A STUDY OF PARTIAL STERILITY IN CERTAIN HYBRIDS

E. M. EAST

Harvard University, Bussey Institution, Forest Hills, Massachusetts

Received February 11, 1921

TABLE OF CONTENTS

INTRODUCTION.....	311
The types used.....	311
The work of the early hybridizers.....	315
Experimental work.....	318
The parental strains and their behavior	318
<i>Nicotiana rustica humilis</i> crossed with <i>Nicotiana paniculata</i> . Experiment 1.....	322
<i>Nicotiana rustica humilis</i> crossed with <i>Nicotiana paniculata</i> . Experiment 2.....	331
Cross between <i>Nicotiana rustica scabra</i> and <i>N. paniculata</i> . Experiment 3.....	340
Cross between <i>Nicotiana rustica brasilia</i> and <i>N. paniculata</i> . Experiment 4.....	342
The descendants of the F ₂ generation of the plants of experiments 1 and 2.....	342
Summary and interpretation of results.....	349
LITERATURE CITED.....	365

INTRODUCTION

In 1909, I made a cross between *Nicotiana rustica* L. and *Nicotiana paniculata* L., in order to observe the behavior of the progeny of a hybrid in which the individuals of the first generation are only partially fertile.

These species were selected because the investigations of several early hybridizers had shown them to be particularly suitable for such a study.

The results obtained seem to be of considerable genetic interest, because of the indication from other sources that a high proportion,—perhaps a majority,—of the domestic plants and animals may have been derived from hybrids where the immediate cross is not completely fertile, in a somewhat similar manner. A short abstract of the work was published in 1915, but activities connected with the war have prevented a thorough consideration of the matter until now.

THE TYPES USED

The strains of *N. rustica* L. under observation were received from Dr. O. COMES through the Office of Plant Introduction of the U. S. DEPARTMENT OF AGRICULTURE. They are evidently descendants of some of the

plants which he used in compiling his monograph on *Nicotiana* published in 1899.

N. rustica is well known as a robust annual, becoming biennial or triennial in sub-tropical regions, having cordate, sub-cordate, or ovate hairy leaves and a more or less branched panicle of greenish yellow flowers. Its origin is unknown, as it is said to have been cultivated over the greater part of North America in the time of COLUMBUS. It was the first species of *Nicotiana* to be taken to Europe, and the first to be cultivated by early Virginia colonists, though soon replaced by varieties of *N. tabacum* obtained from the West Indies and South America.

COMES (1899, p. 20) used practically the same description for the species as DECANDOLLE (*Prodromus* XIII, I, p. 563). This description, however, appears to be a generalization which will include all of the varieties grouped under the name, rather than a distinct type.

COMES accepts a division of the species into six varieties, of which he gives descriptions and figures; but it must be said that neither his words nor his drawings emphasize adequately the differences between them.

The differences are for the most part merely quantitative, it is true, but there are characters which, as extreme deviates, may be said to separate at least four types, *asiatica* to *jamaicensis*, *brasilica*, *texana* to *humilis*, and *scabra*. As a matter of record, I have produced a large number of pure lines from crosses between these types, which, when grown side by side, could easily be distinguished from each other in the field. But, as in most cases where one is dealing with a plant which has been under cultivation for a long period of time, the distinguishing marks bear description only in the case of the extremes.

Whether the varieties actually grown by COMES and sent out under the varietal names noted above, do in truth correspond with the types from which they were originally described, is uncertain. After examining all of the material in the GRAY HERBARIUM and the NATIONAL HERBARIUM, I am of the opinion they do not. But this has little bearing on a genetic investigation. The varieties received under the names *asiatica*, *brasilica*, *humilis*, *texana* and *scabra* were distinct. The variety termed *jamaicensis* was not markedly different from *asiatica*, when grown under optimum conditions; and when grown under poor conditions approached *humilis*.

These observations coincide with those of SETCHELL under Californian conditions, although COMES places *jamaicensis* nearer *texana*.

Those interested in the pure taxonomy of the subject should consult COMES's monograph of 1899, and SETCHELL's comments on plants raised from COMES's seed, published in 1912. I will simply describe the four types used in the experiments.

N. rustica var. *texana* (Naud., hort. Paris.) Comes.—Monographie Nicot., 21, t. IX. This variety seems to be the most generalized type of *N. rustica*. It is moderately branched, with comparatively long internodes, growing under different conditions from 1.25 m to 1.75 m high.

The leaves are slightly rugose, ovate, and sometimes somewhat cordate. The handsome loose panicle is well filled with flowers. The copy of COMES's detail plate (figure 1) shows the characteristics of the flowers and fruit, although the corolla lobes are hardly as deeply cut or as sharply pointed as represented there. Figure 12, b, is representative.

N. rustica var. *brasilia* Schrank (Bot. Zeit. 1807, No. 17, p. 260).—Monographie Nicot., 22, t. XI. This variety compares with *texana* externally as does DE VRIES's mutant *gigas* with its progenitor *Oenothera*

FIGURE 1.—*Nicotiana rustica*, flowers and fruit. (1) *texana*, (2) *jamaicensis*, (3) *brasilia*, (4) *asiatica*, (5) *humilis*, (6) *scabra*. After COMES.

Lamarckiana. It is a heavy-set plant, a *rustica* with the parts telescoped together and enlarged. The stem is thick and angular from the large vascular bundles which go to the huge leaves. The plant is comparatively short, in typical strains hardly reaching 1 m, but the leaves are large, thick, and very rugose and bullate, showing more of a tendency to a cordate base than any other variety. As a commercial plant, it evidently out-yields all other *rustica* varieties, for the broadly ovate leaves are often 50 cm long. The tendency to produce few lateral branches would also seem to be of some advantage in this respect. The stout panicle is so shortened that in the young plant it approaches a head. The flowers like the other parts are short and stout. COMES's detail drawings are corroborated by my own observations. Figure 10, b is characteristic of the young plant, but as the plant becomes older the inflorescence elongates somewhat.

N. rustica var. *humilis* Schrank (Bot. Zeit. 1807, No. 17, p. 260).—Monographie Nicot. 23, t. XIII. This variety may be said to be a small, robust *texana* of very early maturity. Though it seldom grows to more than 50 cm in height, with leaves in proportion, it is an exceedingly vigorous plant. The general habit of growth is very reminiscent of *texana*, but the leaves, flowers and fruit of a typical plant are easily distinguishable

FIGURE 2.—*N. rustica humilis* Schrank.

from those of other varieties. The leaves have a rather bright polished appearance, are somewhat fleshy, and tend toward an orbicular shape. The lobes of the corolla and the shape of the fruit also approach rotundity. Figure 2 is from a characteristic specimen.

N. rustica var. *scabra* (Cav.) Comes.—D. C. Prodr. XIII, I, p. 564.—Monographie Nicot. 23, t. XIV. Variety *scabra* may be considered as

somewhat the opposite of variety *brasilia*. It is tall, sometimes reaching 2.0 m, and though branching freely, is compact because of the decidedly ascendant tendency of the laterals. Leaves and stem are markedly pilose. As in SETCHELL's specimens,

"it lacks glands except on the flowering axes, being clothed elsewhere by a thick and compact covering of white slender hairs abruptly bent in the middle. Above, among the flowers these are mixed with the ordinary stalked, multicellular glands commonly found in the species *Nicotiana*."

The buds and young twigs have a dull purplish color which disappears as they develop. The panicles are loose, and rather delicate. Flowers keep on developing for several weeks even in the axes of mature fruits. The corolla tubes are narrower and the fruits more elliptical than in the other varieties. Figure 11, a, is typical.

The plants of *N. paniculata* L. used in these investigations were obtained from four different sources. One lot came from W. A. SETCHELL, (his number $\frac{10}{07}$): of its origin I am ignorant. A second lot came from O. COMES. Other collections were made by C. T. BRUES and by W. E. CASTLE in Peru. The species does not seem to be variable, as each of these strains was so similar to the others that they might well be taken for samples of the same pure line.

N. paniculata L.—D. C. Prodr. XIII. I, p. 561. R. & P., Fl. Per., t. 129. This plant hardly needs description. It has been grown in all parts of the world since 1752 when it was discovered in Peru. Figure 3 is from a photograph of a typical plant. It is a low plant branching freely at the base, with cordate pubescent long-petioled leaves, and having long loose panicles of tubular flowers. The flowers are yellow-green, gibbous below the limb. The narrow limb is concave, and slightly fine-lobed. It may be reflexed when in full bloom.

THE WORK OF THE EARLY HYBRIDIZERS

Though one cannot be certain as to the varieties of *N. rustica* used by the early hybridizers, there is no question but that the plants which formed the material for KÖLREUTER, SAGERET, NAUDIN, GODRON, GÄRTNER and FOCKE in their experiments with crosses between *N. rustica* and *N. paniculata*, did actually belong to the two species described above.

The cross *N. rustica* × *N. paniculata* has the honor of being the subject of the first investigation in plant genetics. It was made by KÖLREUTER in 1760, and the first hybrid generation studied the following year. The

reciprocal cross was not quite so easy to make, but, after some failures, it was also successfully grown in 1762.

The two species remained favorite subjects for study by the early plant hybridizers. HEDWIG, WIEGMANN, GÄRTNER, NAUDIN, GODRON and FOCKE, made more or less extended experiments with them, crossing and recrossing them in accordance with the methods in vogue before MENDEL's time. I have examined the papers of KÖLREUTER, WIEGMANN, GÄRTNER, NAUDIN and FOCKE, but will follow roughly the compilation made by FOCKE. The papers of HEDWIG and GODRON I have not been able to obtain.

The hybrids of *N. rustica* $\times$ *N. paniculata* were exactly the same as those of the reciprocal cross, although GÄRTNER found the one with *N. rustica* as the mother, to be somewhat more fruitful than the other.

KÖLREUTER and most of the other observers found a high degree of uniformity among the first generation progeny; but GÄRTNER obtained some examples which had shorter and broader flowers than usual, and was able to distinguish several hybrid types. FOCKE's hybrids, also, were variable, but the reason in his case (and possibly with GÄRTNER) was the use of several varieties of *N. rustica* in their production.

KÖLREUTER states that the hybrid plants were practically intermediate between the two parents in all characters, except that they were partially sterile. GÄRTNER, on the other hand, found them to have considerably more resemblance to *N. paniculata* than to *N. rustica*, especially in their rounded leaves, their stickiness and their long corollas. FOCKE's observations were just the opposite of GÄRTNER's. He had great difficulty in distinguishing the hybrids from *N. rustica*, but found little similarity to *N. paniculata* except in the habit of growth and the numerous glands. Measurement of the flowers of FOCKE's hybrids and their parents were as follows:

	<i>N. rustica</i>	F ₁	<i>N. paniculata</i>
	mm	mm	mm
Length of corolla.....	14	19	26
Least diameter of corolla.....	7	6	5
Greatest diameter of corolla.....	9	8	6
Spread of corolla.....	18	15	9

All of the hybridizers found the first-generation hybrids to be very vigorous and of early maturity. FOCKE obtained some dwarf specimens, however, although these blossomed 2 weeks before *N. rustica* and 4 weeks before *N. paniculata* sown at the same time.

FIGURE 3.—*N. paniculata* L.

KÖLREUTER concluded that the plants were more fertile with the pollen of either parent than with their own. He even found a higher degree of fertility with the pollen of a variety of *N. tabacum* known as *N. perennis* than when the plants were mated *inter se*. GÄRTNER's experiments showed the hybrids to be more easily back-crossed with pollen of *N. paniculata* than with that of *N. rustica*.

FOCKE states that he obtained seed by back-crossing with pollen from *N. Langsdorffii*, but the plants coming from these seeds reproduced the original hybrids. Perhaps he met here a phenomenon that I have observed when attempting to cross *Nicotiana* species which seldom produce hybrids. Seed is produced which gives again the maternal species. I have attributed the matter to polyembryony effected through the stimulus of the foreign pollen.

