*, and *repanda*. Nor is the distinctive group, typified by *N. trigonophylla* Dun. and grown here also in a larger-flowered variety received under the name *N. Palmeri* Gray, included. But not all these apparent deficiencies are real, as the following words of explanation will show.

The *corymbosa* group is known to me only from dried material; but I suspect, from its characteristics, that it will prove to have a relatively low compatibility with most of the other species. *Repanda* has been grown but was not used in crosses. This was a real oversight, as it presumably will prove to be compatible with several other groups.

Cavanillesii has not been used by us in a sufficient number of crosses to determine accurately its relationship with other species, but apparently it stands somewhat apart from them. In two combinations, (*suaveolens* $\times$ *glauc*) $\times$ *Cavanillesii* and *rustica* $\times$ *Cavanillesii*, dwarf seedlings covered with tumorous growths were obtained, the plants persisting for several months without increasing much in size. KOSTOFF believes that this reaction (the tumors) is immunological in its nature. Attempts at crosses on *longiflora*, *rustica*, and *Tabacum* yielded only maternals. Using *Cavanillesii* as female, McCRAY (1932) failed with *acuminata* and *caudigera* pollen.

N. trigonophylla Dun., *N. caudigera* Phil., and *N. acuminata* (Grah.) Hooker, might have been included in table 1, as they were tested with all the other species. They were omitted because I possessed no wholly satisfactory material of the few hybrids which had been obtained.

N. trigonophylla was crossed reciprocally with all the species listed in table 1, with no result beyond parthenocarpic development, except that reciprocal hybrids with *Bigelovii quad.* and two hybrids of the combination *trigonophylla* $\times$ *tomentosa* grew to maturity. In so far as the larger-flowered *trigonophylla* (the so-called *Palmeri*) was tested, it gave no hybrids other than on *tomentosa* and on *tomentosiformis*, except that KOSTOFF obtained one hybrid when pollen was used on the combination *rustica humilis* $\times$ *rustica texana*.

N. trigonophylla is possibly one of the components of *N. Bigelovii*, as GOODSPEED suggests. There is also the possibility that it is connected, in some way, with *nudicaulis*. They resemble each other rather closely; and the hybrid *nudicaulis* $\times$ *trigonophylla* is easily obtained, though, in our experience, the reciprocal fails. It is interesting to note the compatibility of *trigonophylla* and the so-called *Palmeri*, North American species, with

the *tomentosa* group, which are South American species with wholly different characteristics.

N. caudigera Phil. was tested reciprocally with all the species of table 1 and with *trigonophylla* and *acuminata*. Reciprocal crosses were obtained in the combinations of *acuminata* and *caudigera*, and the hybrids showed a slight degree of fertility. All other combinations failed; though with *alata*, *Bigelovii*, and *suaveolens* pollen, weak seedlings were obtained which did not flower. It is only fair to say, however, that the number of pollinations made in these attempts at hybridization (3-6) was not sufficient to be certain of the reactions of *caudigera* with other species. It should also be noted that plants of the pure *caudigera* did not grow well under our conditions. The leaves were deciduous, and the plants always died in a month or so after flowering. Possibly these peculiarities affected adversely any hybrids which may have been obtained, though I suspect that, in some degree, they are normal characteristics of the plant.

N. acuminata (Grah.) Hooker was also tested reciprocally with all the plants of table 1, and with *trigonophylla*, *nudicaulis*, and *caudigera*. Besides the hybrids with *caudigera*, already mentioned, no crosses were obtained, though GOODSPEED produced hybrids from the combination *Tabacum* $\times$ *acuminata*. I also find among my records that seeds were obtained with pollen of *glauca* and of *tomentosiformis*. Since no further records were made, and I was absent when these seeds were planted, I must assume that they did not germinate.