The hybridizers all seem to be in agreement that the plants of the second hybrid generation, produced by sib-matings of the partially sterile first hybrid generation, are extremely variable both in morphological characters and in fertility. KÖLREUTER, GÄRTNER, NAUDIN and FOCKE each found that the second-generation plants resembled *N. rustica* rather than *N. paniculata*, and they were also of the opinion that those plants which stood nearest to *N. rustica* were the most fertile. WIEGMANN, on the other hand, observed a similarity to *N. paniculata* in later generations.

NAUDIN obtained 12 second-generation individuals which differed very materially the one from the other, ranging from 30 cm to 120 cm in height. One plant had lanceolate leaves, something never observed in the parent species. These plants were stated to have been more fertile than those of the first hybrid generation. Plants of the third hybrid generation similar to *N. rustica* were found to be the most fertile.

EXPERIMENTAL WORK

The parental strains and their behavior

The first point of genetic importance to which attention should be called in connection with this material is the uniformity of the strains. Each of the four varieties of *N. rustica* and the strain of *N. paniculata* used in the crosses were selfed for two generations before any quantitative data were recorded. Presumably they had been self-fertilized either naturally or artificially for many generations before, if one may judge from the slight variability exhibited. *N. rustica texana* (348) was indeed somewhat variable in height, ranging from 36 to 75 inches (90 to 190 cm); but in habit of growth, shape and texture of leaves, size of flowers and fruit, it was very

uniform. *N. rustica scabra* (352), *N. rustica brasilia* (349), and *N. rustica humilis* (350) were likewise uniform in all characters, even in height. Table 1 shows height measurement on *N. rustica brasilia* and *N. rustica scabra*. The impression of variability given by the measurements is a little misleading, however, since a slight difference in height of the panicles was obliterated in the mind of the observer by the general similarity of the plants in their various characters.

The most uniform variety of *N. rustica* was the small strain known as *humilis*. In one season the range of height measurements on somewhat more than 100 plants was less than 15 cm, and visitors often remarked that they did not know it was possible to obtain a strain of plants so lacking in variability when grown in open field. Similar remarks were made about *N. paniculata* (331). Height measurements on a population of several

TABLE 1

Height of crosses between Nicotiana rustica scabra (352) and N. rustica brasilia (349).

VARIETY OR CROSS	CLASS CENTERS IN INCHES																		TOTAL
	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60	63	66	
349				4	10	22	14	7											57
352										2	1	5	11	16	17	6			58
352 × 349 F ₁													1	3	0	5	5	6	22
349 × 352 F ₁															3	5	2	4	28
(349 × 352)-1 F ₂				2	3	6	2	7	12	8	12	13	12	12	4	15	4	0	114
(349 × 352) 1-1 F ₃	4	9	32	43	17	12	2												119
(349 × 352) 1-2 F ₃									3	4	6	10	9	7	28	13	10	8	101

score of plants usually exhibited a maximum range of less than 12 cm. The plants appeared as if they might have been cut out by a steel die.

Several crosses were made between the *N. rustica* varieties, and their behavior noted through three filial generations. These crosses were *N. rustica scabra* × *N. rustica brasilia*, *N. rustica texana* × *N. rustica brasilia*, *N. rustica scabra* × *N. rustica humilis* and *N. rustica texana* × *N. rustica humilis*. It seems unnecessary to describe this work at length. Three points are to be noted, however, as pertinent to the discussion of the species hybrids described later. (1) The reciprocal hybrids were very similar to each other. (2) The varieties differed from each other by many heritable factors. This is indicated by the greatly increased vigor of the F₁ generations, and by the extreme variability of the F₂ generations. (3) In no case could each of the four *rustica* varieties be reproduced from a single cross.

N. rustica scabra × *N. rustica brasilia*. In table 1, it is shown how much taller the F₁ plants were than the average between the two parents.

The F_1 generation average was even pushed considerably beyond the average of the taller parent (352). The different F_1 generations were very similar to each other in habit of growth, size of leaf, size of flower and size

FIGURE 4.—*N. rustica humilis* $\times$ *N. paniculata*. F_1 plant.

of fruit. Height measurements indicated a slight difference in favor of the cross using *N. rustica brasilia* as the female parent, but the divergence is hardly enough to be significant even in that case.

The variability of one of the F_2 generations is illustrated very well by the height measurements. The possibility of obtaining strains rather uniform in height by selecting extreme F_2 individuals is also manifest.

The most important fact connected with this cross, however, and with the other crosses as well, cannot be demonstrated statistically without covering too much space. It is this: The F_2 individuals, though variable, were what might have been expected by a plant breeder familiar with the two strains used in the cross. They exhibited recombination of the existing characters. Plants similar to *N. rustica texana* were found, but no plants similar to *N. rustica humilis*. Presumably *texana*-like plants arose from the following combinations: Loss of *scabra* hairs; non-appearance of *brasilia* habit of growth, with nevertheless a distinct modification of the *scabra* habit; absence of bullate leaves; and an intermediate size and shape of flowers and fruit, verging toward those of *scabra*. The peculiar smooth fleshy leaves of *N. rustica humilis* did not appear in any members of the F_2 generation.

It might also be mentioned here, that the *gigas*-like habit of *N. rustica brasilia* appeared to be a simple recessive, but it was so changed by numerous modifiers as to make classification difficult.

N. rustica texana $\times$ *N. rustica brasilia*. In this cross the same vigorous F_1 generation and variable F_2 generation appeared. No *humilis*-like or *scabra*-like plants were found. Neither the fleshy leaf of the former nor the characteristic hairs of the latter appeared, though certain individuals had small narrow-tubed flowers and oval capsules much resembling *scabra*.

N. rustica scabra $\times$ *N. rustica humilis*. The height of the F_1 individuals in this cross, unlike that in the first two crosses, was somewhat less than that of the taller parent. The F_2 was again extremely variable. *Humilis*-like plants were regained, and *texana*-like plants appeared; but no plants resembling *brasilia* were found. The appearance of plants similar to *N. rustica texana* was not unexpected, for given the absence of the *scabra* hairs, the characteristics of *texana* are somewhat intermediate between *scabra* and *humilis*.

N. rustica texana $\times$ *N. rustica humilis*. This cross was similar to the preceding one except that the characteristic hairs of *scabra* were absent as well as the *gigas*-like characters of *brasilia*.

The general conclusion from this work is that the four *N. rustica* varieties studied differ the one from the other in a comparatively large number of heritable characters; but that by crossing any two of them, the characteristics of both the others *can not be reproduced*.

Nicotiana rustica humilis crossed with *Nicotiana paniculata*
Experiment 1

Nicotiana rustica humilis (350) crossed with *N. paniculata* (331) was the hybrid selected for particular study.

FIGURE 5.—At left, *N. rustica humilis*; center, *N. rustica humilis* $\times$ *N. paniculata* (F_1); at right, *N. paniculata*.

Hybrids between *N. paniculata* and two other varieties of *N. rustica*, viz., *brasilia* and *scabra*, were made successfully, but were not carried beyond the second hybrid generation. The only point to be emphasized now in regard to them is that they were extremely uniform in all characters.

The reciprocal cross with *N. rustica humilis* was also made. This hybrid was not quite so easy to obtain as when the parentage was reversed, but the only difference noticeable in the reciprocal F_1 populations was a slightly taller plant in the case of *N. rustica humilis* ♀ × *N. paniculata* ♂.

My first F_1 population of the cross *N. rustica humilis* (350) × *N. paniculata* (331) was grown in 1910. In leaf size, shape and texture, and in shape of corolla, the plants were very similar to *N. rustica humilis*. In fact, any taxonomist would have classified them as *N. rustica* plants.

But when examined carefully it was seen that they showed the influence of the *N. paniculata* parent, particularly in the intermediate length of corolla and in the spreading habit of growth,—the branches ending in loose *paniculata*-like panicles. These characteristics are probably better

TABLE 2

Variations in length of corolla tube in cross between *N. rustica humilis* (350) and *N. paniculata* (331).

MATERIAL	CLASS CENTERS IN CENTIMETERS									
	1.125	1.375	1.625	1.875	2.125	2.375	2.625	2.875	3.125	3.375
350, plant 1 (1910).....		24	1							
350, plant 2 (1910).....	1	23	1							
350, 5 flowers each (1910).....	33	168	63							
331, plant 1 (1910).....						2	22	3		
331, plant 1 (1910).....							24	1		
331, 5 flowers each (1910).....						47	178	40		
(350 × 331) F_1 , plant 1 (1910)...				24	1					
(350 × 331) F_1 , 5 flowers each (1910).....		2	9	208	63					
(350 × 331) F_2 , 1 flower each (1911).....	2	7	31	90	76	24	7	3	1	1

shown in figures 4 and 5 than they would be by any amount of description and measurement.

I have already spoken of the uniformity of the two parental types in all characters. The F_1 plants were just as uniform in their traits, though they were about 25 percent taller than *Nicotiana paniculata* (the taller parent). Table 2 gives an illustration of this remarkable lack of variability.

The F_1 plants produced only from 1 to 30 seeds per capsule when crossed *inter se* or when self-pollinated, even though considerable quantities of pollen were applied. This sterility seemed to be equally a sterility of ovules and of pollen for no greater quantities of seed were produced when pollen of the F_1 plants was applied to either parent, or when the pollen of either parent was applied to them.

Matings $331 \times (350 \times 331)$ F_1 and $350 \times (350 \times 331)$ F_1 were somewhat difficult to obtain, but after a score or so of attempts to use F_1 pollen on each of the two pure species, several capsules averaging 10 seeds each resulted. The reciprocal crosses, i.e., the use of the pollen of the pure strains upon the flowers of the F_1 plants, were usually successful, the capsules ranging from 3 to 22 seeds each. Perhaps the very fact that back-crosses with the F_1 pollen were harder to obtain than the reciprocals, shows that ovule sterility was less than pollen sterility. But when they matured at all, the back-crossed capsules of each of the four combinations possible contained about the same quantity of seed. Consequently it seems reasonable to attribute this apparent functional difference between the ovules and the pollen of F_1 plants, to the greater protection against inclement conditions received by the ovules, rather than to inherently greater fertility.

The plants of both $(350 \times 331) \times 331$ and $(350 \times 331) \times 350$ showed the general uniformity of the true F_1 generation, though the former were considerably more variable. The back-cross with *N. paniculata* (331) took on somewhat more of the *paniculata* characters, and that with *N. rustica* (350) resembled *rustica* more than did the F_1 generation. For example, the flowers of the *paniculata* back-cross averaged about 2.3 cm by 65 cm in length and breadth of the corolla tube, while those of the *rustica* back-cross averaged 1.9 cm by 0.9 cm.

By exercising great care in pollinating two F_1 plants *inter se*, a quantity of seed was obtained from which 246 plants were raised in 1911. The percent of germination of the seed is not known.

The F_2 plants were each unlike all the others though in general they resembled *N. rustica* more than *N. paniculata*. In height they varied from 24 cm to 170 cm. Flowers from 21 plants are shown in figure 6 compared with flowers of the two grandparents in the upper right-hand and lower left-hand corners respectively. It is clear that all sorts of combinations between the long narrow corolla of *N. paniculata* and the short broad corolla of *N. rustica* are in evidence, but the point of particular interest is that the extremes exceeded those of each parent. The same point is emphasized by figure 7, in which the largest leaves of a number of F_2 plants are shown on the same scale.

A good many measurements of the various characters of this F_2 generation were made, but reports of series of measurements on particular characters serve to confuse rather than to clarify the points which seem important.

For this reason a number of photographs of F_2 plants are submitted in place of detailed measurements.

Though nearly all of the plants of this generation would have been grouped as *rustica* plants, there were seven which resembled *paniculata* rather closely. Of these, four retained traces of *rustica* descent. The other three plants duplicated *N. paniculata* exactly. Figure 8 shows one of these segregates. By comparing it with figure 3, a representative of the pure species, one can see that it is no exaggeration to use the word exactly.

In every feature of habit of growth, of leaf and of flower, these plants were wholly *N. paniculata*.

Naturally the suspicion arose that these plants were *paniculata* individuals which had been included by mistake, or had arisen as volunteers. Nevertheless there was some evidence this was not the case. In the first

FIGURE 6.—Flowers from 21 plants of F_2 generation *N. rustica humilis* $\times$ *N. paniculata*. Upper right, *N. paniculata*; lower left, *N. rustica humilis*.

place, the seeds from which the plants were grown, were planted in sterilized soil, and the seedlings transplanted very carefully and tagged in the field. Some misplacement was found, of course, for no one can carry on extended experiments without error. But on examining the field of some 10,000 plants, the traceable misplacement was found to be about 1 plant per 2000, while here were three plants in a lot of 246 for which to account. In the second place, though two of these *paniculata*-like plants apparently were as fertile as the pure-line *paniculatas*, one was completely sterile. The fact that two of these supposed segregates were so fertile is admittedly a somewhat suspicious circumstance, but the appearance of a sterile plant duplicating *paniculata* characters seemed to clinch the claim of that particular individual as a segregate from the original cross.

Seed from self-fertilized flowers of one of the fertile plants was grown the next year, and the progeny bred true to the *paniculata* characters.

FIGURE 7.—The largest leaf on each of the 21 plants of F_2 generation *N. rustica humilis* $\times$ *N. paniculata* of which the flowers are shown in figure 6. A, *N. paniculata*; B, *N. rustica humilis*.

Not a trace of influence of *N. rustica* appeared. This fact combined with the others mentioned made me so apprehensive of the whole matter that the experiment was repeated in its entirety. This second investi-

gation of the *rustica-paniculata* cross is described later in the paper. Suffice it to say now, that I am convinced from it that the three *paniculata*-like plants of the F_2 generation under discussion were in fact segregates.

Several reasons for accepting this view will appear later. We will mention here only the reason why the complete fertility of the supposed segregate need not be taken as a particularly suspicious circumstance. It is this: Though the F_1 plants were very slightly fertile, their fertility was uniform. The F_2 plants, on the other hand, showed as great a variability in the fertility exhibited as in any other character complex. Several plants could not be induced to set a single seed in the limited number of trials made, either with their own pollen, with pollen of any of their sibs, or with pollen from either of the species entering into the cross. In others, there were varying degrees of fertility up to complete fertility as far as the quotas of seed set are concerned, though some small percentage of pollen abortion was found in the best.

Among the plants of the F_2 generation, 6 or 7 were found which reproduced the characters of *N. rustica humilis*, one of which is illustrated in figure 9. None of them was markedly sterile, though varying degrees of fertility were exhibited. But the fact that *N. rustica humilis* was reproduced as a segregate need not astonish one. The astonishing fact was that, though variety *humilis* entered into the cross, and though variety *humilis* crossed with other varieties of *N. rustica* did not throw segregates duplicating varieties *brasilia* and *scabra*, nevertheless *these varieties were duplicated* in the segregates when *humilis* was crossed with *paniculata*.

Figure 10 shows, an F_2 segregate of this population at the left, compared with a *brasilia* plant at the right. Though the photographs do not emphasize the points of similarity, in the field they were very marked. This plant could only have been classified as a *brasilia* plant, though the leaves were not so bullate as in the particular pure-line *brasilia* plant with which we have chosen to compare it. On the other hand, several other pure lines of *brasilia* plants have been grown which do not exhibit such a bullate appearance as the one here shown; and further, the particular way the sunlight fell on this plant while the photograph was being taken, rather obscures the amount of leaf-wrinkling it possessed.

In figure 11 a typical plant of variety *scabra* is shown at the left, while a *scabra*-like segregate appears at the right. In this picture, one who is familiar with the appearance of *scabra* will be struck with the fact that the segregate seems to be more strikingly *scabra*-like than the plant from the pure line. This is because of the fact that the photograph of the true *scabra* plant is so sharply focussed that the grayish appearance due to the

FIGURE 8.—F₂ segregate, *N. rustica humilis* × *N. paniculata*, duplicating characters of *N. paniculata*.

hairs is partly obliterated. The habits of growth may seem somewhat different but this is not really true. The segregate is a little more vigorous than the *scabra* plant, and though the photographs were taken when the plants were of the same age, the lateral branches of the *scabra* plant had not developed to the same extent. In another week the pictures would have been practically identical, for the two plants tallied character by character even to the characteristic dull purple in the young flower buds.

Again, we have a third segregate in the left-hand plant of figure 12 which might have been die-cut as a representative of variety *texana* (the right-hand plant). The similarity of the two plants is obvious even in the photographs taken under different weather conditions. Notice specially the size and shape of the flowers, the method of branching, and the character of the panicles. The leaves are the same shape, size and texture, and are set at the same angle on the stalks.

A few other extreme plants are interesting in this connection. A rather gigantic form is pictured in figure 13. It is not extremely tall, as can be seen by comparing it with the foot-rule, but the leaves are far larger than any we have examined in the larger cultivated types of *N. rustica*. The largest leaf measured possessed a blade 75 cm $\times$ 60 cm. Contrast this huge plant with the little one shown in figure 14, where the whole plant is only 25 cm high and has a maximum leaf size of 9 cm. Oddly enough the size of the flowers is not noticeably correlated with the size of the plant as a whole. The flowers of the lilliputian of figure 14 are almost as large as those of the giant sister of figure 13. Another peculiar type is illustrated in figure 15. In texture and shape of leaf, this plant is much like the *brasilia* variety; but the inflorescence is unique. The lateral branches combine with the main stem to produce the appearance of one large corymb. In marked contrast to this type is one shown in figure 16 where the prominently white-veined leaves curl at the edges and the inflorescence is such that the plant has a pyramidal appearance. Again in figure 17, a plant is shown which is quite different from any described variety of *N. rustica*. The sparse leaves are lanceolate in shape and very delicate in texture; the flowers are long and narrow; and the general character of the panicle is much looser than in the other types described, although approached by *N. rustica scabra*.

The photographs of the few F_2 individuals shown in the figures, represent some of the extreme types. Now in imagination, picture graded series of intermediates between these particular plants—hardly two of similar appearance—and you have a fair idea of the F_2 generation of this cross.

It is useless to try to show frequency distributions of particular characters. They fail to serve the purpose, for it is the *combination* of *characters* which makes every plant distinct from the other. At the same time the measurements of height of plant, length and breadth of leaves, size of flower and of fruit, show clearly four facts which are important for

FIGURE 9.—F₂ segregate, *N. rustica humilis* × *N. paniculata*, duplicating characters of *N. rustica humilis*.

the theoretical discussion which is to be given later. (1) In an F₂ series of less than 300 plants, nearly every individual *seems* to have an hereditary constitution different from all others. (2) In height of plant and in size and shape of leaves, the range is far beyond that of either grandparent; but in size and shape of flower, though combinations of the grandparental

characters are found, they do not pass far beyond those actually existent in the pure species. (3) Nearly all of the plants resemble *N. rustica*. Those which resemble *N. paniculata* at all, are almost identical with it. In other words, a series of combinations presumably possible, appears to have been omitted. (4) The fertility of the plants varies from a condition where almost all of the pollen is abortive and few if any seeds are produced (limited number of trials) with sib pollen or with pollen of either of the grandparental species, to a condition where every ovule (full capsules) seems to function with "own" pollen (though from 20 percent to 30 percent of this pollen is abortive).

Nicotiana rustica humilis crossed with *Nicotiana paniculata*
Experiment 2

Owing partly to the suspicion that the *paniculata*-like segregates in the F_2 population of the cross just described might have been "volunteers," and partly to the desire to make some observations that had been omitted, a repetition of the cross between *N. rustica humilis* (350) and *N. paniculata* (331) was made.

The F_1 generation was practically identical with that described under experiment 1. The plants were slightly taller and the flowers slightly larger, but this difference may be attributed to the rich soil upon which they were grown.

Some considerable time was spent studying the fertility of this generation, with the following results. From 1 percent to 7 percent of the ovules were proved to be functional, by careful pollinations with "own" pollen, with sib pollen and with pollen of the two parental species. This ratio was determined by comparing the number of seeds actually produced with the number found in full capsules of *N. paniculata*, as a determination of the number of ovules in a flower of the plants under consideration was difficult. There is some inaccuracy in these figures, therefore, but it is probably not very great. The variation found is due, in part, to manipulation difficulties and in part to the small samples involved; and one may take it that under the best conditions 3 or 4 percent of the ovules of the F_1 generation on an average will produce seeds, and that these will germinate in from 35 to 55 percent of the cases.

Samples of the pollen of F_1 , taken from mature anthers, likewise showed a variable ratio of grains apparently perfect. The variation here was from less than 3 percent to about 10 percent, and seems to be due largely to the great difficulty of obtaining random samples. When pollen from anthers

FIGURE 10.—At right, *N. rustica brasilia*; at left, F_2 segregate, *N. rustica humilis* $\times$ *N. paniculata*, duplicating characters of *N. rustica brasilia* with the exception of the bullate leaves.

just opened is shaken out, the count is distorted because the perfect grains shake out more easily. When the pollen is scraped out with a scalpel the results are a little more uniform, but even with this method they are not to be relied upon. Paraffine sections of mature unopened anthers gave a lower ratio—from 1 to 3 percent—of grains perfect in appearance; but this method also has its drawbacks. Sections from anthers of different phases of maturity, indicate that *many of the pollen grains which appear to be functional in the young unopened anther, dry up as soon as the latter opens*. Furthermore, germination tests made in stigmatic fluid and in sugar solutions, showed that from 20 to 70 percent of the grains which appeared to be perfect would not extrude pollen tubes. It may be argued that such a test does not preclude the possibility of a high percentage of germination of the "good" grains on the plant stigmas. This is true. But experience with the pollen of *N. rustica humilis* and *N. paniculata* in hanging-drop cultures, leads me to believe that over 80 percent germination can be obtained by this means, if the pollen is really functional.

The general conclusion from observations on the pollen of the F_1 generation of this cross, is that less than one-tenth of one percent of the tetrads which the pollen mother cells *start* to form, ever succeed in functioning as pollen.

One other set of observations should be mentioned in this connection, though the conclusions may be revised after more extended investigation. Examination of slides of *N. rustica humilis*, of *N. paniculata*, and of the F_1 , indicates that each species has 24 chromosomes as the gametic number, and that a high percentage of breakdown occurs at the heterotypic division of the pollen mother cells.

Of the F_2 generation, 300 plants were raised to maturity. These plants were studied rather carefully, and it is hoped that some day the data may be punched on cards and all of the statistical relations made out with a Hollerith machine. For the present purpose too voluminous citation of these details regarding separate characters would simply result in obscuring the important points.

The plants were again extremely variable. For example, the height frequencies in decimeter classes, for the 288 plants which had reached their maximum height at the time of measurement, were as follows:

Class centers in dm.....	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Frequency.....	1	2	1	2	7	10	11	13	19	35	39	35	31	29	17	15	13	2	3	2	1

When one realizes that nearly all of the F_1 plants are included in *one* of these decimeter classes,—less than 10 percent falling into contiguous classes,—this range of the F_2 generation from 3 decimeters to 23 decimeters is tremendous. A similar variation occurred in habit of growth and time of maturity (range of 30 days), in shape, color, leafiness and hairiness of stem, in size, shape, thickness, bullation and hairiness of leaves, and in character of inflorescence (from the head-like panicle characteristic of variety *brasilia* to a loose panicle resembling a raceme). The fruit also showed extreme variation, ranging from a pumpkin shape, one and one-half times as broad as long, to an ovoid type nearly twice as long as broad, and from a length of 3 mm to a length of 18 mm. It is probable, however, that variation in fertility distorted these records. In flower shape and size, variation was not so marked. A few plants had *rustica*-shaped flowers longer than those characteristic of *paniculata* and a few had *rustica*-shaped flowers about one-half the size of those of *N. rustica humilis*; but only one plant approached *N. paniculata* flower characters.