Let us sum up these extraneous observations independently of the more consistent records found in table 1. The *corymbosa* group appears to have been differentiated rather markedly from most of the other Nicotianas, while the *repanda* group appears to have had less differentiation and will probably be found to exhibit compatibility with at least a few species in our selected group; but since these conclusions are drawn solely from the taxonomic evidence, they are uncertain. The *Cavanillesii* group is another highly differentiated branch of the family from which no mature hybrids were obtained in combination with other species. Dwarf seedlings resulted in two cases which, in their chromosome relationships, were $(16 \times 12) \times 12$, and 24×12 . The *trigonophylla* group appears to be closely related to *N. Bigelovii* S. Wats. and also to *N. nudicaulis* S. Wats., but the latter hybrid is obtained only when the cross is 24×12 . Two other hybrids were *rustica* $(24) \times$ *trigonophylla* (12) and *tomentosa* (12) or *tomentosiformis* (12) $\times$ *trigonophylla* (12). *Caudigera* and *acuminata* evidently belong together and are not closely related to the other species tested. Such relationship as is shown has little apparent connection with chromosome number. GOODSPEED gets 24×12 (*Tabacum* $\times$ *acuminata*). We obtained weak seedlings

in combinations 12×9 (*caudigera* $\times$ *alata*), 12×16 (*caudigera* $\times$ *suaveolens*), and 12×24 (*caudigera* $\times$ *Bigelovii*); while no seedlings were obtained in the reciprocals. More extensive tests would perhaps change the significance of these results.

The compatibilities shown in table 1 are reasonably dependable, since in no case were fewer than 25 pollinations made; but they are not strictly comparable, as in several instances over 1000 pollinations were made.

The table was made up in the following way. Two compartments have been used for each combination. In the first, the usual occurrence is entered; in the second, the less frequent occurrence is shown. Thus X (no stimulation), P (parthenocarpy), WE (weak embryos,—that is, non-germinating seeds having embryos), or WS (weak, non-flowering seedlings), may be entered in compartment one; while HL (large hybrids showing great heterosis), HM (hybrids close to the average size of the parents), HS (small hybrids), or HD (dwarf hybrids) may be entered in compartment two. This system makes it possible to compare the compatibilities of any two species at a glance, provided one exercises a little caution in assigning meanings to the choice of compartments and to the use of the designation X. If a hybrid is assigned to compartment one, it means that roughly two capsules with a fair amount of seed will be obtained out of three pollinations. The use of compartment two, however, means that the hybrid is really difficult to make. The use of the sign X, on the other hand, includes two phenomena. If the pollen tubes reach the ovary, some sort of stimulation usually takes place (see KOSTOFF, 1930), though this is not always the case. Complete failure, therefore, may represent either true incompatibility or deficiency in pollen-tube length.

Fifty-two flowering hybrids were produced. [EAST and HAYES (1912) record hybrids in the cross *Langsdorffii* $\times$ *Bigelovii*; yet no herbarium specimen or photograph of the plants was preserved. The only note regarding the matter now available is a record card giving the germination percentage of the seeds and a statement about the vigor of the plants. I suspect that the card itself was made out when the seed was obtained, and that the other entries should have gone on the card of some other hybrid. At all events, numerous attempts to produce such hybrids during succeeding years have failed]. They will be discussed in the second paper of this series. Here I wish to deal only with compatibility.

It has been suggested that if two related species differ in chromosome number, it is easier to obtain a hybrid when the species with the larger chromosome number is used as female (THOMPSON 1930, WATKINS 1932, MÜNTZING 1933). Our data tend to confirm this idea. There are 18 crosses where no reciprocals were obtained. Among these cases there are 5 where the chromosome numbers of the parental species are the same. Of the 13

remaining examples, 11 are cases where the female parent has the higher number. The exceptions both involve *paniculata*. There are 17 instances where reciprocal hybrids were obtained, but the combinations *rustica* with *paniculata* and *Sanderæ* with *Langsdorffii* may be omitted, because it is known that the difficulty of obtaining hybrids when the short-styled plant is used as the male is largely one of insufficient pollen-tube growth. Four others, *longiflora* with *glauca*, *glauca* with *tomentosa*, *tomentosa* with *sylvestris*, and *suaveolens* with *Bigelovii*, show no discernible differences in reciprocal compatibility, although two of these crosses are between parents of unlike chromosome numbers. There are 11 examples where the cross is easier to make one way than it is the other. *Bigelovii* × *Tabacum* and *rustica* × *Tabacum*—both combinations where $n=24$ —are easier to obtain than the reciprocal combinations; but in the remaining 9 combinations the pairs in question differ in chromosome number, and the cross is invariably easier to make where the female has the higher number. It does not appear from table 1 that *paniculata* × *Sanderæ*, *Tabacum* × *Sanderæ*, and *glauca* × *Langsdorffii*, are easier to combine than in the reciprocal manner, but such is the case.