The characters of the plants considered together showed that many of the plants could be classified as resembling varieties *humilis*, *brasilia*, *scabra*, and *texana* as in experiment 1. There were others which resembled those figured previously, as well as a few new types. Only one plant resembled *paniculata* at all closely, though certain features possessed by many individuals, length of corolla, hairiness, cordate leaves, etc., may have been hereditary contributions from this source. The one *paniculata* segregate, curiously enough, was only about two-thirds the size of a normal *paniculata*, though all of the size relations appeared to be the same,—that is, leaves and flowers were smaller in their proper ratios. The plant was 66 cm in height; the leaves were the exact shape, texture and hairiness of *paniculata*; the flowers also were exactly what one might imagine to be characteristic of a dwarf *paniculata*,—19 mm long and 3 mm broad, flaring at the end to 6 mm. About 20 percent of the pollen grains appeared to be normal, but the many attempts at self-pollination were all failures.

The appearance of this plant in this population, is to some extent a confirmation of the results of experiment 1. It gives more confidence in the supposition that the three plants apparently identical with *N. paniculata* found in that F_2 population, were in truth segregates from the F_1 generation.

From the standpoint of genetic theory, the questions connected with the fertility of this family seemed important, and a serious effort was made to discover the facts. Since the inquiries were subject to the limitations already described, the effort was not all that might be desired; nevertheless,

I believe that the data have a decided value, and that the conclusions drawn from them are satisfactory from the standpoint of statistical theory.

First, the pollen from 52 plants was examined microscopically. Representatives from all of the more distinctive types were included. The pollen was examined dry, in water and in glycerin. The general results are as follow:

(1) Judging from the amount of pollen formed and from the percentage of aborted grains, it appears uncertain that any F_2 plants are more sterile

FIGURE 11.—At left, *N. rustica scabra*; at right, F_2 segregate, *N. rustica humilis* $\times$ *N. paniculata*, duplicating characters of *N. rustica scabra*.

than the plants of the F_1 generation. In nine plants out of 52 examined, the proportion of pollen formed and the proportion of grains apparently viable were comparable with the proportions found in F_1 . The grains apparently viable were between 3 percent and 10 percent of the total.

In one other plant, only aborted pollen grains were found, but it is assumed that this condition was not due wholly to heredity because the plant formed about 25 percent of the number of seeds expected normally when pollinated with sib pollen.

(2) Ninety percent of the plants produced much greater complements of good pollen than those of the F_1 generation, and the percentage of grains apparently viable ranged from 10 percent to 80 percent. No plants in this generation produced a proportion of "good" pollen equal to that of the pure species (95 percent to 98 percent), but three plants of the F_2 generation described in experiment 1 had over 96 percent "good" pollen. The modal condition was around 65 percent "good" pollen.

FIGURE 12.—At right, *N. rustica texana*; at left, F_2 segregate, *N. rustica humilis* $\times$ *N. paniculata*, duplicating characters of *N. rustica texana*.

(3) No decided correlation between plant characters and fertility as determined by this method could be discovered.

(4) No pollen germination tests were made on this population, either in stigmatic fluid or in sugar solutions, but judging from the complements of seed formed naturally (probably sib-pollination), many of the "good" pollen grains did not function. This conclusion was drawn from the fact that no seed was obtained on several plants producing some apparently

"good" pollen. It might be concluded that this result came about because of a greater proportion of sterility among the female gametes, but the general evidence is against such a conclusion.

The shortcomings of the pollen-examination method of estimating fertility are obvious. Because of them another method was used. It has its faults, but I believe that by its use a reasonably accurate comparison of the fertility of the F_1 and F_2 generations can be made, and this is the essential point.

Each plant was examined carefully at the stage when the seeds are just beginning to turn brown. The flowers had had a fairly equal chance of being pollinated naturally with sib pollen; and the seeds formed had matured sufficiently to give a fair idea of those retaining some measure of vitality, since abortive seeds had begun to shrivel. The procedure was as follows. The percentage of capsules formed per plant having been given weight in the judgment, several capsules from each individual were carefully "peeled" and an estimate of the fertility made from the seeds therein. The plants were thrown into eleven fertility classes ranging

TABLE 3

Estimated fertility of the F_2 generation (350×331) through an examination of the capsules.

Class.....	0	1	2	3	4	5	6	7	8	9	10
Frequency.....	4	4	11	23	34	20	41	32	56	28	17

from 0 to 10. It may be assumed that classes 5 to 6 represent about a half complement of seeds, class 10 represents complete fertility on the female side, and class 0 represents complete sterility. The results of an examination of 269 plants are given in table 3.

The curve is not as smooth as it would have been, had not the records been taken in classes numbered 1, $1\frac{1}{2}$, 2, $2\frac{1}{2}$, etc. The tendency was to enter the records in the integral classes. But this fact does not interfere with the main result. On this scale the plants of F_1 belong uniformly in class 2. In other words, the F_2 plants were variable in fertility, but 97 percent of them showed greater fertility than those of the F_1 generation. At least 12 percent of the plants gave full complements of seed which seemed perfect in every way.

On the other hand, one *can not* conclude that a single plant—through heredity—was less fertile than the F_1 generation, *though such may be the case*. Let us look at the matter a little more carefully. Suppose it be admitted that the F_1 plants showed a slightly greater fertility than class 2. This would leave 19 plants out of 269 F_2 plants apparently less fertile

than those of the F_1 generation. At the same time it must be remembered that the F_1 plants were pollinated very carefully. A great deal of pollen was applied, and hundreds of flowers were tested. A similarly extensive test on each of these F_2 plants was impossible. Each plant self-pollinated (162) had only 5 flowers manipulated (with the exception of the *paniculata*-like plant), taking the usual precautions, though the capsules pollinated naturally were examined carefully. To the best of our knowledge and belief, therefore, evidence for a fertility less than that of F_1 exists for only the 8 plants of the first two classes, and this evidence is not conclusive, except possibly in the above-mentioned *paniculata*-like individual. In the first place, each of these plants might have produced some seed if pollinated as carefully and extensively as were our F_1 plants, since each produced as great a percentage of apparently viable pollen as the average F_1 plant. In the second place, in any family of plants individuals appear occasionally that are completely sterile for reasons which seem to be unconnected with heredity. Some of these plants may be of this type. It seems to me, therefore, that the following conclusion is in accordance with all the facts. *Ninety-seven percent of the F_2 plants produced in the cross between $N. rustica humilis$ and $N. paniculata$ were more fertile than the F_1 plants, and it is not certain that the remaining 3 percent were less fertile.*

One hundred sixty-two of these F_2 plants were self-pollinated. In almost every case 5 flowers were used. Unfortunately the prevalence of aphid and an excess of rain and wind during the remainder of the season ruined a very large part of this work. Only 94 plants matured seed, and of this number only 70 produced 75 or more seeds. Seed was collected from 203 plants where natural pollination had taken place, however, and the germination of each lot tested. One hundred seeds from each plant were counted, placed in separate Petri dishes on moist blotting paper, and kept in a rather warm greenhouse. The results were rather interesting. While most of the seeds—particularly the seeds of those plants which showed a high percentage of germination—sprouted after 4 or 5 days, a number of the dishes showed a continuous germination week by week,—several seeds germinating after 63 days. It would have been decidedly worth while to have traced the life history of some of these plants germinating at different periods to see if there were marked differences in their characters, but this was impossible.

Table 4 is a correlation table made up from the germination tests of 67 plants from which both selfed seed and open-pollinated seed were obtained. To those who know how uniform germination tests made from duplicate samples of seed usually are, it may seem strange that the corre-

lation between the two sets was only 0.33 ± 0.07 . But this is not really so odd. The conditions under which the selfed seed matured were bad, but not uniformly bad. Some bags had aphides under them, others were free from aphides; some were wet on the inside, others stood up well and were only wet on the outside. Nevertheless, the results here and in table 6, show that many of these plants were in a rather balanced condition as regards maturing seed. Little changes in environment evidently affected

TABLE 4

Correlation in percent of germination between open-pollinated and self-pollinated seed produced by F_2 plants of cross between *N. rustica humilis* and *N. paniculata*.

		Percent open-pollinated									
		5	15	25	35	45	55	65	75	85	
Percent self-pollinated	5	1	3	1		2	1	1	1		10
	15	2	2	2	3	1	2	2			14
	25		1		3	4					8
	35	1	1	1		3					6
	45		2		1	2		2			7
	55		1	1		1	2	1			6
	65	1	1	1	1			2			6
	75		1			2		3	1	3	10
		5	12	6	8	15	5	11	2	3	67
$r = 0.33 \pm 0.07$											

$$r = 0.33 \pm 0.07$$

TABLE 5

Percent of germination of open-pollinated seed produced by plants of the F_2 generation of cross between *N. rustica humilis* and *N. paniculata*.

Percent germination.....	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90
Frequency.....	16	17	14	17	19	12	13	15	17	9	13	10	9	11	5	1	3	2

the maturing capsules much more than similar changes would have affected pure lines, since several pure lines under observation at the same time gave germination tests of over 90 percent. Thus there is pretty good evidence for supposing that zygotes were eliminated with a high degree of frequency by external conditions; and it seems to me that it is more than a guess to say that there was a selective elimination of plants with particular hereditary complexes.

Table 5 gives the percent of germination of the open-pollinated seed.

Cross between Nicotiana rustica scabra and N. paniculata. Experiment 3

Before continuing to describe the descendants of the F_2 generation of the cross between *N. rustica humilis* and *N. paniculata* (experiment 2) two crosses between *N. paniculata* and other varieties of *N. rustica* should be discussed for the sake of comparison. The first of these is one in which *N. rustica scabra* was used as the female.

Both when the plants are considered as units and when specific characters are examined, the F_1 population compares in uniformity favorably with those already described. For example the height frequencies of 49 plants in decimeter classes starting with 7.5 are 6, 15, 19 and 9. Again the flower-length frequencies in millimeter classes beginning with 19 are 4, 20, 22 and 3.

The fecundity of the plants seemed to be slightly greater than in the other crosses, in that a larger percentage of self-pollinated flowers produced some seeds. But since the number of seeds per capsule obtained was about the same, it is not certain that the gross fertility exhibited was any greater.

The 257 F_2 plants raised to maturity showed again an extreme variability, ranging from 30 cm to 210 cm in height, and reproducing *brasilia*, *scabra*, *texana*, *asiatica*, *humilis*, *nana* and *gigas* types as before.

Three differences between the plants of this cross and those of the cross into which variety *humilis* entered were found, however, and these differences appear to be important. (1) *All* of the plants leaned decidedly toward the *rustica* side of the house. Not a single plant was found that one could say truthfully was *paniculata*-like, although traces of possible *paniculata* influence could be seen in fully half of the population. (2) Small plants with a starved appearance were found in considerable numbers, (38 out of 257 plants). In the cross between *N. rustica humilis* and *N. paniculata* scarcely two plants were of the same type, yet here 15 percent of the population were dwarfs that were fairly uniform in appearance. These plants resembled the one reproduced in figure 14, except that few seed capsules were formed. Flowers pollinated carefully by hand, as well as flowers pollinated by insects, fell off after 3 or 4 days. Toward the end of the season a few capsules were formed, but they contained no normal seed. A few collapsed seeds in which no embryo could be discovered, were all I obtained for my trouble. Pollen from these plants examined in glycerin appeared to have as high as 3 percent of normal grains, but there was no evidence that this appearance was a true criterion of vitality. *A part of these plants was tested carefully, and I believe it safe to say that they were less fertile than those of the F_1 generation.* (3) Among the other plants of the population a varying degree of fertility was found, with the

tendency toward high fertility as in experiment 2; but none showed decided evidence of being less fertile than the plants of the F_1 generation. Thus there was a high correlation between a physical type (the sickly dwarf), and sterility. Perhaps the phenomenon met here is due to a lethal factor simi-

FIGURE 13.—Giant F_2 segregate *N. rustica humilis* $\times$ *N. paniculata*.

lar to those described by MORGAN for *Drosophila melanogaster*; at least one may suppose it is not the same type of absolute sterility found to be questionable in the F_2 generation of experiment 2. This experiment was carried no further.

Cross between Nicotiana rustica brasilia and N. paniculata. Experiment 4

The cross in which *N. rustica brasilia* entered as the maternal parent, was very similar to that in which variety *humilis* was used as the *rustica* parent. Time for studying this cross in detail was not available, but 26 plants of the F_1 generation and 57 plants of the F_2 generation were grown and examined. The uniformity in physical appearance and in fertility characteristic of the other F_1 generations was again found here. Similarly also the F_2 generation was extraordinarily variable, and produced individuals resembling *brasilia*, *humilis*, *texana*, *scabra* and "nana." Nothing can be said concerning the fertility of the plants except that it was variable. Judging from open-pollinated capsules, the majority of the plants were more than 50 percent fertile, but whether any plants were less fertile than those of the F_1 generation is unknown.

The descendants of the F_2 generation of the plants of experiments 1 and 2

A considerable number of F_2 plants from experiments 1 and 2, *N. rustica humilis* $\times$ *N. paniculata*, were selfed, and the descendants followed for several generations. The behavior of the families of each experiment was so similar that there is no advantage in describing more than one set. In table 6, therefore, the salient features of 51 F_3 families arising from F_2 plants of experiment 2 are set forth.

The important question to be answered by these data is that of variability, and it was not an easy matter to make an estimate which would be quantitative and at the same time practical. Fifty-one families were raised, giving me a real task in trying to cover the point at issue without covering an interminable number of pages. If, for example, frequency distributions were made of a number of characters in each family, there would be an apparent accuracy in the results. But this accuracy would in truth be only apparent. What we wish to know is the variability of the families as entities. With series of data for separate characters, it would be necessary to try to keep the figures for each character in mind, and to weight these figures mentally in making a general average. Instead of doing this on paper after taking the data, therefore, it was done in the field. For this reason the figures of table 6 may be subjected to the criticism that they are distorted by the personal equation of the writer. This is true. But, on the other hand, all figures are somewhat distorted by personal equations, and I believe the data of table 6 give a better idea of the actual situation than would have been given by a more voluminous set.

The first column in the table gives the number of the F_3 family. In the second column is the number of plants under observation. When the plants showed a distinct type, it is listed by name in the third column. Under height, leaves, flowers and fertility, the measurements of the extremes are given in the first column. In the second column under each heading a number is given which represents the variability of the character in question on a scale of 10 where 1 represents the wonderful uniformity of the pure line of *N. paniculata* described previously and 10 represents the inordinate variability of the F_2 generation of experiment 2. Finally column 4 gives the general variability of each family on the same scale. This figure is frankly an estimate. But it was made in the field after the estimates had been made of the variability in height, in size and shape of leaf, in size and shape of flower and in fertility. It gives weight to these determinations, but it also considers the general characteristics of the plants in a manner that could only have been done by an observer in the field.

Owing to the small number of plants grown, families 52, 101 and 176 should be omitted. Four other families, 67, 199, 228 and 234, may also be omitted because it was so difficult to estimate their variability on a scale comparable to the others. Family 199 contained 48 plants. All but 5 of these plants were typical *N. rustica humilis* plants with a variability scarcely greater than that of the maternal great grandparent. The 5 aberrant individuals were tall plants resembling *N. rustica texana*. Omitting the *texana*-like plants the general variability of the family would stand at 2, but its rating with them included is questionable. Similar remarks might be made for the other three families. Perhaps it is better to rest our conclusions on the 44 families remaining after the omission of these seven.

Two facts stand out impressively in this array of data:

(1) The families are all rather high in fertility and low in the variation in fertility within each group. Several families showed scarcely any discernible variation in fertility and No. 131 should probably be ranked the equal of its great-grandparents. Every ovule was capable of being fertilized, and the amount of abortive pollen (3 to 5 percent) was so small one would hardly suspect the origin of the strain. (2) The uniformity of the families, visualized as relative homozygosity of each parental F_2 individual, is much greater than one would expect. Twenty-two families, just one-half of the total, were rated 3 or less in general variability. When it is recalled that *N. paniculata* our No. 1 in the scale is the most uniform strain ever raised in our experimental garden, whether of *Nicotiana* or of

TABLE 6
Characteristics of the F₃ families of cross between N. rustica humilis and N. paniculata.

F ₃ FAMILY NUMBER	NUMBER OF PLANTS	TYPE	GEN- ERAL VARIA- BILITY	HEIGHT		LEAVES		FLOWERS		FERTILITY		NOTES
				Range	Varia- bility	Range	Varia- bility	Range	Varia- bility	Range	Varia- bility	
24	16	sh. <i>tex.</i>	3	80-100	3	15×12-23×22	3	21×16-22×18	2	8-9	2	1 type only
30	20	var.	6	50-110	7	9× 5-30×21	8	15×15-18×15	3	6-9	5	
33	51	<i>scabra</i>	2	110-135	2	20×15-24×18	2	18×16-19×17	2	8-9	2	1 type only
49	49	<i>texana</i>	4	80-150	5	20×12-32×23	4	17×15-19×16	3	9-10	2	1 <i>bras.</i> type
52	7	var.	6	70-110	5	20×14-23×16	3	20×13-21×19	5	7-9	4	
56	34	<i>hum.-tex.</i>	5	50-160	7	12×10-24×22	6	19×14-22×19	3	6-8	5	
64	30	sh. <i>tex.</i>	3	100-140	3	14× 8-25×16	3	20×16-22×15	2	5-8	4	1 type only
67	27	<i>scab.-tex.</i>	4	110-150	3	15× 9-25×22	5	18×15-19×15	2	8-9	3	4 plants 60 cm.
76	37	<i>texana</i>	4	70-160	6	13× 8-23×20	4	18×16-23×20	5	7-8	4	Few lvs.
79	38	Var.	6	70-150	6	15×10-25×19	6	18×13-22×18	5	7-9	3	lvs. obl.-lanc.
83	51	<i>humilis</i>	2	100-120	3	18×13-20×16	2	18×16-19×17	2	9-10	2	lvs. thick
85	51	<i>scabra</i> ?	2-	130-170	2	23×16-25×18	1	18×18-19×19	1	8	1	late maturing
87	50	<i>hum.-tex.</i>	4	60-120	3	15×10-28×22	4	17×13-19×19	4	7-8	2	
88	35	<i>texana</i>	4	90-160	4	20×15-32×28	5	18×17-19×18	2	8-9	2	lvs. <i>bras.</i>
89	28	sparsely lvd.	2	105-130	2	25×20-30×22	2	18×15-21×20	3	7-8	2	
90	25	new type	2	100-140	2	25×20-31×28	3	16×15-18×17	2	7-8	2	habit uniform 1
93	25	<i>hum.-tex.</i>	3+	80-120	4	20×12-28×24	4	16×14-20×18	3	7-8	2	
98	30	var. <i>tex.</i>	5	60-180	6	20×12-30×26	5	19×18-20×18	2	7-8	3	
101	3	tall <i>tex.</i>		130		26×22		18×15-18×16		8		
102	57	<i>texana</i>	4	80-150	4	15×10-28×22	5	18×14-20×18	4	7-8	3	
104	56	new type	6+	100-150	5	20×20-30×12	6	17×16-20×19	3	7-8	3	like No. 90
108	33	new type	4	50-150	5	20×12-28×24	4	18×17-21×20	4	7-8	2	tall laterals
110	21	<i>texana</i>	2	120-160	2	25×18-28×23	2	18×16-19×16	2	7-8	2	tall type
111	50	tall <i>bras.</i>	1+	100-120	1	24×19-28×26	2	18×15-18×16	1	8	1	late
114	44	<i>texana</i>	3	60-160	4	15×10-32×25	4	18×16-19×16	2	6-7	3	
115	52	new type	2	110-140	1	25×20	1	18×18-19×18	2	7-8	2	few lvs.

TABLE 6 (Continued)

122	36	<i>texana</i>	5	60-150	6	17×10-30×22	4	16×12-20×16	5	8-9	3	like No. 111
124	52	tall <i>bras.</i>	2	120-150	2	18×14-30×19	2	18×18-19×18	1	7-8	3	
131	40	tall <i>bras.</i>	1+	100-115	1	25×20-28×26	1	19×16	1	9	1	
139	50	<i>texana</i>	5	70-120	4	14×10-30×25	5	17×13-19×16	4	5-7	4	very large caps.
145	50	<i>scab.-tex.</i>	5+	60-190	6	15× 8-30×24	6	18×17-19×17	2	8-9	2	
146	35	<i>humilis</i>	3	50-90	3	15× 8-26×18	3	18×15-19×16	3	7-9	3	
149	52	tall weak	3	130-160	3	28×26-34×29	4	16×15-17×16	3	4-9	5	
150	104	tall <i>bras.</i>	2-	110-150	2	25×16-27×18	1	18×14-19×15	1	8-9	1	
159	53	<i>texana</i>	3	100-160	3	25×18-28×18	3	19×15-21×16	3	8-9	3	
163	54	sparse <i>tex.</i>	2	120-180	3	24×20-26×22	3	20×16-21×17	2	7-8	2	small type
166	47	<i>bras.-tex.</i>	4	90-130	3	26×20-28×24	3	18×16-19×17	3	5-8	4	
167	44	<i>texana</i>	5	35-140	6	8× 6-30×26	6	14×12-20×18	6	8-9	2	
168	25	elong. <i>hum.</i>	5	50-120	6	20×18-28×24	5	18×15-19×15	4	8-9	3	5 <i>texana</i>
176	11	6 tall <i>bras.</i>	4	110-130	3	18×16-26×22	4	18×15-19×16	4	7-9	3	
191	46	var.	5	40-150	7	15× 8-28×21	6	16×14-21×20	6	8-9	2	
192	43	var.	6	60-160	6	16×15-28×26	7	16×16-18×16	6	8-9	3	also lvs. 28×14 5 <i>texana</i> excluded
199	48	<i>humilis</i>	2?	60-100	2	12× 8-20×15	2	18×15-19×18	3	7-8	4	
213	59	<i>texana</i>	5	70-190	6	17× 8-34×26	6	18×13-20×18	3	7-8	3	
214	47	var. <i>hum.</i>	4	50-130	5	10× 6-25×20	5	18×18-20×18	3	7-8	4	lvs. sparse lvs. round 3 with small lvs. also 2 small pl.
217	50	var.	5	80-130	5	16×12-25×20	5	16×15-17×15	4	7-8	3	
219	43	new type	3	50-110	5	15×13-25×25	3	16×16-18×17	2	8-9	2	
228	45	<i>tex.-scab.</i>	3	110-180	3	28×20-32×25	2	18×15-20×16	2	8-9	2	
234	42	<i>tex.-scab.</i>	2+	160-180	1	20×16-28×22	2	18×16-19×16	2	8-9	2	
248	38	<i>texana</i>	3	100-180	5	18×16-30×26	3	19×17-21×19	3	6-8	3	
250	25	<i>humilis</i>	3	60-120	4	16× 8-20×16	3	16×15-17×15	2	8-9	2	

any other genus, the fact is extremely suggestive. Our varieties *texana*, *jamaicensis* and *asiatica* raised from stock self-fertilized for several years and presumably inbred for a long period of time, would have to be rated nearly 3 in variability.

*Fifty percent of the F_2 plants of this cross, therefore, were so homozygous as to produce populations comparing favorably with those found in the so-called pure varieties of *N. rustica* which had been collected from various sources.*

FIGURE 14.—Very small F_2 segregate *N. rustica humilis* $\times$ *N. paniculata*.

Now we hope there will be no misunderstanding of the position taken here. It is not maintained that each of these 22 families was as uniform as the "pure" varieties of *N. rustica* they most resembled. But it is believed that none exceeded the "pure" strains by more than one-half point on our scale of 10. In other words both the eye and the measuring-stick told us that the variability of the "pure" strains and the segregate strains was of the same general order of magnitude.