In addition to these facts, I believe that some weight may be attached to the differences in behavior where no mature hybrids were obtained. One may, perhaps, omit the case of *paniculata*-*Sanderæ*. Mature hybrids were obtained with difficulty in both combinations, but more seeds germinated giving weak seedlings in the combination *Sanderæ* × *paniculata* (9×12) than in the reciprocal. In each case, however, all three types of reaction were observed. There are seven examples where weak seeds, having embryos but not able to germinate, were obtained when the parental species having the higher chromosome number was used as the female, while no stimulation was observed when they were used as the male. In addition, *Tabacum* × *longiflora* yielded weak seedlings, while *longiflora* × *Tabacum* yielded seed with embryos too weak to germinate. The one exception to the rule is *paniculata* × *suaveolens* (12×16). This combination gave weak seedlings, while the reciprocal gave only parthenocarpic seeds.

DISCUSSION

Probably no single explanation will suffice for all these facts and for similar facts that have been adduced in other genera. In order to simplify matters, therefore, it seems desirable to omit all cases where it is known that pollen tubes of a short-styled species do not reach the ovary of a long-styled species. Here the difficulty in question is one having to do either with a fairly definite ultimate length of pollen tube or of a slower growth-rate. At all events, when opportunity for fertilization is presented, hybridization frequently takes place—as, for example, in *Sanderæ* × *Langsdorffii*

and in *paniculata* $\times$ *rustica*. Nevertheless it is also possible that true differences in compatibility are present in addition to the pollen-tube difficulties. This may be the case in combinations of *rustica* and *paniculata*. Other physiological difficulties connected with pollen-tube growth are also known. The bursting of tetraploid *Datura* pollen tubes in diploid tissue is an example.

Omitting the above cases without prejudice as to whether the ultimate cause may not often be the same in both groups, there are three explanations of the rather generally greater compatibility of species unions when the species having the higher chromosome number is used as the mother. [KIHARA and NISHIYAMA (1932) have suggested that differential development of hybrid seeds in reciprocal crosses is due to different potencies of the male nuclei in activating the egg and the fusion nucleus. If the chromosome number of the male gamete is the larger, rapid growth of endosperm and embryo are said to result; if it is smaller, the reverse is true. I have not been able to convince myself that the facts accord with this view. It is generally believed, and with considerable reason, that the female, through the cytoplasm of the egg, controls all early divisions in the filial generation. Some time is necessary before the filial genes become active in directing development]. They are: (a) cytoplasmic differences, (b) disturbed endosperm relations, and (c) specific immunity reactions.

These interpretations, the first two of which have been considered minutely by THOMPSON, WATKINS, and MÜNTZING, and the last by KOSTOFF, have been thought to be quite independent of one another. It is true that they could turn out to be different and independent of one another had we an exact knowledge in physiological terms of what goes on at fertilization and during early embryology. As they stand, however, they are mere statements in vague terms that there are broader possibilities for harmonious chemical reactions during early development when the embryo sac machinery is controlled by the parent with the larger number of chromosomes than there are when it is controlled by the parent with the smaller number of chromosomes. The only difference between them concerns the type, or possibly the location, of the chemical action. The only matter warranting discussion, then, is whether there are any facts which delimit the problem somewhat more precisely.

When KOSTOFF (1929, 1930) suggested that certain inhibition phenomena manifested during the early development of embryo and endosperm in hybrids resembled those interpreted as antigen-antibody reactions by immunologists, it was not well received. It was thought to be a speculation having no basis of fact, because, it was said, the proteins necessary for the production of such reactions can not pass through living membranes. During the past four or five years such criticisms have become untenable.