No descendants of the F_3 families of experiment 2 were carried further, but from the F_3 families of experiment 1, which, it will be recalled, were in every respect similar to those of experiment 2, several strains were carried through the F_4 , F_5 and F_6 generations,—selfed seed being used in each instance. There were eight families in the F_4 generation, and several of these were split up in the F_5 and F_6 generations. This series of strains does not include the progeny of one of the *paniculata*-like individuals of the F_2 generation, which as we have already stated, was kept under observation. This plant produced a population resembling pure *N. paniculata* in every detail. The family showed no trace of inheritance from *N. rustica*, and was as uniform as a population of the *paniculata* great-grandparent. The individual plants seeded freely. The capsules were full of seed and various samples of the pollen showed it to be from 96 to 100 percent perfect, morphologically.

The remaining strains clearly belonged to the *rustica* group. No systematist would have suspected that they were the descendants of segregates arising from a cross where *N. paniculata* was used as one of the parents. Six of the strains were similar to certain *N. rustica* varieties obtained from Dr. COMES. One was clearly *N. rustica humilis*, a second was *N. rustica asiatica*, and a third was *N. rustica brasilia* in appearance. Two other strains were unlike any plants of *N. rustica* we have seen, but nevertheless would have been classified as members of that species. The first of these was a giant form, figure 13 giving a good idea of its characteristics. The second was a small type in which the plants all resembled figure 14. The remaining strains were from variable F_3 families resembling combinations of COMES's varieties.

These strains were grown in order to try to answer two questions which are important in any theoretical interpretation of the results from the experiments considered as a whole. The first question concerns the variability of the families. The second question pertains to the fertility of the individuals.

The behavior of the F_3 families demonstrated conclusively that a much greater number of the F_2 plants were homozygous in the majority of their characters than one would have any right to expect had there been such recombination of hereditary factors as is usually found in varietal crosses, with no selective elimination of gametes and of zygotes. It is important then to know whether this apparent homozygosis is in truth a homozygosis of all the hereditary factors possessed by the individual, or whether recombination with the ultimate attainment of a greater degree of homozygosis is possible. Selfed individuals from the variable populations of F_3 ,

gave populations more uniform than those from which they came. This is probably what should have been expected. Again, two of the selfed populations from F_3 generations rating 2 in general variability, produced

FIGURE 15.— F_2 segregate *N. rustica humilis* $\times$ *N. paniculata* showing habit of growth not found in either grandparent.

populations in which leaf size in one case and habit of growth (this means somewhat more than mere height) in the second case were distinctly less variable than in the parental population. In no other instance could one

be certain that the 5 near-homozygous F_3 families tested had produced populations less variable than themselves, but it must be remembered how uniform these families were in the F_3 generation. Conversely, in no instance was a variability greater than that of the parental population observed.

In the case of fertility, not only was the variability of the parental population never exceeded by the progeny, but no individual of a population resulting from the selfing of a particular plant was ever less fertile than its parent. The succeeding generations always *increased* in actual fertility and in uniformity in the production of functional gametes and zygotes. In this series, only the two *paniculata*-like plants of F_2 , already described, seemed to have perfect gamete-producing mechanisms, although by the word perfect it is not meant to exclude the possibility of an occasional aborted pollen grain. In the remainder of the F_2 plants, and in their descendants, many plants were found in which the ovules were all functional, but it is probable that none reached the percentage of perfect pollen grains characteristic of pure *N. paniculata* and pure *N. rustica*. In these two pure species, individuals have been found where 100 percent of the pollen grains seemed to be morphologically perfect, but in general there was from 2 percent to 3 percent of abortive grains. Whether this low percentage of aborted grains is due wholly to environment or not I am unable to say. I suspect this to be true, however, because I have found at least that high a percentage of aborted pollen grains in some individuals of every "pure" species of plant I have ever examined. The most fertile plants among the descendants of this cross, excluding the two *paniculata*-like segregates, had about 4 percent of the pollen abortive, with the one further exception of a *brasilia*-like plant where about 2 percent of the pollen was imperfect. Thus while many plants which came out of this cross compare favorably in a general way with the fertility of the two pure species that entered into it, adequate statistical proof that they equaled the pure species in fertility is not available. I believe the general experience with the cross leads one to expect the ultimate production of individuals wholly as fertile as the pure races, but definite proof has not been forthcoming.

SUMMARY AND INTERPRETATION OF RESULTS

Those who have borne with us thus far no doubt will have been impressed with the fact that a very large amount of quantitative data has been more or less roughly lumped together in a descriptive manner. The danger in such a procedure is fully realized. It makes it difficult for the reader to

make up his mind as to whether the separate conclusions drawn are really established by adequate and critical data, or whether they are merely tentative. At the same time it must be clear to every one that the essential facts are rather broad and general in their nature, and could have been presented in no other way. I have dealt with the variability of the plants considered as units, rather than the variability of separate characters. In arriving at decisions, it was necessary to deal with characters separately, of course; but in weighting these data on the separate characters in order to accomplish the main objective,—an estimate of general variability,—personal equation necessarily enters. I believe the results justify the procedure, however, for two reasons. In the first place, if each plant character had been studied with all the refinements of modern statistical methods, it still would have been impossible to have judged the plants as units by an impersonal objective summation of these data. The subjective element would have been masked in a quantity of detail, but it would have entered into the final result. Furthermore, I believe that in such work as this the investigator who lives with his plants in the field, who uses all the quantitative data at his command, but who nevertheless brings to his aid all the somewhat intangible facts that intimate experience gives him, is able to come to a better realization of the truth than one who works over cold data obtained by others.

Our position in the matter having been made clear, let us endeavor to pick out the established facts which appear to be essential from the standpoint of genetic theory.

The two species, *N. rustica* and *N. paniculata*, species giving extremely uniform progeny when selfed, have produced reciprocal first-hybrid-generation populations almost identical with each other, and as uniform in all their characters as the parental populations themselves.

The proportion of fertile ovules formed by each F_1 plant was about the same; that is to say, careful manipulation would result in an average seed complement of about 3 to 4 percent of the normal. Each plant seemed to have this figure as a possibility. One might obtain no seed or any proportion of seed up to 8 percent after a pollination, but the variation was as great on one plant as another, and this variation may be taken largely as a mark of failure to provide the best conditions for fertilization plus the variation always existent in random sampling. At least one-half of these seeds will germinate.

An investigation of F_1 pollen formation leads me to believe that less than 0.1 percent of the possibilities of the pollen mother cells in the formation of pollen grains is ever realized, yet a study of mature anthers only

indicates that 2 to 3 percent of the pollen grains found there are functional. The histological results on pollen show that one is often seriously misled if conclusions as to what has happened during gamete formation are drawn from a casual examination of the contents of mature anthers. The pollen-germination studies showed further that apparent morphological perfection in a pollen grain is not to be construed as positive evidence of ability to function.

Taken at their face value the data on gamete formation seem to indicate that the formation of female gametes is some 40 times greater proportionately than the formation of male gametes. This result, however, can not be taken to mean that 40 functional female gametes per thousand and only 1 functional male gamete per thousand may be formed because of incompatible hereditary elements entering into the remaining non-functional gametes which do not come to maturity or do not function at maturity. A great part of this differential proportion in the formation of male and female gametes may be due wholly to factors of environment. In other words, the greater size of the cells, the larger amount of cytoplasm, or in general the better mechanism for gamete production in the ovary, make slight changes in temperature, osmotic pressure, etc., less effective in stopping gamete formation in the ovary than in the anther. There is considerable evidence for the truth of this statement in the fact that comparable percentages of seed were produced in each of the following cases, self-pollination, sib-pollination, use of pollen of either pure parental species on the flowers of the F_1 generation, and use of the pollen of the F_1 generation on the flowers of either parent.

The characters of the F_1 plants rather blended those of the two parents, but the resemblance to *N. rustica* was greater than to *N. paniculata*. The plants produced by back-crossing F_1 with each of the two parents, partook of more of the characteristics of the parent used. The variability of the back-crosses was considerably greater, statistically speaking, than that of the true F_1 generation, but botanically the plants were like true F_1 generations in that they were of one general type with much less variability than the F_2 generation in all characters.

The F_2 generation was inordinately variable. With the possible exception of certain flower relations, variation in all characters transgressed those of the grandparents markedly.

Numerous character combinations, predictable with random mating and no selective elimination of gametes or zygotes, were omitted. This statement is made after very careful consideration and a full realization of the theoretical implications. Combinations approaching *N. rustica* were much

more frequent than those approaching *N. paniculata*. It is not largely a question of salient characters being masked or of group heredity (i.e.,

FIGURE 16.—F₂ segregate *N. rustica humilis* × *N. paniculata*. Peculiar habit of growth.

linkage), for the characters have been considered separately as well as in groups. *N. paniculata* differs from *N. rustica* in numerous characters.

Some few *paniculata*-like characters can be located in a large percentage of the F_2 population. But when one tries to find combinations of these characters, he comes to an *impasse*. There is a gap between *rustica*-like plants with a few traceable *paniculata* characters, until plants appear which are indistinguishable from *N. paniculata*.

Segregation of determiners for fertility occurs in F_1 , resulting in certain F_2 plants giving full capsules of viable seed. With seed production taken as the criterion of fertility, the F_2 generation ranges from that of the F_1 generation to that of the pure strains. It may be that individuals inheriting less fertility than those of the F_1 generation appear, but there is no critical evidence that this is true. What may be taken as the facts of the case from the present evidence, is that a frequency curve of fertility on the female side runs from the 3 or 4 percent characteristic of the F_1 generation, to 100 percent, with a marked abmodality toward the side of high fertility. Judgment of the fertility of F_2 by examination of the pollen does not change this general conclusion materially. Adequate evidence of the production of perfect pollen equal to that of either parent,—98 percent maximum, shall we say,—has not been forthcoming, but plants having 96 percent of "good" pollen have been found.

Varietal distinction might be accorded almost everyone of the plants of the F_2 generation in the crosses where *humilis* was used as the *N. rustica* parent. The parental forms were recovered once in every 100 to 200 plants. It should be noted also that of the four plants resembling *N. paniculata* obtained, two were very fertile (one reproducing a characteristic *N. paniculata* population), and two were so sterile that no viable seed was obtained.

Reproductions of all of the named varieties of *N. rustica* were obtained in the F_2 of the cross with *N. paniculata*, though these forms could not all be obtained by intercrossing any two of the varieties themselves.

In the cross between *N. rustica scabra* and *N. paniculata*, about 15 percent of the F_2 generation consisted of delicate dwarf plants of similar type with lack of fertility seemingly correlated with delicate constitution. In the other crosses there were no noticeable correlations between fertility and other plant characters.

Observations on generations later than the F_2 were made only in the case of the cross between *N. rustica humilis* and *N. paniculata*. Three points stand out rather impressively: (1) As judged by F_3 progeny, the F_2 plants show a *much greater degree of homozygosity* than is the general Mendelian expectation in varietal crosses. (2) A selfed plant of the F_2 or of later generations produces progeny with equal or greater fertility

than itself. The few cases where a slightly less fertile daughter was obtained, probably may be attributed to physiological reasons unconnected with heredity. (3) Plants almost wholly fertile produce progeny heterozygous for many allelomorphs though the variability of the resulting population, in my experience, is always less (presumably it may be the same) than that of the population from which the parent plant was selected.

In my opinion these are the salient points which should be taken into consideration in seeking a theoretical interpretation of the experiments.

Though certain data were gathered in the years 1916, 1917 and 1919 which make the problem a little clearer, most of the facts reported here had been collected by 1915. In that year I gave a paper before the AMERICAN PHILOSOPHICAL SOCIETY which was reported in abstract in the Proceedings (54: 69-72). A provisional hypothesis (A) was rejected.