Detailed studies of reactions in pregnancy and of allergic manifestations have shown that small quantities of proteins sufficient to cause marked reactions can pass living animal membranes. Further, a large and growing literature tends to show that the situation in plants is similar (see complete review by CHESTER 1933). Thus one must conclude that there is an opportunity for maternal tissue to react harmoniously or inharmoniously on developing endosperm and embryo.

Now, since it is an immunological tenet that various kinds of proteins may be produced and enter into specific reactions within a given reaction system (blood stream or cell), it is suggested that there is ordinarily (in cases where extreme segregant gametes are formed, this statement would not be true) a similar distribution of probabilities for harmonious or for inharmonious reactions, whether one considers fertilization and the subsequent changes of early development as matters wholly dependent upon the reactions between embryo sac constituents and male nuclei, or whether one admits the possibility of immunological reactions between maternal and filial tissues.

In allopolyploidy, if A and B are genoms, and x and y represent gene changes, then the polyploid is $A+x+B$, while the simple constituent is $A+y$. The mutations x and y must be harmonious with A if the organism is to persist; but x may not be harmonious with y. It happens frequently, therefore, that species $A+x$ and $A+y$, derived from the same stock, will not cross. By similar argument, $A+x+B$ and $A+y$ may be incompatible reciprocally. But there is the possibility of a broader series of cytoplasmic products being initiated under the control of $A+x+B$ than there is under the control of $A+y$. Thus it seems reasonable to assume greater likelihood of harmonious development of a hybrid when the mother is a pure species of some type of allopolyploid origin than if the mother is one of the constituents. And the same argument holds in case one admits the possibility of protein reactions similar to those designated as antigen-antibody reactions.

A further development of this idea also has some plausibility. It is known that when hybrids show a border-line fertility, the eggs are more likely to be functional than the sperm. In animals, there is an enormous amount of egg cytoplasm manufactured under the influence of the entire maternal complement of chromosomes, while there is practically no sperm cytoplasm. In plants, the disproportion in the amounts of cytoplasm available in the embryo sac and in the male gametes is less. I suggest that the third cell-division in the embryo sac is an activity associated with the provision of adequate materials of proper type and quantity for the filial generation.

Information is not plentiful on the reactions of autopolyploids with con-

stituent diploids, but that which is available also indicates that the cross is easier to make when the form with the higher chromosome number is used as the mother. Where this difference is not one of pollen-tube growth, and hence possibly a distinct problem, may it not be that greater quantity of cytoplasmic products rather than a larger number of types is the explanation?

It has been emphasized, particularly by MÜNTZING, that disturbed seed development in hybrids of the type we are discussing is due to a change in the relationship between maternal tissue, endosperm tissue, and embryo tissue which normally have the chromosome relationships $2n:3n:2n$. How does this view fit in with the idea that breadth of chemical possibilities is the chief causal agent in determining differences in reciprocal compatibilities? An exact answer can be given only when we are certain of the facts in each particular case. MÜNTZING, and also WATKINS, assume $3N$ endosperm development in these hybrids without proof. Such assumptions are possibly at variance with the facts, for KOSTOFF (1930), working here with *Nicotiana* hybrids, found N and $2N$ endosperms as well as $3N$ endosperms. But assuming that $3N$ endosperms develop, it still seems reasonable to suppose that the excess chromosomes coming from the female parent will furnish more harmonious environment for the young embryo and more possibilities of nutriment in the germinating seeds if the species with the higher number of chromosomes is the mother.

SUMMARY

An extensive survey of the compatibility between species in the genus *Nicotiana* corroborates the idea that, in general, greater compatibility is shown when species having higher chromosome numbers are used as females.

It is suggested that conflicting views as to the probable explanation of this phenomenon can be harmonized if one assumes that where two species of a given genus have different chromosome numbers, some type of allopolyploidy may be presumed, and that the parent with the higher chromosome number presents broader possibilities for harmonious chemical reactions during early development if the embryo sac machinery is controlled by the parent having the larger chromosome number.

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