"(A) Through multipolar spindles, mating of non-homologous chromosome pairs at synapsis, or other mitotic aberrations at the reduction division, the 24 chromosomes characterizing each of the two species may be distributed irregularly at gametogenesis. If some of these irregular gametes may function, the majority of the experimental data are satisfied, but there are reasons which there is not time to consider which make this scheme improbable."

Obviously any peculiar case of heredity *may* be due to a rare or even a unique distribution of the chromosomes. There may be a doubling of chromosomes as in *Oenothera gigas* and the *Primula* investigated by GREGORY. An extra chromosome may be found as in *Oenothera lutea*. There may be the phenomenon of non-disjunction discovered by BRIDGES in *Drosophila*. These special cases of inheritance are important, however, only in so far as they show what peculiar results may be brought into line with the chromosome theory of heredity when it is found that the chromosomes do not behave in the usual manner. Such results make ridiculous the attitude of a certain type of scientist, who, when he obtains data he does not understand, immediately proclaims from the housetop that the whole structure of modern genetics has fallen. A special-mechanism hypothesis in interpreting the data presented here has been rejected for four reasons. (1) A special mechanism should never be invoked until a sustained effort has been made to interpret the facts by the method of chromosome distribution we have come to look upon as the *characteristic* type for both the higher animals and the higher plants. (2) Cytological observations in the hybrid plants under consideration warrant us in believing that the machine breaks down in certain cases, but that when it runs it runs in the usual manner. (3) Repeated crosses involving these species having given

similar results, and the behavior of other partially sterile species crosses that I have had the opportunity to observe (for example, the wheats) having been of the same general type, it seems likely that a rather general phenomenon is involved. (4) The facts can be given a "regular" Mendelian interpretation with a little subsidiary explanation.

The interpretation favored by the writer and submitted for consideration in 1915 was as follows:

"(B) On the other hand the facts may be interpreted without assuming irregularities of chromosome distribution if (1) there is a group of chromosomes in each parent that cannot be replaced by chromosomes from the other parent; if (2) there is a group of chromosomes from each parent, a percentage of which may be replaced by chromosomes from the other parent, but where functional perfection of the gametes varies as their constitution approaches that of the parental forms; if (3) there are other chromosomes that have no effect on fertility and therefore can promote recombinations of characters in the progeny of fertile F_2 plants; if (4) a naked nucleus entering the normal cytoplasm of the egg in the immediate cross can cause changes in the cytoplasm that will affect future reduction divisions; if (5) this abnormally formed cytoplasm is not equitably distributed in the dichotomies of gametogenesis in the F_1 generation; if (6) it follows from (4) and (5) that F_2 zygotes may be formed which are less perfect in their gamete-forming mechanism than those of the F_1 generation; and if (7) the heterotypic division of gametogenesis does not necessarily form two cells alike in their viability."

This conception of the matter satisfied the requirements of the data. Each part was added only after careful consideration. But perhaps the statements were too concise to be understood easily. At least no comments, either favorable or adverse, were made on them by other geneticists; and a year later (1916) GOODSPEED and CLAUSEN published an interpretation of the results of some crosses between *Nicotiana sylvestris* and *N. tabacum*, in which they used a conception practically the same as my (1) and (2). This conception they called a Reaction System. The data on which they based their ideas, were briefly as follows. The argument is taken from BABCOCK and CLAUSEN (1918).

Nicotiana sylvestris, a species belonging to the Petuniodes section of the genus, and showing no marked variations after years of cultivation, crosses freely with *N. tabacum* varieties. In any case the F_1 hybrids are uniform in their characters. They are very strong vigorous plants in which the influence of *N. sylvestris* may be seen, but this influence is almost hidden by the dominance [?] of *N. tabacum* characters. Furthermore the hybrids resemble the particular variety of *N. tabacum* used in the cross.

The hybrids are almost sterile, and it is doubtful whether any seeds can be obtained by self-fertilization or sib-crosses. When *N. sylvestris* pollen is used on the hybrids, they set about 1 percent of the normal complement

of seeds. The sesqui-hybrids thus obtained show diversity of type; but about 10 percent of them resemble *N. sylvestris* very closely, are almost completely fertile, and give rise to individuals which are seemingly pure

FIGURE 17.—F₂ segregate *N. rustica humilis* × *N. paniculata*, showing characters not found in either grandparent.

N. sylvestris. Similarly a cross with *N. tabacum* pollen gives a low percentage of variable sesqui-hybrids approaching *N. tabacum* in character, and those approaching *N. tabacum* most closely are the most fertile.

In passing it may be said that the writer made this cross in 1908, and the results obtained were very similar to those of GOODSPEED and CLAUSEN.

According to the Reaction-System conception, the normal functioning of a set of hereditary factors depends upon the harmonious interrelations which the factors maintain with each other. In the F_1 hybrids, the resemblance to *N. tabacum* is thought to indicate a more or less complete dominance of the *N. tabacum* factors as a complete Reaction System.

"Developing the reaction system hypothesis, it would appear that, if the *N. tabacum* and *N. sylvestris* systems display a high degree of mutual incompatibility, any gamete containing elements derived from both systems would give a reaction system subject to profound disturbances incident upon the inharmonious relations set up between the *N. tabacum* and *N. sylvestris* elements. If the admixture be relatively slight, the inharmonious elements may not greatly affect the workings of the reaction system and there would result individuals showing practically the entire set of characters of one or the other parent, and such individuals would be fully fertile. A slightly greater proportion of inharmonious elements in the reaction system would result in such profound disturbances in its functioning as to produce the abnormal individuals of various kinds which make up so large a proportion of the progeny from such parentage. When the proportions of inharmonious elements in the gametes become still greater, they fail to function at all. It is upon the formation of such non-functional gametes or the attempt to produce them, that the partial sterility of the hybrid depends, and since in this particular case these form by far the greater proportion of gametes, the hybrid is very nearly completely sterile."

BABCOCK and CLAUSEN have given a diagrammatic illustration of the scheme involved here by a table showing the recombination series in the gametes of F_1 . It is based on the fact that the haploid number of chromosomes in *N. tabacum* is 24, and on the assumption that the number in *N. sylvestris* is the same. It is reproduced here (table 7) because the number of chromosomes both in *N. rustica* and *N. paniculata* also appears to be 24, and the table will serve as a diagram in our further discussion. The gametic recombinations are given by the expansion of the expression $(1 + 1)^{24}$, the sum of the coefficients being 16,777,316. No crossing over is assumed. The diagram is not meant to be more than a possible illustration of the actual state of affairs in the germ-cells of the hybrid under discussion.

The real issue which GOODSPEED and CLAUSEN wished to call to the attention of geneticists is one to which the latter could scarcely have objection. It is one which has been discussed casually as an *obvious* genetic conception, and yet it has been the basis of criticisms directed against genetic workers by biologists who have been foolish enough to attribute to geneticists the naïve idea of a particulate inheritance in which unit characters, having no better working relation with each other than

bricks in a hod, are shuffled and combined at random. GOODSPEED and CLAUSEN deserve thanks for making it clear that geneticists have no such conception, that their real idea is that of a kind of reaction system in which the parts are parts of a smoothly working whole. Organisms may be likened to machines fitted for similar work. The same fuel, the same lubricants, the same paint may be used indiscriminately on either of two samples chosen at random without making much difference in the working

TABLE 7

Recombination series of gametes of F₁ of Nicotiana species crosses. (After Babcock and Clausen).

CONDITION OF GAMETES	<i>Tabacum:</i> <i>sylvestris</i> CHROMOSOMES	PROPORTIONATE NUMBER OF GAMETES	PROGENY WHEN POLLINATED WITH <i>N. tabacum</i>	PROGENY WHEN POLLINATED WITH <i>N. sylvestris</i>
Functional	24:0	1	Plants resembling the <i>N. tabacum</i> parent and of various degrees of fertility	Plants resembling the F ₁ and abnormal plants, but all nearly completely sterile
	23:1	24		
	22:2	276		
	21:3	2,024		
	20:4	10,626		
	19:5	42,504		
Non-functional	18:6	134,596	No viable seeds	No viable seeds
	17:7	346,504		
	16:8	736,321		
	15:9	1,307,504		
	14:10	1,961,256		
	13:11	2,496,144		
	12:12	2,705,456		
	11:13	2,496,144		
	10:14	1,961,256		
	9:15	1,307,504		
	8:16	736,321		
	7:17	346,504		
	6:18	134,596		
Functional	5:19	42,504	Plants resembling the F ₁ hybrid and nearly completely sterile	Abnormal, infertile plants and fertile plants closely re- sembling <i>N. sylves-</i> <i>tris</i>
	4:20	10,626		
	3:21	2,024		
	2:22	276		
	1:23	24		
	0:24	1		

efficiency. But let one try to interchange carburetor parts, engine parts, or driving mechanisms, and it becomes a question of whether these parts are closely enough related to be exchanged without upsetting the mechanism in its entirety. Just one misfit of an essential part, and the machine no longer goes.

With this idea we are wholly in agreement, but we wish to go a little further and try to picture the reproductive mechanisms of the plants

involved, in such a manner as to satisfy the known facts of our investigations.

In the first place let us consider the F_1 generation of the cross between *N. rustica humilis* and *N. paniculata*. The two parental strains having shown a very high degree of uniformity, one must assume that each is homozygous for practically all hereditary factors, that every gamete of each type has the same constitution, and that all of the F_1 zygotes are therefore alike. These assumptions fit in with the fact that the F_1 population showed no more variability than the parental populations. Again, one must assume that the cytoplasm of the germ-cells of the parents has little effect on the actual characters of the F_1 generation, because nearly all, and possibly all of the cytoplasm is furnished by the maternal parent, and the reciprocal F_1 generations are to all intents and purposes identical. But there is no reason why one cannot suppose that the cytoplasm helps lubricate the machinery of the reduction division, so to speak.

Turning now to the gamete production of the F_1 generation, let us glance at table 7. Manifestly the actual production of gametes falls far short of the theoretical production possible when none is aborted. But do the pedigree-culture facts and the cytological observations give us any reason for concluding how and why this abortion occurs? Let us see. Clearly the obvious suggestion is the one we have given in B (4) to the effect that there are "chromosomes in each parent that cannot be replaced by chromosomes from the other parent." As GOODSPEED and CLAUSEN would express it, certain chromosome combinations will fail to function because the reaction system has been upset. The simplest diagram of the situation is that suggested in table 7 where the 6 chromosome combinations at each extreme carrying 19 to 24 chromosomes of one parent and 1 to 6 chromosomes of the other parent are the only ones functioning, thus providing for about 7 functional gametes per thousand. The difficulty with this diagram is that it is too simple. The proportion of functional gametes is at least 30 per thousand, judging from the fertile ovules. In individual capsules it ran up to about 100 per thousand, but this high figure may have been a distorted result caused by insufficient samples. With due regard for the theory of random sampling, however, 30 per thousand is somewhere near the maximum limit. Of course the proportion of functional gametes appeared to be much less, but part of the sterility exhibited there may have been due to causes that had little to do with selection according to hereditary constitution. It seems to me that this is a fair deduction from the fact that reciprocal back-crosses produced about the same amount of seed. To fit these facts, therefore, one must suppose that

from 20 to 24 chromosomes of one of the parents must be present in the functional gametes produced by the F_1 generation.

Now the above assumptions will account for the sterility found in the F_1 generation, for the variability exhibited in the F_2 generation, and the frequent appearance of types like the grandparents which appear there. If a differential compatibility is assumed at the ends of the table, i.e., if more of the *paniculata* chromosomes must be present on the one hand than of the *rustica* chromosomes on the other hand, in order to produce functional gametes, the *rustica*-like appearance of all but a few F_2 individuals is accounted for. In fact one should assume functionality in the first case only when either 23 or 24 chromosomes of *paniculata* are present. But with these simple assumptions one does not fully satisfy the following conditions found in the F_2 generation, viz., the appearance of plants identical with other *rustica* varieties, and the appearance of homozygous individuals with great frequency.

These two phenomena seem to be related phenomena of considerable significance. Do we not have here an addition to the steadily accumulating evidence on simple Mendelian unit characters, that changes in the germ-plasm are largely changes in organization which affect only an end result? Here, for example, one finds in *N. rustica humilis* a variety capable of producing plants having certain definite characters. But when crossed with *N. paniculata*, the other well known varieties of *N. rustica* appear in the F_2 generation. Is this not an indication that in the germ-plasm of *N. rustica humilis* there exist the potentialities of producing all of the other varieties waiting only the chance for a particular reorganization which comes when the cross with *N. paniculata* is made? To the writer's mind the simplest interpretation of the appearance of these types and their appearance in relatively homozygous conditions, is that *N. rustica* and *N. paniculata* in their evolutionary divergence from each other have not varied in all of their chromosomes. One might suppose, for example, that among the 24 chromosomes of the two species, 12 are identical in composition, and therefore in function; 6 others differ in composition (unit factors) very slightly; while considerable evolutionary divergence has come about in the remainder. It is not necessary to draw a concrete picture of the results to be expected from these assumptions. It is clear that any degree of gametic sterility necessary to satisfy the experimental evidence can be obtained, that one can obtain many different types in F_2 and that many of these types will be to a high degree homozygous.

With these facts in mind, the postulates of B (1), B (2) and B (3) will stand, provided that in B (3) some of the chromosomes having no effect on

fertility are assumed to be identical in the two species. The postulates B (4) to B (7) were made to interpret what at that time was thought to be a fact,—recombination which gave plants of the F_2 generation *less* fertile than those of the F_1 generation. There is a grave difficulty in the way of interpreting such behavior on the assumption that difference in genetic constitution is the basic cause of sterility. The greatest degree of genetic difference, heterogeneity if you will, exists in the F_1 generation. Recombinations, whether by the shuffling of whole chromosomes, or by crossing over, increases homozygosity. *A less degree of genetic difference in the F_2 generation, therefore, cannot be the cause of greater sterility than was exhibited by the F_1 generation.* For these reasons, we interpreted the matter by assuming some sort of a mechanical control of the process of reduction by the cytoplasm, a cytoplasmic lubrication of the process, so to speak. Building on this foundation, increased sterility in the F_2 generation can be explained very clearly by the four postulates B(4) to B(7). Under our original presumption of fact, all of them are necessary, and none is on its face improbable. But since the evidence of lessened fertility in any of the individuals making up the F_2 generation is inadequate, and the proportion of individuals where a possible lessened fertility exists is very small, these hypotheses may be discarded as unnecessary. Lessened fertility in F_2 , if it exists at all, may be the result of special conditions unconnected with our assumption of the general correlation of sterility with a high degree of genetic heterogeneity. It can be interpreted by the assumption that special factor combinations enhance sterility. For example chromosome pair $\frac{AB}{AB}$ in one parent and chromosome pair $\frac{ab}{ab}$ in the other parent may function in such a way as to provide for fertility. The F_1 combination $\frac{AB}{ab}$ may also be fertile. But the combinations $\frac{Ab}{Ab}$ and $\frac{aB}{aB}$, possible after the formation of crossover gametes, may be sterile.

If we are to make a general summary of the results of this work, then, it will be an extension of certain conclusions reached by EAST and HAYES in 1912. In this paper a series of crosses between various *Nicotiana* species were discussed from the standpoint of their behavior as first-generation hybrids. Vigor of heterosis was seen to accompany hybridization far beyond that point of difference between the species entering into the combination which produced sterility. Then came a degree of difference which accompanied a sudden drop in vigor to a point below that of either parental type. The conclusion was that at a certain point differentiation in gametic

structure is so great that the reduction division with the formation of all the possible types of gametes does not take place in the normal manner. At this point of differentiation, however, ontogeny may go on normally. With a still greater differentiation in gametic structure even ontogenetic cell division does not proceed in the usual manner, and a marked lowering of vigor occurs. Accompanying a constant increase in the constitutional differences exhibited by the species entering into the hybridization, as assumed to exist from the behavior of the crosses, comes a constantly greater deficiency in ability to go through the life cycle, the last stage before total incompatibility is reached being the production of seeds having a partly developed endosperm but no embryo.

These experiments carry one of the ideas expressed at that time a little further. Numerous matings were made between two species in which differentiation had gone just far enough to cause the great majority of the gametes to cease to function; yet in all the behavior of the pedigree cultures and in all of the cytological observations, the evidence is that inheritance proceeds in a normal Mendelian manner. With similar gametes uniting to produce an F_1 generation, great uniformity results. Ontogenetic division has proceeded regularly in spite of the unlike constitutions of the two haploid sets of chromosomes possessed by each nucleus. When gametogenesis occurs in the immature germ-cells of the hybrids, the evidence is that it also takes place in a wholly normal manner, but that only a part of the resulting gametes are functional. What is more natural than to suppose that the gametes eliminated are those in which chance has thrown together such a nearly balanced lot of chromatin of different constitution that proper reorganization in preparation for their essential gametic functions is impossible? And the evidence of the pedigree cultures bears out this view.

Other inductions may be made from the behavior of the cultures, however, without drawing upon the imagination. In the first place, it is clear that if the assumption as to the cause of sterility be granted, more chromosomes of *N. paniculata* are necessary for proper functioning of the germ-cells when they are in the majority than when those of *N. rustica* provide the excess. Secondly, the variability of the F_2 generation, the peculiar types produced by a small number of F_2 seeds, and the behavior of the F_3 generations produced by self-fertilized F_2 individuals, make it seem probable that certain heterozygous combinations are formed but are eliminated as zygotes. It may be asked why, if the F_1 individuals go through their development normally when they must of necessity possess the greatest degree of heterozygosity, F_2 individuals should be eliminated? The ques-

tion is pertinent, but not unanswerable. The gametes of the parental strains are formed under natural conditions. They are perfect gametes. When fertilization occurs ontogeny proceeds in an orderly manner, and abnormal conditions are manifested only at gametogenesis. At gametogenesis some of the gametes expected are formed, others are eliminated. Does it not seem probable then, that a certain proportion of those functional gametes formed by the F_1 plants are still so abnormal in structure that when fertilization occurs development is difficult? Now it seems wholly reasonable to assume that difficulty of development is in this instance correlated with heterosis, i.e., with unlike chromosomes, just as difficulty in gametogenesis is similarly correlated in the F_1 generation.

It is further probable from the ratios obtained, that a considerable percentage of the chromosomes of the two species have not been differentiated in constitution, or have been differentiated very slightly. In fact this last assumption appears to be the simplest way of accounting for the occurrence of the duplicates of varieties *brasilica*, *scabra* and *texana*, in the cross with *N. paniculata* where only *N. rustica humilis* was used.

Finally, there is a rather peculiar circumstance for which to account in the comparatively low variability of the back-crosses of the F_1 generation with either parent. In varietal crosses similar matings give a less degree of variability than a true F_2 generation, but the extreme variability of type of the F_1 gametes is unquestionably brought out. Here the variability, though considerably greater than that of the true F_1 generation, is not such as would appear to be possible if all of the F_1 gametes functioning in the production of a true F_2 generation were to unite with the gametes of the pure species and produce viable zygotes. Confessedly, this phenomenon is somewhat of a puzzle. I can see in it only a further elimination of the extreme gametes of the F_1 , but just why such a peculiar thing should occur, is not clear. Perhaps our observations are distorted in that we had become accustomed to an inordinate variability in the combinations of gametes forming the F_2 generations, and had expected a greater variability than was proper when a series of like gametes mated with those of the F_1 generation. Possibly a variability standing half-way between that of the F_1 generation and the F_2 generation was not to have been expected. Certainly the variability exhibited by the back-crosses was more than that of an F_1 generation than one would suspect would be the midpoint between the F_1 generation and the F_2 generation. But this may simply be an erroneous idea.

Such, then, are the theoretical conclusions to be drawn from the data. But there is also a conclusion of practical significance which ought not to

be neglected. Not only these experiments but other critical experiments on plants indicate that the greatest opportunity for plant improvement and possibly for animal improvement as well, lies in crossing together types which have become as far differentiated from each other as is possible without producing complete sterility in the hybrids. When species differing in a large number of allelomorphs can be crossed, and the resulting hybrids are completely fertile, there is a wonderful chance for desirable recombinations occurring in the F_2 generation. In cases of this kind that we have studied, however, the appearance of the F_2 individuals and their behavior on further breeding indicate a large number of *minor* variations in the chromosomes. The variability of F_2 is great, it is true, but recombination of a great number of factors having a minor effect on the morphology or the physiology of the individual, gives a graded series difficult to distinguish the one from the other. Opportunity for selection is there, but with so many individuals massed in the intermediate classes, practical use of selection is rather difficult. On the other hand, when two species are still further apart in their constitution, so far indeed that partial sterility becomes characteristic of the F_1 individual, the chromosome differences produce marked results even though the great majority of the gametes and zygotes is eliminated. That is to say, the greater the differentiation of the protoplasm the greater the chance for extreme differentiation in the individuals which do pass through the selective sieve that eliminates so many. Therefore one may assume that the greatest opportunity for obtaining new and desirable domestic forms is by crossing types as different from each other as is possible with some degree of fertility in the F_1 generation.

Again, we believe that just those families and genera that have varied widely in nature, yet have retained that necessary physiological compatibility which allows them to cross and to produce more or less fertile hybrids, are the ones—the only ones—that have been *really important* as domestic plants and animals. Some of the genetic evidence, and most of the historical evidence relating to the important cultivated species indicates a polyphyletic origin. Such critical data as we have, therefore, indicate that it was possible to domesticate, to improve and to use the forms man has utilized, just because nature had prevented too great sexual incompatibility from accompanying the physical differentiation that had arisen in certain genera through natural selection. In fact we believe this conclusion has the dignity of a natural law; viz., the desirability of a type for domestication varies directly with the amount of physical variation and inversely with the amount of sexual incompatibility accompanying such variation. We

believe that plant breeders and animal breeders should emphasize this more and more in a practical way, that they should never lose sight of the fact that conscious selection of valuable types of organisms is a useful tool only when there is material for selection. And crossing, wide crossing, furnishes the greatest opportunity to obtain this material.

It has been the writer's experience that the evidence as to the polyphyletic origin of the great groups of domesticated plants and animals, based on historical and anthropological grounds, is not fully appreciated. When this evidence is compared with the genetic evidence of compatibility at hand, there is really an extremely good case *a priori* for asking the government to undertake practical plant breeding and animal breeding through hybridization on a much greater scale than is going on at present.

LITERATURE CITED

- BABCOCK, E. B., AND CLAUSEN, R. E., 1918 Genetics in relation to agriculture. xx + 675 pp. New York: McGraw-Hill Book Co.
- COMES, O., 1899 Monographie du genre *Nicotiana*. 80 pp. Naples: Topographie Coopérative.
- EAST, E. M., 1915 An interpretation of sterility in certain plants. Proc. Amer. Philos. Soc. 54: 70-72.
- EAST, E. M., AND HAYES, H. K., 1912 Heterozygosis in evolution and in plant breeding. U. S. Dept. Agric. Bur. Plant Ind. Bull. 243, pp. 58.
- FOCKE, W. O., 1881 Die Pflanzen-Mischlinge. iv + 569 pp. Berlin: Borntraeger.
- GÄRTNER, C. F. v., 1849 Versuche und Beobachtungen über die Bastarderzeugung im Pflanzenreich. xvi + 790 pp. Stuttgart: Hering.
- GOODSPEED, T. H., AND CLAUSEN, R. E., 1916 Mendelian factor differences versus reaction-system contrasts in heredity. Amer. Nat. 51: 31-46, 92-101.
- GOODSPEED, T. H., AND CLAUSEN, R. E., 1917 The nature of the F_1 species hybrids between *Nicotiana sylvestris* and varieties of *Nicotiana tabacum*. Univ. of California Publ. Botany 5: 301-346.
- SETCHELL, W. A., 1912 Studies in *Nicotiana* I. Univ. of California Publ. Botany 5: 1-86.